CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Bisphenol A (BPA) is a long- and well-known endocrine disruptor (ED), that has increasingly been receiving a considerable amount of attention from the scientific community as well as the general public, mainly because of its ubiquity in our environment and uncertainties about its effects on humans. For the most part, BPA enters the body by the ingestion of contaminated food or beverages. It leaks from polycarbonate plastics, which are used to line food and drink containers such as bottles and cans. Further minor ways of penetrating into the body are through the skin e.g. contact with thermal receipts (Ehrlich *et al.*, 2014, Liao and Kannan 2011a) or inhalation e.g. cigarette smoke or dust (Braun *et al.*, 2011a, He *et al.*, 2009).

Bisphenol A being an endocrine disruptor, can mimick the body's own hormones and may lead to adverse health effects (Okada *et al.*, 2008). It is commonly used in the manufacture of polycarbonates (PC) and epoxy resins used in consumer products which include food cans and beverage bottles. BPA is just one of many chemicals that leach out of food can linings (Shanthanagouda *et al.*, 2014). Due to its major applications in the production of plastic food or beverage containers and the coating of food cans, people of different ages are inevitably exposed to BPA in daily life. BPA is an estrogen mimic compound which results in an array of health impacts that are hormone related, including prostate and breast cancer (Pupo *et al*., 2012). The adverse effects of BPA are largely related to its estrogenic activity (Kurosawa *et al*., 2002). However, BPA has other effects such as inflammatory cytokine dysregulation (Ben-Jonathan *et al*., 2009), and mitochondrial mediated apoptosis in the hepatic tissue (Xia *et al*., 2014).

Studies in animal models have also shown that exposure to BPA leads to metabolic deficits, including disruption of glucose homeostasis (Alonso-magdalena *et al.,* 2010), altered mitochondrial function (Jiang *et al.*, 2014), and changes in growth rate during early development (Ryan *et al.,* 2010). Evidence for endocrine-disrupting chemicals interfering with lipid metabolism, free fatty acid (FFA) balance and transport, and oxidative stress is available in various species (Silins and Hogberg 2011). BPA augments the inflammatory response in rodents (Hugo *et al.*, 2008) and is associated with oxidative stress (Yang *et al.*, 2009).

1.2 STATEMENT OF THE PROBLEM

Studies have shown that BPA was toxic to laboratory animals at doses close to human exposures (Mikołajewska *et al*., 2015; EFSA, 2006; USEPA, 2011) and that the chemical causes toxic effects that are on the rise and very common in people (FAO/WHO, 2010). These disturbing facts raised questions about the extent to which current, widespread exposures to BPA are contributing to the burdens of human diseases. Epidemiological studies have reported positive correlations between increased BPA exposure and human pathologies, including metabolic disorders. The ubiquity of BPA and the range of target organs BPA impacts emphasize the need for a comprehensive evaluation and assessment of the health risks stemming from effects of BPA exposure and expansion of investigations to endpoints of relevance to various systems and their integrative functions.

With the decline in birth rate and the increases in infertility and mean age of women at first birth (Lim, 2011) reproductive health is becoming a major public health challenge in the Federal Republic of Nigeria. Accumulating evidence from animal studies has also suggested that BPA potentially cause a wide range of reproductive abnormalities (Lee *et al.*, 2003). With the growing epidemic of disease worldwide and the extensive use of consumer goods containing BPA, there is risk of BPA acting as a potential triggering compound in diseases. Many of the mechanisms known to exist in disease pathophysiology also appear to exist with immune reactivity from BPA exposure. In addition, severe oxidative stress resulting from exposure to BPA could lead to DNA damage and mutation of tumor suppressor genes (Trasande *et al.*, 2012).

1.3 AIM AND OBJECTIVES

1.3.1 AIM

The aim of this study was to unveil and establish the possible integrative effects and physiological disposition of Bisphenol A on sex and thyroid hormones, interleukins, inflammatory and oxidative stress markers in female wistar albino rats.

1.3.2 OBJECTIVES

The objectives of this study were:

i. To determine the LD_{50} of Bisphenol A in albino rats using the oral and subcutaneous route.
ii. To determine the effect of Bisphenol A on weight of rats
iii. To establish the effect of Bisphenol A on sex and thyroid hormones following sub-chronic exposure.
iv. To monitor the sub-chronic effect of orally administered Bisphenol A on interleukin profiles.
v. To monitor the effect of Bisphenol A on inflammatory biomarkers at sub-chronic exposure doses.
vi. To assay for oxidative stress biomarkers such as endogenous antioxidants, lipid peroxidation, peroxiredoxin, glutathione and thioredoxin systems.

1.4 RELEVANCE OF THE STUDY

This study will give a feature of epidemic of diseases that have their etiology either fully or partly from BPA exposures. These parameters assayed are part of the main regulators of the human physiology in several areas, for instance interleukins and nitric oxide are known to be cellular mediators.

1.5 SIGNIFICANCE OF THE STUDY

The significance of this study is, following the observation and monitoring of the parameters assayed, if it is confirmed that disease etiology arises fully or partly from BPA, then the study will help to readjust and change the attitude and behavior of the population to health care management. Also, it is anticipated that observing the toxicity profile at higher than normal exposure rate will assist in deducing the exact nature and pattern of low exposure dose toxicity. Moreso, in Nigeria, the use of BPA and BPA containing products are without any restriction.

CHAPTER TWO

LITERATURE REVIEW

2.1 CHEMISTRY OF BISPHENOL A

Bisphenol A, is an organic compound that has two phenol functional groups. It is prepared by the condensation reaction of acetone, and two equivalents of phenol (Liu *et al.,* 2013) with hydrochloric acid (Silver *et al.,* 2011). Typically a large excess of phenol is used to ensure full condensation.

$$(CH_3)_2CO + 2C_6H_5OH \longrightarrow (CH_3)_2C(C_6H_4OH)_2 + H_2O$$

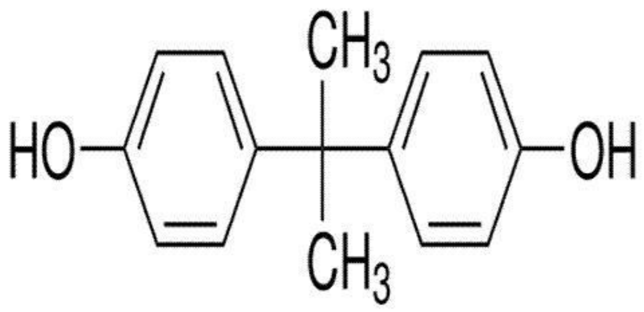

Fig. 1: Structure of Bisphenol A.

2.2 PROPERTIES OF BISPHENOL A

Bisphenol A is a white solid crystals or flakes with a mild phenolic odour at ambient conditions; its melting point is 155˚C and specific gravity is 1.060-1.195g/cm^3. BPA is fairly soluble in water with a solubility of 300g/m^3 at 25˚C. BPA has a relatively high boiling point of 398˚C at 760mmHg, and low vapour pressure of 5.3×10^{-6} Pa at 25˚C. The pKa value of BPA is between 9.59 and 11.30. BPA is considered as a hydrophobic compound of octanol-water partition coefficient (K_{ow}) of $10^{3.4}$ with a slight polarity due to the two hydroxyl groups, It has very high value of octanol-air partition coefficient (K_{oA}) of 2.6×10^{12} which suggests that BPA in gaseous form will sorb strongly to solid surfaces (Wang *et al.,* 2012). Its air-water partition coefficient (K_{aw}) is very low 10^{-9}, hence, BPA is unlikely to evaporate from aqueous solution. It is soluble in non polar solvent such as acetic acid and very soluble in ethanol, benzene, and diethyl ether (Vandenberg *et al.,* 2013). BPA molecules have a fairly strong fluorophore due to the conjugated Π-electrons in the two benzene rings. Its chromophore is relatively weak and sensitivity of UV detection is much low. Aerobic biodegration is the dominant loss process for BPA in river water and soil; and its biodegradation half life is about 4.5 days (Teppala *et al.*, 2012). Its loss process in the atmosphere is due to the rapid reaction with hydroxyl radicals and the photo-oxidation half life for BPA in air is about 4hours (Vandenberg *et al.,* 2010).

2.3 USES OF BISPHENOL A

Bisphenol A (BPA) is a monomer used primarily in the production of polycarbonate (PC) resins and epoxy resin widely used in consumer products (Trasande *et al.,* 2012), with other uses that include the synthesis of flame retardant, unsaturated polyester resin, polysulfone (PS) resin and polyetherimides (PEI) (Ehrlich *et al.,*, 2014). Another use for bisphenol A is in the plastic and rubber industries, as plasticizer and as a polymerization inhibitor in polyvinyl chloride (PVC). BPA is a preferred colour developer in thermal paper (Kinch *et al.,* 2015). BPA-based products are also used in foundry casting and for lining water pipes (EPA, 2010)

It is used in many consumer products as lacquers applied as food can linings and coating on metal lids for glass jars and bottle (Erickson 2010). Poly carbonate plastics, is used to make a variety of common products used in food contact materials such as infant feeding bottles, tableware, microwave ovenware, food containers, water bottles, milk and beverage bottles, processing equipment and water pipes, sport equipment, medical and dental devices, dental fillings and sealant, eye glass lenses, compact disk (CDs), digital visual disk (DVDs) and house hold electronics (NTP, 2008). It is used in products like adhesive and flooring materials and in paints and varnishes. Bisphenol A, no doubt is one of the world's highest production volume environmental chemical (Ritter 2011)

2.4 HUMAN EXPOSURE TO BISPHENOL A

It is known that the major exposure route to BPA is via the diet. Exposure to BPA during infancy directly occurs through breast feeding, PC bottle feeding and infant formula feeding. The global population is subject to repeated exposure to BPA, primarily through packaged food, drinking and bathing water, dental sealants, dermal exposure, and inhalation of household dusts (Lakind *et al.*, 2012) with detectable amounts of metabolites in the urine of >90 % of the population worldwide (Ye *et al.*, 2008). Daily dietary intake has been estimated to be about 0.2μg/kg body weight in breast-fed babies, 2.3μg/kg body weight in formula-fed babies using non-PC bottles, 11μg/kg body weight in formula-fed babies using PC bottles, and 1.5μg/kg body weight in adults. Assessment of daily human exposure to BPA in the general population by biomonitoring of urinary excretion of BPA metabolites has been estimated to be up to 0.16μg/kg body weight in the USA, and 0.04-0.08μg/kg body weight in Japan. (Xiaoqian and Hong-Sheng 2014).

Bisphenol A is well absorbed by the oral route. Due to its properties, bisphenol A can be easily released from the polymer product, in which it is present, and migrate into the environment. The ester bond connecting the BPA molecules in polycarbonates or epoxy resins is hydrolyzed during heating or in acidic or alkaline medium. As a result, free BPA is released and it migrates into the food, beverages and into the environment. In addition, migration is enhanced by repeated washing with detergents, rubbing and sterilization (EFSA, 2006).

Based on the studies conducted at multiple research centers worldwide (see Table 1-11), the tolerable daily intake of this compound has been set at 0.05 mg/kg body weight/day (Table 2 and 6) as a safe dose for humans (EFSA, 2006). In a study on food stored in cans coated with epoxy resin under conditions corresponding to the sterilization process (canning), significant amounts of BPA, 70–90 μg BPA per 1 kg of the medium were detected in the preserved foods or model (food-simulating) liquids (EFSA, 2006). Goodson *et al.,* (2002) found that the average content of BPA in meat products was 110 μg BPA/kg of meat (17–380 μg/kg) (Goodson *et al.*, (2002)). Thomson and Grounds (2005), who studied foods in market found that concentrations of bisphenol A in fish ranged from < 20–109 μg/kg. In individual samples, bisphenol A concentrations reached 109 μg BPA/kg of tuna fish, while for beef meat the corresponding value was 98 μg BPA/kg of canned beef (Table 11). In the drinks, BPA concentration was below 10 μg BPA/kg. Brede *et al.,* (2003) evaluated BPA migration into model (food-simulant) liquids from the bottles intended for infant feeding (Table 2). The authors subjected the bottles to a variety of procedures, including: multiple washing, cooking and brush abrasion. After the bottles had been filled with water (100°C) and stored for 1 h, mean BPA concentration determined in the new

bottles was 0.23 µg BPA/l of the liquid (range: 0.11–0.43–µg/l), while for the bottles subjected to 51 washing cycles in the dishwasher, the average concentration was 8.4 µg BPA/l (range: 3.7–17 µg BPA/l), while after 169 washing cycles the corresponding value was 6.7 µg BPA/l (range: 2.5–15 µg BPA/l) (Table 2). Ehlert *et al.,* (2008) also reported concentrations ranged from below 0.1 to 0.7 µg/l. Maragou *et al.,* (2008) after 12 bottle sterilization cycles noted decreased BPA migration to water (2.4–14.3 mg/kg). Exposure of infants to BPA that migrated from feeding bottles into the water or milk ranged from 0.2–2.2 mg/kg b.w./day (Table 2).
Bisphenol A concentrations in soft drinks and foods stored in cans with epoxy coating also have been analyzed (Table 11). The determined BPA concentrations are greater than 7 µg BPA/l of liquid. However, the average concentration of BPA in canned foods was 40 µg BPA/kg (Table 11). Based on that data, value of the migration level of BPA was estimated to be 10 µg/l and the upper level was estimated at 50 µg BPA/l. Significantly higher BPA levels, 100 µg/l, were determined for infants 0–6 months of age due to predominance of the liquid dairy products fed from plastic bottles capable of releasing significant amounts of bisphenol A (see Table 7-9) (EFSA, 2006).
There has been research done on dietary exposure to BPA (Table1-4 and Table 7-9), depending on the age and the source of food intake (EFSA, 2006). According to data reported by EFSA (EFSA, 2006), breast milk fed infants are at the lowest risk of the dietary exposure: 0.2 µg/kg b.w./day. In the 3-month-old infants fed milk from bottles made of polycarbonate plastic, the exposure is 4 µg/kg b.w./day for normal levels of migration, or 11 µg/kg b.w./day for the high levels of migration. Bisphenol A exposure of 6–12-month-old infants varies depending on the type of food intake, particularly of milk and other beverages, including water, fruit juice and other foods stored in plastic containers, with the possible risk of exposure to 8.3 or 13 µg BPA/kg b.w./day (Table 7-9), depending on the scenario of migration (EFSA, 2006). In addition, taking into account the highest level of BPA in model (food-simulating) liquids (5 µg BPA/kg), exposure from this particular source is low, 0.3 µg of BPA/kg b.w./day (Mikołajewska *et al*., 2015). The highest values of exposure to BPA have been determined among infants and young children due to their frequent contact with feeding bottles, toys and other items containing BPA (Table 7 and 10). Under normal conditions, migration of bisphenol A from plastic bottles into the water or milk for infants is estimated to range from below 10 to 20 µg BPA/l of liquid (Mikołajewska *et al*., 2015).
As can be seen from the data below, exposure to this compound through a diet changes with age, and in children aged 1.5 years it is 5.3 µg BPA/kg b.w./day (EFSA, 2006), while in adults it is 1.45 µg BPA/kg b.w./day, after taking into account all the factors that may affect these values such as body weight, the amount of food eaten and beverages drunk and the levels of BPA due to migration of this compound to beverages and foods (EFSA, 2006).
Data on the toxicological reference values and dietary exposure to BPA are summarized in Tables 1–4.

Table 1. Reference values of bisphenol A (BPA) determined from research results

Parameter	**Tolerable value of BPA**	**Reference**
Tolerable Daily Intake (safe dose for humans)	0.05 mg/kg b.w./day (50 µg/kg b.w./day)	USEPA (2011)
Specific Migration Limit for European Union	3 000 µg/kg food	EFSA (2006)
Concentrations of BPA migration to foods and beverages	10 µg/l 50 µg/l (high concentration)	EFSA (2006) IHCP (2003)
Concentrations of BPA migration for 6-month infants	100 µg/l	Mikołajewska *et al*., (2015)

Table 2. Concentrations of bisphenol A (BPA) determined in different matrices

Matrice	**Concentration of BPA**	**Reference**
Canned foods	40 µg/kg (70–90 µg/kg food or simulants)	EFSA (2006)
Canned beverages	< 7 µg/l simulants	EFSA (2006)
Meat products	110 µg/kg meat (17–380 µg/kg meat)	Goodson *et al.,* (2002)
Beef	98 µg/kg	IHCP (2003)
Fish	< 20–109 µg/kg	Thomson and Grounds (2005)
Beverages	< 10 µg/kg	Thomson and Grounds (2005)
Migration to water from plastic bottles for infants	< 10–20 µg/l	Kersting *et al.,* (1998)
Migration to water from plastic bottles for infants (water 100°C)	0.23 µg/l (0.11–0.43 µg/l)	Brede *et al.,* (2003)
Migration to water from plastic bottles for infants after multiple washing in the dishwasher	2.5–17 µg/l	Brede *et al.,* (2003)
Migration to water from plastic bottles for infants after heating in microwave oven	< 0.1–0.7 µg/l	Ehlert *et al.,* (2008)
Migration to water from plastic bottles for infants after sterilization	2 400–14 300 µg/kg	Maragou *et al.,* (2008)
Dust	< 0.5–10 200 µg/kg	USEPA (2011)
	553 µg/kg dust (117–1 486 µg/kg dust)	Völkel *et al.,* (2008)
	1 460 µg/kg dust (535–9 730 µg/kg dust)	Geens *et al.,* (2009)
	820 µg/kg dust	Rudel *et al.,* (2003)
	6 530 ng/g (4 865–8 380 µg/kg) in offices	Geens *et al.,* (2009)
Air	0.734 ng/m3 (0.1–1.8 ng/m3) (inside)	USEPA (2011)
	< 0.1–2.5 ng/m3 (outside)	Wilson *et al.,* (2001)
	0.004–17.4 ng/m3	Fu and Kawamura (2010)
	4.55 ng/m3 (0.2–17.4 ng/m3)	Fu and Kawamura (2010)
	1.04–4.51 ng/m3	Wilson *et al.,* (2001)
Drinking water	0.0011 µg/l (0.0005–0.002 µg/l)	Kuch and Ballschmiter (2001)
River water	0.0047 µg/l (0.0005–0.014 µg/l)	Kuch and Ballschmiter (2001)
Industrial and municipal wastewater, leachate from landfill	0.016 µg/l	Kuch and Ballschmiter (2001)
	41 µg/l (28–72 µg/l)	Fürhaker *et al.,* (2000)
	18 µg/l (2.5–50 µg/l)	Fürhaker *et al.,* (2000)
	21 µg/l (10–37 µg/l)	Fürhaker *et al.,* (2000)
	n.d.–5.8 µg/l	Vinggaard *et al.,* (2000)
	< LOD–2.5 µg/l	Fürhaker *et al.,* (2000)
Paper	40 µg/kg paper	Vinggaard *et al.,* (2000)
	550–24 100 µg/kg	Vinggaard *et al.,* (2000)

Table 3. Bisphenol A (BPA) intake by adults from different sources.

Source of intake	Daily intake of BPA for adults	Reference
General intake	0.0004 µg/kg b.w./day	Geens *et al.,* (2009)
General exposure to BPA from different sources	0.008–1.5 µg/kg b.w./day	Geens *et al.,* (2009), NTP (2008)
Dietary exposure (Canned food and beverages)	0.008 µg/b.w./day	Thomson and Grounds (2005)
	max 0.29 µg/kg/day	Thomson and Grounds (2005)
	0.57–6.9 µg/day	Thomson and Grounds (2005)
	1.7–2.7 µg/person/day	Dekant and Völkel (2008)
	1.45 µg/kg b.w./day	(EFSA, 2006)
	1.56–10.453 µg/day	von Goetz *et al.,* (2010)
Daily exposure to BPA through inhalation	0.008–0.014 µg/person/day	USEPA (2011)
Dust	0.029–0.244 µg/day	Geens *et al.,* (2009)
	0.0084–0.109 µg/day	Loganathan and Kannan (2011)
Dental	0.215 µg/day	von Goetz *et al.,* (2010)
Exposure to BPA from thermal paper / other than thermal paper	71 µg/day (exposure by 10 h/day)	Biedermann *et al.,* (2010)
	0.017–0.541 µg/day (general population)	USEPA (2011)
	1.303–40.59 µg/day (occupationally exposed)	USEPA (2011)
	0.0001 µg/day (other paper)	USEPA (2011)
	0.0001–1.41 ng/day (paper currencies)	Liao and Kannan (2011a)
	0.0007–14.1 ng/day (paper currencies – occupational)	Liao and Kannan (2011b)

Table 4. Bisphenol A (BPA) consumption by children from different sources

Source	Daily intake of BPA for children	Reference
Dietary exposure	1.088–4.992 μg/day	von Goetz *et al.*, (2010)
	1.7–2.7 μg/day	Wilson *et al.*, (2007)
General exposure to BPA of infants fed from plastic bottles	0.2–2.2 μg/kg b.w./day	Maragou *et al.*, (2008)
Exposure to BPA of infants fed breast milk	0.2 μg/kg b.w./day	EFSA (2006)
Exposure to BPA of 3-month infants fed from polycarbonate bottles	4 μg/kg b.w./day (according to its normal concentration of migration)	EFSA (2006)
	11 μg/kg b.w./day (according to high migration)	USEPA (2011)
Exposure of 6–12-month infants to BPA, depending on food intake	8.3 μg/kg b.w./day (according to its normal concentration of migration)	EFSA (2006)
	13 μg/kg b.w./day (according to high concentration of migration)	USEPA (2011)
Exposure of 1.5 year old children to BPA, depending on intake of commercially available foods and beverages	5.3 μg/kg b.w./day	CEC (1993)
Exposure to BPA from different sources	0.043–14.7 μg/kg b.w./day	Geens *et al.*, (2009),
Dental materials	0.215 μg/day	von Goetz *et al.*, (2010)
Inhalation of airborne dust	0.0078–0.014 μg/day	Wilson *et al.*, (2007)
Dust	0.042–0.435 μg/day	USEPA (2011)
	0.073–0.975 μg/day	Geens *et al.*, (2009)

Table 5. Values of daily intake of bisphenol A (BPA) with soil, dust, and soil+dust (USEPA 2011 and 2008)

Exposure factor	BPA intake (mg/day) children/teenagers				adults
	6 weeks < 1 year	1 < 6 year	3 < 6 year	6 < 21 year	
Soil	30	50	200	50	20
Dust	30	60	100	60	30
Soil+dust	60	100	200	100	50

2.4.1 Environmental exposure

a. Dust and air

Bisphenol A may be present in the dust. It is believed that dust may be important in the case of exposure of children, who are playing on the floor and frequently happen to put their hands into their mouth (Mikołajewska *et al*., 2015). However, because of the low vapor pressure of bisphenol A (5.3×10^{-9} kPa at 25°C), inhalation exposure to this compound is likely to be a small part of the overall exposure to BPA (IHCP, 2003). Current tolerable values for daily soil/dust ingestion for children differ, depending on age. Table 5 and 2 shows the central tendency and the high end recommendations for tolerable daily ingestion (in mg/day) of soil, dust or soil + dust for children, and also for adults (USEPA, 2011). The estimated daily exposure to BPA is 0.008–0.014 µg/person/ day, while the daily exposure to BPA by eating foods contaminated with BPA is 1.7–2.7 µg/person/day (Mikołajewska *et al*., 2015), so the inhalation exposure is about 200 times lower than the dietary BPA intake with food. According to Geens *et al.,* (2009), the average intake of BPA by an adult human is 0.4 ng BPA/kg b.w./day.

Exposure to BPA from other sources is 0.008–1.5 µg/kg b.w./day for adult person and 0.043–14.7 µg/kg b.w./day for 1.5–6-year-old children, in whom the value of exposure to dust is negligibly low (Table 3,4). Widespread use of BPA in household products (carpeted floor, boards, adhesives, paints, electric devices, etc.) (Mikołajewska *et al*., 2015), has increased the interest in the dust as a matrix to study BPA content. Völkel *et al.*, (2008) detected BPA in dust house samples (N = 474) at concentrations from 117 to 1486 µg/kg dust. Similar concentrations of BPA in house dust samples (USA, Massachusetts) were recorded by Rudel *et al.,* (2003). The average concentration was 820 µg/kg dust, but the analysis was carried out in 118 samples. In turn, higher concentrations of bisphenol A in dust collected from 18 houses and 2 offices in Belgium were reported by Geens *et al.,* (2009). Bisphenol A concentrations ranged from 535 to 9730 ng/g, whereas in the 2 samples of office dust they were 4.5 times higher – 6530 ng/g dust (4865–8380 ng/g) (Geens *et al.,* 2009). In 56 dust samples, Loganathan and Kannan (2011) detected BPA at concentrations from < 0.5 to 10 200 ng/g. In 44 samples of dust collected in houses from 2 other localizations in 2006 and 2010, the concentrations ranged < 0.5–2320 ng/g. Wilson *et al.,* (2001) studied the content of BPA in the indoor air of the daily care facilities in North Carolina, USA. The concentrations determined there were from < 0.1 to 1.8 ng/m^3 (0.734 ng/m^3) in the air inside the premises and comparable concentrations of BPA, < 0.1–2.5 ng/m3, were assayed in the air outside the facilities. Simultaneously, concentrations in floor dust samples were a bit higher (1.04–4.51 ng/m^3).

In urban areas of India, Japan, China and New Zealand, Fu and Kawamura (2010) detected much higher concentrations of BPA in the air, from 0.004 to 17.4 ng/m3, i.e., much higher than those reported by Wilson *et al.,* (2001). BPA concentrations in rural areas of China were 0.030–0.240 ng/m^3 (N = 5). The highest level of average BPA concentration in the air 4.550 ng/m^3 (0.2–17.4 ng/m^3; N = 49) was reported in India (Cennai, India, 2007). Wilson *et al.,* (2007) examined environment of 257 children at the age of 1.5–5 years, in randomly selected households and care facilities in North Carolina and Ohio, USA. They studied BPA concentrations in environmental samples (soil, indoor and outdoor air, house dust) and personal samples, inter alia hand wipes. Wilson *et al.,* (2000) also analyzed solid and liquid foods, and drinking water. Moreover, BPA was detected in children's liquid and solid food samples, in below 70% and over 80% of the samples, respectively. In addition, BPA was detected in over 50% of the samples of food preparation surface wipes, hard floor surface wipes, indoor air and transferable residue samples. They found BPA almost in all the samples from hand wipes of children (Wilson *et al.,* (2007).

b. Water

Bisphenol A is often found in water at concentrations of the order of a few ppm (1 ppm corresponds to 998.859 μg/l). Bisphenol A is one of the most frequently detected endocrine disruptors in the environment (Mikołajewska *et al*., 2015). Bisphenol A, and also other endocrine disruptors, are not completely eliminated from the effluents during their processing in the waste water treatment plants (Table 2). Moreover, bisphenol A may be released to the environment from BPA-contaminated waste buried in soil. Bisphenol A may be also released from the soil and contaminate ground water (Kuch and Ballschmiter (2001). Kuch and Ballschmiter (2001) tested samples collected from waste water treatment plant, surface waters (river, lake) and drinking water in South Germany. Bisphenol A was detected in all the tested samples of drinking water, with the mean value of 1.1 ng/l (range: 0.5–2 ng/l). In the river water the concentration of BPA was 4.7 ng/l (range: 0.5–14 ng/l), whereas the waste water contained 16 ng/l (4.8–47 ng/l) of BPA. The highest concentration of BPA, 28–72 μg/l, was observed by Fürhaker *et al.,* (2000) in the samples of waste collected from a paper manufacturing plant. Samples of waste from chemical industry contained 18 μg/l (range: 2.5–50 μg/l, N = 4); BPA content of household and municipal influent was 21 μg/l (range: 10–37 μg/l, N = 7). Bisphenol A concentrations in the waste water treatment plant effluent ranged from non-detectable (n.d.) to 2.5 μg/l (household, food industry, hospital, washing and cleaning up company).

2.4.2 Other sources of exposure

- **Dental materials**

Other source of BPA (see Table 2-4 and Table 7 and 10), which may influence the risk of exposure includes dental materials. It is likely that bisphenol A, as a pollutant formed during the synthesis of dental fillings, can be released to the human saliva through enzymatic hydrolysis by esterases found in saliva (Mikołajewska *et al*., 2015). Fung *et al.,* (2000) observed that BPA is detected in saliva 1 h after filling the defect, and becomes non-detectable after 3 h, whereas in serum it is not detectable after 24 h. In saliva, the concentration of BPA ranged from 5.8 to 105.6 ppb (Table 3). This is 250 times lower than in the studies carried out by Olea *et al.,* (1996), where the concentrations in saliva ranged from 3.3 to 30.0 ppm (Olea *et al.,* (1996), Fung *et al.,* (2000). These differences could be due to different quantities of dental sealant used to fill the defect. Still, BPA intake from a dental sealant is much lower than the dose of BPA from the dietary intake.

Arenholt-Bindslev *et al.,* (1999) analyzed saliva collected at 3 stages: immediately after filling, 1 h and 24 h after placement of a dental sealant. Average fill weight was 38±3 mg. Bisphenol A concentration determined in the test samples immediately after placement of the sealant was 1.43 ppm (0.3–2.8 ppm), whereas after 1 h and after 24 h the concentrations of BPA in saliva were $\leq$ 0.1 ppm (below the limit of detection). An estrogenic activity was observed in saliva samples only

immediately after placement of the dental sealant, and it was significantly different ($p < 0.05$) from the concentration in saliva control samples (morning saliva on fasting).
Olea-Serrano *et al.*, (1999) observed slightly higher amounts of BPA (90–865 μg) in saliva collected after 1 h from applying the sealant (50 mg). Samples containing the highest amounts of BPA showed estrogenic activity in the proliferation assay. These values provide useful data for the assessment of risk to people.

- **Paper**

BPA, the exposure does occur, especially through dermal contact with products containing BPA (e.g., thermal printer paper) (Mikołajewska *et al*., 2015). People who are particularly exposed include those who have frequent contact with thermal paper, for at least 10 hours daily (Table 10). In such instances, the possible exposure to BPA in cash register attendants may reach the value of 71μg/day. Ozaki *et al.*, (2004) observed higher concentrations of BPA in the recycled paper (0.034–0.36 mg/kg) than in the virgin materials (0.19–26 mg/kg). Lopez-Espinosa *et al.*, (2007) analyzed paper containers for storing take away foods in terms of content of hormonally active chemicals, e.g., BPA. In the food packaging materials they determined concentrations of BPA from 0.00005 to 1.81 mg/kg paper. Whereas Vinggaard *et al.*, (2000) determined higher concentrations of BPA than Lopez-Espinosa *et al.*, (2007) in extracts of virgin paper ranging 0.03–0.1 mg/kg and 0.6–24.1 mg/kg in recycled paper (kitchen rolls) (Lopez-Espinosa *et al.*, (2007), Vinggaard *et al.*, (2000). Some cigarette filters may contain up to 25% of BPA, and may be an important source of exposure to this compound, especially in pregnant female smokers (Mikołajewska *et al*., 2015).

Table 6. Evolution of the reference values: TDI and SML related to BPA in plastic food contact materials

Year	TDI (mg/kg bw/day)	SML (mg/kg food or food simulant)	Reference
1986	0.05	3.0	SCF (1986)
2002	0.01	0.6	SCF (2002)
2006	0.05	0.6	EFSA (2006)
2010	0.05	0.6	EFSA (2010)
2011	0.05	0.6*	CD (2011)
2015	0.004 (4 μg/kg bw/day)	0.6*	EFSA (2015.)

* **Restriction**: BPA not to be used for the manufacture of polycarbonate infant feeding bottles.

Table 7. Summary table on average (A) and high (H) exposure (µg/kg bw/day) from dietary and non dietary sources to BPA in the different age groups of the general population. Dermal doses are expressed as equivalent oral doses.

Age group	Exposure level	Dietary Oral Food and beverages	Non-dietary Oral Dust and toys	Dermal Thermal paper	Cosmetics	Sum of non-dietary
Infants 1-5 days (breastfeed)	A	0.225	-	-	-	-
	H	0.435	-	-	-	-
Infants 6 days-3 months (breastfeed)	A	0.165	0.009	-	-	0.009
	H	0.600	0.015	-	-	0.015
Infants 4-6 months (breastfeed)	A	0.145	0.009	-	-	0.009
	H	0.528	0.015	-	-	0.015
Infants 0-6 months (formula fed)	A	0.030	0.009	-	-	0.009
	H	0.080	0.015	-	-	0.015
Infants 6-12 months	A	0.375	0.009	-	-	0.009
	H	0.857	0.015	-	-	0.015
Toddlers 1-3 years	A	0.375	0.007	-	-	0.007
	H	0.857	0.012	-	-	0.012
Children 3-10 years	A	0.290	0.003	0.053	0.008	0.064
	H	0.813	0.005	0.424	0.016	0.445
Adolescents 10-18 years	A	0.159	0.002	0.113	0.015	0.13
	H	0.381	0.003	1.036	0.029	1.068
Adults 18-45 years	A	0.126	0.0006	0.071	0.012	0.084
	H	0.388	0.001	0.650	0.024	0.675
Other adults 54-65 years	A	0.126	0.0006	0.071	0.012	0.084
	H	0.341	0.001	0.650	0.024	0.675
Elderly 65 years and above	A	0.116	0.0006	0.071	0.012	0.084
	H	0.375	0.001	0.650	0.024	0.675

Source: EFSA (2015)

Table 8. Dietary exposure to BPA estimates for different age groups of the general population (EFSA, 2015.)

Population	**Average exposure (µg/kg bw/day)**	**High exposure 95% percentile (µg/kg bw/day)**
Infants (1 - 5 days) - *breastfed*	0.225	0.435
Infants (6 days - 3 months) - *breastfed*	0.165	0.600
Infants (4 - 6 months) - *breastfed*	0.145	0.528
Infant (0-6 months) – *formula fed, non-PC bottle*	0.030	0.080
Infants (6 - 12 months)	0.375	0.857
Toddlers (1-3 years)	0.375	0.857
Children (3-10 years)	0.290	0.813
Adolescents (10-18 years)	0.159	0.381
Adults (18-45 years)	0.132	0.388
Adults (45-65 years)	0.126	0.341
Elderly (65 years and over)	0.116	0.375

Table 9. International dietary exposures to BPA estimates from model diets for different age groups of the population (FAO/WHO, 2010)

Population	Mean exposure (µg/kg bw/day)	High exposure (95th percentile) (µg/kg bw/day)
Infants (0-6 months) *exclusively breastfed*	0.3	1.3
Infants (0-6 months) *Formula (powder-liquid), PC bottle* *(best case - worst case)*	2.0 - 2.4	2.7- 4.5
Infants (0-6 months) *formula (powder-liquid), no PC bottle* *(best case - worst case)*	0.01 - 0.5	0.1-1.9
Toddlers (6-36 months) *breastfed + solid food* *(best case - worst case)*	0.1	0.3-0.6
Toddlers (6-36 months) *formula, PC bottle + solid food* *(best case - worst case)*	0.5 - 0.6	1.6 - 3.0
Toddlers (6-36 months) *formula, no PC bottle + solid food* *(best case - worst case)*	0.01-0.1	0.1-1.5
Children (over 3 years old) *(fruits, vegetables, meat, soups, carbonated drinks etc.)* *(best case - worst case)*	0.2-0.7	0.5-1.9
Adults *(fruits, vegetables, grains, meat, soups, carbonated drinks, tea, coffee, alcoholic beverages etc.*) *(best case - worst case)*	0.4-1.4	1.1 -1.2

Table 10. Non-dietary exposure to BPA (µg/kg bw/day) in different age groups of the general population (EFSA, 2015)

Source and route of exposure	**Age group of population**	**Average exposure (µg/kg bw/day)**	**High exposure (µg/kg bw/day)**
Thermal paper (dermal)	Infants (0-1 year)	not applicable	not applicable
	Toddlers (1-3 years)	not applicable	not applicable
	Children (3-10 years)	0.069	0.550
	Adolescents (10-18 years)	0.094	0.863
	Adults	0.059	0.542
Cosmetics (dermal)	Infants	0.005	0.009
	Toddlers	0.003	0.005
	Children	0.002	0.004
	Adolescents	0.003	0.005
	Adults	0.002	0.004
Dust (oral/ingestion)	Infants	0.009	0.015
	Adults	0.0006	0.001
Toys (oral/ingestion)	Infants	0.0002	0.0006
	Toddlers	0.00001	0.00001
Air (inhalation)	Infants & toddlers	0.0007	0.0014
	Adults	0.0002	0.0003

TABLE 11. BPA Levels (ng/g ww) in Canned and Plastic Wrapped Human Foods

Food	pH	Package	BPA level (ng/g ww)
Del Monte fresh cut green beans	6	Can	65.00 59.90 26.60 22.70 19.70
Progresso light homestyle vegetable and rice soup	6	Can	15.60 11.70 10.80
Progresso classics vegetable soup	5	Can	7.28 10.70 8.81
Progresso classics tomato basil soup	4	Can	8.17 7.05 6.34
Campbell's condenced chicken noodle soup	7	Can	4.46 5.59 5.12
Hormel chilli with beans	6	Can	3.47 5.04 4.44
Chef Boyardee spaghetti and meatballs	5	Plastic	4.31 3.97 2.74
Kroger sweet peas garden variety	6	Can	2.65 3.77 3.47
Enfamil premium LIPIL infant formula milk based	7	Can	0.97 1.31 0.92
Beach cliff sardine in water	7	Can	0.81 1.23 0.77
Campbell's chicken noodle soup 3% fat	6	Can	0.76 0.88 0.85
Swason's white premium chunk chicken breast	7	Can	0.82 0.80 0.74
V8 100% vegetable juice	4	Can	0.71 0.49 0.47
Tree top 100% apple juice from concentrate	4	Can	0.46 0.78 0.54
Summer crisp whole kernel golden sweet corn	7	Can	0.37 0.32 0.25
Hormel SPAM	7	Can	<0.20
campbell's chicken noodle soup 2.5%	6	Can	<0.20
Similac advanced infant formula	7	Can	<0.20
Similac isomil advance soy infant formula	7	Can	<0.20
Pinepple chunks in pineapple juice	4	Can	<0.20
Gerber graduates spaghetti rings in meat sauce	5	Can	<0.20
Chef Boyardee mac and cheese	6	Can	<0.20

Chicken of the chunk light tuna in water	6	Can	1.66 4.16 2.30	Kroger slimlite ultimate creamy chocolate milk	8	Can	<0.20
Kroger mixed vegetables	6	Can	2.29 2.00 1.50	Bumble bee chunk light tuna in water	6	Can	<0.20
				Sprouts organic cinnamon apple sauce	4	Plastic	<0.20
Campbell's chunky savory pot roast	6	Can	1.46 1.71 0.81	Carnation evaporated milk vitamin D added	7	Can	<0.20
Kroger canned beef	6	Can	0.80 1.24 1.00	Hunts 100% natural tomato paste.	5	Can	<0.20

2.5 METABOLISM OF BISPHENOL A

2.5.1 ABSORPTION

BPA is rapidly and extensively absorbed from the gastrointestinal tract and dermal absorption is relatively high. Morck *et al.*, (2010) reported 13% absorption via the human skin. Marquet *et al.*, (2011) measured an *in vivo*percutaneous absorption flux of 0.4µg/cm^2/hour in rats. Zhai *et al.*, (2014), on the other hand, observed absorption of 46% via the human skin. A penetration of 8.6 % with a maximum penetration rate of 0.022 µg/cm^2/hour was measured (Demierre *et al.*, 2012). Research published in 2002 shows that when bisphenol A enters the body, it is rapidly absorbed in the gastrointestinal tract and is metabolized in the liver and the intestine (Kurebayashi *et al.*, 2002). Some of the ingested BPA is excreted with the urine, sweat or feaces in a form of inactive metabolites of BPA; bisphenol A sulfate (Völkel *et al.*, 2002).

2.5.2 DISTRIBUTION

BPA accumulates into adipose tissue (Rubin 2016) and is present in body fluids of the normal population. BPA has been detected in the human placenta (Schonfelder *et al*., 2002), cord blood (Wan *et al*., 2010), amniotic fluid (Yamada *et al*., 2002; Nishikawa *et al.*, 2010), fetal liver (Cao *et al*., 2012) and breast milk (Sun *et al*., 2009; Carwile and Michels 2011), and in fetus (Nishikawa *et al.*, 2010). BPA is well distributed in a wide range of organs, in the following order, predominantly detected in the lung, followed by kidneys, thyroid, stomach, heart, spleen, testes, liver, and brain. Ratios of the organ to serum BPA concentrations for the organs ranged from 2.0–5.8, except for brain ratio, 0.75, (Vitku *et al.*, 2015). Balakrishnan *et al*., (2010) recently reported that BPA easily passes through the placental barrier. BPA concentration in maternal milk is about 1-3µg/L.

Fig.2: Food packaging, metal food and beverage cans, and plastic bottles; The largest exposure humans have to BPA is by mouth from such sources as food packaging, the epoxy lining of metal food and beverage cans, and plastic bottles.

2.5.3 METABOLISM

After oral dosing, BPA is removed rapidly from the blood by first pass metabolism in the liver (Taylor *et al.*, 2011,). BPA metabolism is dominated by phase II conjugation reaction in the hepato-intestinal tract (Rodríguez-Ramírez and Reyes-Romero 2013). BPA undergoes substantial presystemic phase II metabolism primarily in the gut (Mourad and Khadrawy, 2012), and the liver (Rodríguez-Ramírez and Reyes-Romero, 2013), to BPA glucuronide (BPA-G) and BPA sulfate (Rezg *et al.*, 2014). Bisphenol A is glucuronidated by liver microsomes and the glucuronidation is mediated by UDP-glucuronosyltransferase (Xiaoqian and Hong-Sheng, 2014). The system keeps recycling the BPA glucuronide, after glucuronidation in the liver, some the resultant glucuronide is excreted into the bile (Inoue *et al*., 2001). while the BPA sulfate that is now water soluble gets excreted in urine, faeces and sweat.   This first-pass metabolism limits the internal exposures to aglycone BPA. Kharrazian (2014) proposed a scheme to illustrate the hepatic biotransformation of Bisphenol A (see fig. 3)

2.5.4 EXCRETION

The main route of excretion is via faeces. The available data indicate that the percentage of the administered dose of BPA recovered in the faeces is in the range of 5–18%. Urinary excretion is secondary, with 13–22% of the administered dose, recovered in the urine (Rodríguez-Ramírez and Reyes-Romero 2013). In adults, based on study, the estimated daily removal of bisphenol A with urine was 0.21–1.4 mg/day. This corresponds to < 0.003–0.023 μg BPA/kg/day intake. Urinary BPA excretion in healthy adults between consecutive days (N = 5) varied from < 0.058 to 0.13 μg/day (Arakawa *et al.*, 2004).

Because BPA is well absorbed into the body by ingestion, pregnant women, infants and young children are particularly vulnerable to BPA. The risk of adverse health effects may be due to the increased absorption and decreased excretion of BPA from the body (Mikołajewska *et al*., 2015), and also due to several factors, such as e.g., body weight, metabolic rate.

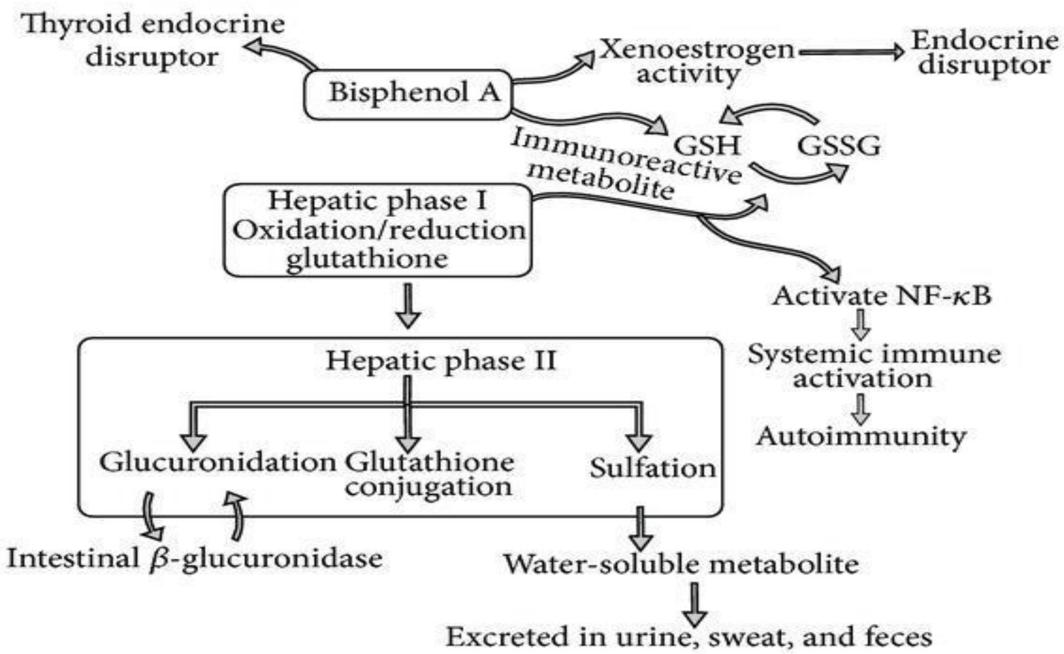

Fig.3: Illustration of hepatic biotransformation of bisphenol A (Datis Kharrazian(2014).

2.6 TOXICITY

Environmentally relevant doses (10µg-50µg/kg/day) of BPA can cause effects on human development and reproduction. Reports have shown that BPA causes; increases in weight and size of the prostate gland, decreases in sperm efficiency, and earlier puberty in female offspring of exposed mouse mothers (NTP., 2008). Low doses of BPA caused abnormalities in the oocytes (Durando *et al*., 2007). Invernizzi (2013) showed that *in utero* exposure to BPA trigers ductal and alveolar structures proliferations, development of ductal hyperplasia (Murray *et al*., 2007), modifications of the mammary gland architecture (Moral *et al*., 2008), mammary carcinogenesis (Jenkins *et al*., 2009), inflammatory cytokine dysregulation (Ben-Jonathan *et al*., 2009), and mitochondrial mediated apoptosis in the hepatic tissue (Xia *et al*., 2014).

Reported health implications associated with BPA exposure include diabetes (Lang *et al.*, 2008), cardiovascular disease (Melzer *et al.*, 2010), altered liver enzymes activities (Oguazu *et al.*, 2015) and obesity-promoting effects (Oguazu and Ezeonu, 2017). BPA alters glucose homeostasis, increased pancreatic insulin content and induced insulin resistance (Alonso-Magdalena *et al.*, 2012), BPA induces oxidative stress (Mourad and Khadrawy, 2012), coronary artery disease (Melzer *et al.*, 2012), activates Maxi-K ion channels in coronary smooth muscle cells (Asano *et al.*, (2010), increased blood pressure (BP) and decreased heart rate (HR) (Bae *et al.*, (2012), increased risk of hypertension (Erickson, 2010), decreased efficiency of sperm production (Mourad and Khadrawy 2012) and increased ovarian cancer cell proliferation in a dose-dependent manner (Park *et al*., 2012).

BPA is capable of inducing toxic effect on non-reproductive vital organs; several studies have reported that absorption of BPA has cause extensive damage to the liver and kidney (Ezeonu *et al.*, 2015; Oguazu *et al.*, 2015), formation of multinucleated giant cells in liver hepatocytes, DNA adduct formation and induced the production of free radicals in hepatocytes *in vitro* (LaKind *et al.*, 2012). Indeed, this chemical compound may be involved in adipose tissue dysfunction, metabolic/endocrine dysfunctions, cancer and fertility problems (Shankar and Teppala*et al.*, 2011, Wang *et al.*, 2012), impaired plasma glucose (Teppala *et al.*, 2012), involved in insulin resistance (Alonso-Magdalena *et al.*, 2010), causes permanent chromosomal damage linked to recurrent miscarriage and birth defects (Vom-saal and Hughes., 2005), spur both the formation and growth of fat cells (Braun *et al.*, 2011).

The potential of the lowest doses of BPA can inhibit microtubule polymerization, affect the spindle apparatus and produce aneuploidy (NTP, 2008), congression failure, chromosomal misalignment on the meiotic spindle, and aneuploidy in oocytes (Popa *et al*., 2014), Synaptic abnormalities and recombination aberration increased in oocytes (Mourad and Khadrawy, 2012), and alterations of various brain nuclear receptors, alongside, increased progesterone receptor immunoreactivity (Braun *et al.*, 2011), and enhanced antagonism at thyroid receptors (Jung *et al*., 2007).

BPA provoked an increase in body weight (Oguazu and Ezeonu, 2017), and adipose tissue weight (Miyawaki *et al*., 2007), alteration in adipogenesis and an increase in white adipose tissue and over expression of some adipogenic genes (Mourad and Khadrawy, 2012) and increase lipid accumulation in the differentiating adipocytes and upregulates the expression of adipocyte proteins through the activation of glucocorticoid receptor (Invernizzi, 2013). BPA suppresses low glucose-induced intracellular calcium oscillation on α-cells *Ex vivo* (Alonso-Magdalena *et al*., 2010), increase the activation of the transcription factor CREB (Braun *et al.*, 2011), abnormal levels of the liver enzyme γ-glutamyl-transferases, alkaline phosphatase and lactate dehydrogenase (Oguazu *et al.,* 2015; Lang *et al*., 2008).

According with this, concerns have been raised also on the developmental neurotoxicity and neurobehavioural effects of BPA. Miyagawa *et al.,* (2007) reported impaired memory, increased aggressiveness (Jones and Watson, 2012), alters anxiety (Stump *et al.,* 2010), loss or reduction of sexual dimorphisms (Christensen *et al.,* 2012), loss of sex difference in corticotrophin-releasing hormones (Funabashi *et al.,* 2004), reduced the number of tyrosine by dioxylase-immunoreactive (Rubin *et al*., 2016).

Neonatal exposure to BPA significantly increased the number of TH-ir cells (Patisaul *et al*., 2013), decrease the volume of the substancial nigra and fewer dopaminergic neurons (Tando *et al*., 2007), reduction of TH immuno reactivity, increased apoptotic cell death decreased gene expression of mesencephalic dopamine transporter (Ishido and Suzuki 2010), accelerates neuronal differentiation, migration and abnormal thalamocortical projections (Nakamura *et al*., 2010), accelerates formation of the dentate gyrus of hippocampus, decreased the proliferation of neural progenitor cell (NPC), cytotoxicity, increased number and length of neuritis and levels of β- III-tribulin (Kim *et al*., 2009). Neuronal cell death was induced by BPA (Lee *et al*., 2007), promotion of neuronal differentiation (Kim *et al*., 2009), and suppression of proliferation of neural progenitor cell (Kim *et al*., 2007). Ishido and Suzuki, (2010) found inhibitory effect of BPA on neural stem cell migration and proliferation, activation of protein Kinase Pathways via the extracellular signal-regulated kinase (ERK), with neurotoxicity that is cell death (Lee *et al*., 2008).

There are also evidences indicating that developmental exposure to BPA perturb the neurotransmitter; BPA alter the dopaminergic system either at presynaptic or at post synaptic level (Ritcher *et al*., 2007), decrease in brain expression levels of TH, the rate-limiting enzyme for DA synthesis (Christensen *et al.,* 2012), changes in gene expression of dopamine transporter (Miyagawa *et al*., 2007), altered dopamine metabolism (Honma *et al*., 2006), and activation of dopamine receptor mediated G-proteins (Narita *et al*., 2007). The cholinergic system is a target for BPA (EFSA, 2010), such that *In vitro* studies have suggested that BPA affect gamma-amino butyric acid type A receptor (GABA A) (Choi *et al*., 2007), inhibited the expression of ERβ protein and increased aromatase (Xu *et al*., 2010), increase in norepinephrine, decreases in DOPAC in hippocampus and increases 5-hydroxyindole-3-acetic acid (5-HIAA) in brain stem, and 3-4-dihydroxyphenylacetic acid (DOPAC) in striatum (Matsuda *et al*., 2010), increased levels of DOPAC in the candate and dorsal raphe nucleus, increased 5-HIAA in the caudate, dorsal raphe nucleus, thalamus and substantia nigra (Nakamura *et al*., 2010), suggesting BPA exposure might perturb the neurotransmitter system (Padgett *et al.,* 2013).

Also, BPA exposure has been associated with autoimmune pathophysiology. BPA disruption of cytochrome P450 enzymes (see fig. 4) by downregulation, and alterations in immune function, is a potential mechanism for autoimmune pathophysiology induced by BPA exposure (Lee *et al.,* 2012).

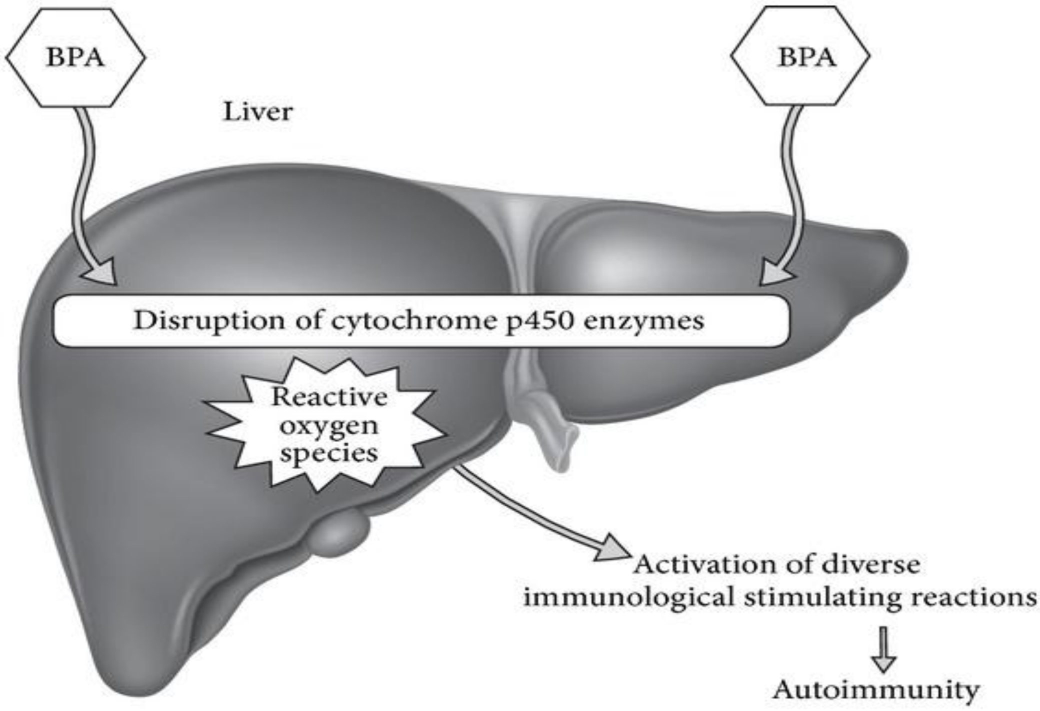

Fig.4:BPA disruption of cytochrome P450 enzymes (Datis Kharrazian(2014)

It was reported that female and male health has been seriously threatened, and the environmental pollution was thought to be the main reason of this phenomenon (Wilcox and Bonde 2013). BPA is a common environmental estrogen with endocrine Interference effect, and can affect multiple organs of human (Shankar *et al.,* 2013; Li *et al.,* 2012; Holladay *et al.,* 2010). One research revealed that BPA can affect the function of thyroid gland and may disrupt the function of nuclear hormone receptors and their cofactors to disturb the internal hormonal environment (Moriyama *et al.,* 2012). Braun *et al.,* suggested that prenatal exposure of BPA may be associated with externalizing behaviors in children (Braun *et al.,* 2011b). Gestational BPA exposure affects behavioral and emotional regulation domains in children (Braun *et al.,* 2011b). An animal experiment reported that BPA exposure promotes a podocytopathy with proteinuria, glomerular hyperfiltration and podocytopenia (Olea-Herrero *et al.,* 2014), raises the levels of many kinds of cytokine and induce developmental immunotoxicity (Holladay *et al.,* 2010). As an environmental estrogen, BPA can affect the functions of reproductive system and lead to infertility. Fujimoto *et al.,* suggested that BPA exposure interfere with oocyte quality (Fujimoto *et al.,* 2011), influence semen quality parameters (Knez *et al.,* 2014), decrease sperm count and quality, and DNA damage in somatic and germ cells (Dobrzynska and Radzikowsha 2013). BPA up regulated cluster in expression in atrophic prostate epithelial cells and induced lipid peroxidation and DNA fragmentation in spermatozoa (De-flora *et al.,* 2011). Although the body of ED research is continuously expanding, there still exist uncertainties in the process of BPA degradation in the body. It has been thought that BPA is rapidly metabolized in the liver and excreted in the urine within hours (Volkel *et al.,* 2002, 2011). But a recent study showed that during fasting BPA levels did not decline rapidly, suggesting a substantial non-food-related exposure and accumulation of BPA in tissues (Stahlhut *et al.,* 2009).

2.7 HORMONAL INFLUENCE OF BISPHENOL A

2.7.1 SEX HORMONES

The ability of BPA to alter responses to sex hormones in both male and female organism has been investigated. Environmentally relevant BPA levels (10µg – 50µg/kg/day) has adverse effects on testicular function by decreasing pituitary luteinizing hormone (LH) secretion and reducing leydig cell steroidogenesis (Akingbemi *et al.,* 2004). Serum testosterone concentration was decreased (Rogers *et al.,* 2013), suppression of Testosterone production via decreased luteinizing hormone secretion, BPA interfere with luteinizing hormone (LH) receptor ligand binding (Rajakumar *et al.*, 2015). (Deng *et al.*, 2004) found that plasma free testosterone levels were dramatically decreased, decrease in Testosterone levels, (Mendiola *et al.,* 2010; Tohei *et al.,* 2001) and plasma concentrations of luteinizing hormone were increased (Tohei *et al.,* 2001). Low dose BPA decreased the expression of steroidogenic enzymes and testosterone production *in vitro* (Nanjappa *et al.,* 2012). Similarly, BPA decreased testosterone levels in human, mouse, and rat fetal testes cultured *in vitro* (N'Tumba-Byn *et al.,* 2013). The *in vitro* study of Zhang *et al.,* (2011), reported that BPA suppressed estradiol (E2) catabolism but without altering aromatase activity. On the other hand, studies from *in vitro* and *in vivo* experiments focused on the impact of BPA on aromatase activity have yielded results (Castro *et al.,* 2013). BPA exposure impaired the hippocampal synaptogenic response to estradiol (Leranth *et al*., 2008) and prevent testosterones synthesis (Leranth *et al*., 2008). Li *et al.,* (2010) reported reduced sexual desire, erectile or ejaculation difficulty, and reduced sexual satisfaction. Li *et al.,* (2011) reported decreasing sperm concentration, total sperm count, sperm vitality and motility. Cha *et al.*, (2008) reported decreased testosterone levels and increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels. Hanaoka *et al.,* (2012) showed decreased FSH levels. Maohua *et al.,* (2015) reports higher prolactin (PRL) level with BPA. BPA can also bind to membrane estrogen receptor (mER), and these receptors are capable of nongenomic steroid actions (Peretz *et al.,* 2014). Low level of BPA exposure produces a calcium flux (Ezeonu *et al.,* 2015) which may lead to prolactin release (Mok-Lin *et al.*, 2010). BPA can also induce prolactin gene expression and cell proliferation (Zhai *et al.,* 2014).

In an animal study, BPA resulted in an increase in serum PRL levels (Peretz *et al.,* 2014), which is also known as hyperprolactinemia as seen in fig. 7 (Goloubkova *et al.,* 2010). Maohua *et al*., (2015) reports increase progesterone and E2 level. Altered E2 among exposed workers (Zhai *et al.,* 2014), low FSH and high LH (Maohua *et al.,* 2015). Perinatal (Xi *et al.*, 2011) and postnatal (Fernández *et al.,* 2010; Tan *et al.*, 2013) low dose BPA exposure increased serum estradiol levels, testosterone and progesterone levels (Fernández *et al.,* 2010; Tan *et al.*, 2013), disruption of estrous cyclicity (Adewale *et al.,* 2009), increased testosterone production (Fernández *et al.*, 2010), and ovarian cysts (Newbold *et al.,* 2009). Multiple studies have shown a relationship between BPA exposure and steroid hormone production (Peretz *et al.,* 2014). BPA exposure caused a decrease in peak serum estradiol levels (Ehrlich *et al.*, 2012; Bloom *et al.,* 2011), with increased testosterone and androstenedione levels in women (Kandaraki *et al.,* 2011).

Although epidemiological studies on BPA suggests that both low and high doses of BPA increase plasma estradiol, testosterone, and corticotropin releasing hormone levels (Tan *et al.,* 2013), luteinizing hormone excess (Hampton 2013), increased testosterone, androstenedione levels and insulin resistance (Kandaraki *et al.,* 2011); Low dose BPA decreased progesterone levels (Berger *et al.,* 2008), decreased estradiol, testosterone, aromatase, and steroidogenic acute regulatory protein (Lee *et al.,* 2013), decreased expression of estrogen and progesterone receptors, (Berger *et al.,* 2008), inhibition estradiol, testosterone, androstenedione, estrone, dehydroepiandrosterone, and progesterone production (Peretz *et al.*, 2011; Ziv-Gal *et al.*, 2013). However, BPA, increases testosterone synthesis (Zhou *et al.*, 2008), decreased progesterone synthesis (Zhou *et al.*, 2008), increased estradiol levels, while higher doses ranging between 1 and 10 µM decreased estradiol levels.

In male species, most researches reported a decrease in testosterone levels and steroidogenic enzymes (Castro *et al.*, 2013; El-Beshbishy *et al.*, 2012). Zang *et al.*, (2016) observed decrease testosterone synthesis *in vivo* and suppress sexual functions, testis weight and relative testis weight (Zang *et al.*, 2016), high serum Androstenedione concentration (Zhou *et al.*, 2013). Androstenedione is the precursor of testosterone; hence, decreased Androstenedione levels may reduce the conversion to testosterone (D'Cruz *et al.*, 2012b). Also, BPA decreased production of Androstenedione *in vitro* (Zhang *et al.*, 2011). In another study, Zhou *et al.*, (2013) observed a positive association between serum BPA concentration and the serum sex hormone-binding globulin SHBG level. Experimental studies indicate that BPA exposure decreases hormone levels in male. Rodent and *in vitro* studies have suggested that BPA has both estrogenic and antiandrogenic effects (Dcruz *et al.,* 2012a). Studies have reported that BPA exposure is associated with a variety of adverse effects on reproductive system including decreased steroidogenesis in the testis, and decreased serum follicle stimulating hormone (FSH) and testosterone levels (Fenichel et al 2013). Hanaoka *et al.*, (2012) reported an inverse correlation between BPA level and serum FSH concentrations in men. Meeker *et al.*,(2010) observed urine BPA concentrations were positively associated with the FSH level and inverse relationships between urinary BPA and the free androgen index (FAI) and estradiol (E2). Mendiola *et al.*, (Mendiola *et al.,* 2010) found an inverse association between urinary BPA concentration and FAI levels as well as a significant positive association between BPA and sex hormone-binding globulin (SHBG) levels. Galloway *et al.,* (Galloway *et al.*, 2010) found that higher daily BPA excretion was associated with a higher total testosterone concentration. Takeuchi and Tsutsumi (2012) also found significant positive correlations between serum BPA concentrations and total testosterone and free testosterone (FT) levels. Zhou *et al.*, (2013) study observed significant inverse associations between serum BPA concentration and serum AD, FT, and FAI levels. The serum BPA concentration was positively associated with the serum SHBG level. Epidemiological studies have investigated the association between BPA exposure and serum hormone levels in men. Increased BPA exposure, increased serum testosterone (Galloway *et al.,* 2010), decreased free androgen index (FAI), the ratio of FAI to luteinizing hormone, and the ratio of free testosterone to luteinizing hormone in male (Mendiola *et al.*, 2010), increased serum levels of follicle stimulating hormone, but decreased levels of ratio of estradiol to testosterone (Meeker *et al.,* 2010). These suggest that BPA can alter steroid hormone pathways also in men.
Low dose of BPA exposure decreased testosterone levels (Xi *et al.*, 2011). Urinary BPA was reported to be positively associated with plasma E2 (Lassen *et al.*, 2014). Vitku *et al.*, (2015) observed, increases of E2 and E1, decreased dihydrotestosterone levels (Sánchez *et al.,* 2013), increased the plasma estradiol to testosterone ratio (Nicholson and Ricke 2013), increased androgen receptor, aromatase, and estrogen-related receptor gamma gene expression (Arase *et al.*, 2011). Zhou *et al.*, (2013) also observed an inverse association between the urinary BPA concentration and free androgen index (FAI) levels. Zhou *et al*., (2013), showed that serum BPA concentration was also inversely associated with the serum FT level. Mendiola *et al.*, (Mendiola *et al.,* 2010) found a suggestive inverse correlation between creatinine-adjusted urine BPA concentrations and FT (Mendiola *et al.*, 2010). However, Takeuchi and Tsutsumi (Takeuchi and Tsutsumi 2012) reported positive correlations between the serum BPA concentration and the FT level by use of combined data for men and women.

Collectively, these data indicate that BPA exposure alters sex hormone levels in male and female species (Qiu *et al.,* 2013), but strain and species as well as other confounders such as time and route of exposure, and age at analysis, may modulate the sensitivity to BPA effects.

2.7.2 THYROID HORMONES

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are synthesized and stored in the thyroid gland and circulate in the blood stream mostly bound to the plasma protein, thyroxine binding globulin (TBG), thyroxine binding pre-albumin (TBPA) and albumin. Approximately 0.03% of T4 is the free unbound state in blood at any one time. Triiodothyronine T3 circulates in blood almost completely to a carrier proteins, mostly TBG.

The effect of BPA varies at different levels of the thyroid system. BPA have shown inhibitory activity on the TPO enzyme, which is a key enzyme involved in the synthesis of T3 and T4 (Schmutzler *et al*., 2007). BPA have several *in vitro* effects on the thyroid receptor β (TRβ), such as repressing the transcription of the thyroid receptor β (Sheng *et al.,* 2012), and having an antagonistic role on the thyroid receptor β. There is an interference on the negative feed-back that the thyroid hormones carry out on TSH release (Zoeller *et al*., 2005), accelerated embryonic development and advanced hatching through its effect on the thyroid receptor (Ramakrishnan and Wayne 2008), and interfered with T3 action during metamorphosis processes (Iwamuro *et al*., 2006). There is increasing evidence that exposure to BPA, impair normal thyroid function, reduced bound circulating and tissue thyroid hormone concentrations (Godey *et al.,* 1995, Morse *et al.,* 1993).

BPA alter thyroid hormone status by epidemiological investigations (Koopman-Essenboon *et al.,* 1994). Epidemiologic studies have revealed an association between BPA exposure and altered thyroid hormones (Wang *et al.,* 2013), increased thyroid function (Wang *et al.,* 2013). The NHANES study also reported a suggestive inverse relationship between urinary BPA and total T4 and TSH (Meeker and Ferguson 2011). Another survey observed a significantly negative correlation between serum BPA and FT4 level, but BPA was associated with TSH (Sriphrapradang *et al.,* 2013). BPA exposure up-regulate genes involved in the synthesis of thyroid hormones in the thyroid follicle (Gentilcore *et al*., 2013), reduced bound T4 women and decreased TSH in male (Chevrier *et al.,* 2013), decrease in serum bound T4 level (Du *et al.,* 2014) and increase in free T4 levels (Zoeller *et al.,* 2005). Studies have shown that BPA can compete with T3 to bind with the thyroid plasma transporter Transthyretin which could drive to a decrease in plasma bound T3 (Ishihara *et al*., 2003). BPA impair thyroid hormone action by inhibiting T_3 binding to the TR and by suppressing its transcriptional activity. Heimeier *et al.,* (2009), showed that doses of BPA affected the gene expression that is controlled by T3 hormone. BPA altered the expression of many genes known to be turned on by thyroid hormone. BPA has the potential to affect genes that are regulated by thyroid hormone during human development. BPA also altered T3 gene expression. Also, according to Heimeier *et al.,* (2009), BPA represents a serious risk to human development through disruption of T3 signaling pathways.

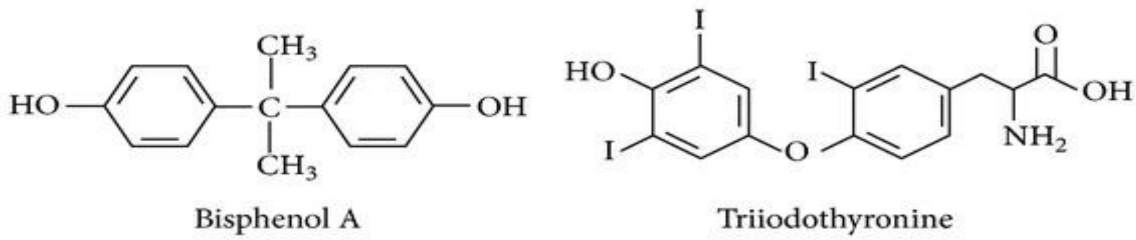

Fig.5; Structure of BPA and Triiodothyronine

Increasing concerns over the effects of BPA highlight the need for screening of the effects of other endocrine disruptors on thyroid receptors to assess potential disruption of the endocrine system. Finally, bisphenol A have shown high affinity for TTR and prealbumin, competing with thyroid hormones for these plasma transporters and decreasing plasma bound thyroid levels (Hamers *et al*., 2006).

2.8 BISPHENOL A AND INTERLEUKINS

Interleukins that are involved in Inflammation are characterized into two (see fig 6):

- Interleukin involve in acute Inflammation
- Interleukin involve in chronic Inflammation

And there are two (2) classes of interleukin

- Proinflatory interleukins
- Anti-inflammatory interleukins

Interleukins involved in acute inflammation: Several interleukines play key roles in mediating acute inflammatory reactions, they includes IL-1, IL-6, IL-11, IL-8. Of these, IL-1 (α and β) are extremely potent inflammatory molecules: they are the primary interleukins that mediate acute inflammation induced in animals (fig 6).

Interleukines involved in chronic inflammation: Chronic inflammation may develop following acute inflammation. The interleukine known to mediate chronic inflammatory processes can be divided into those participating in humoral inflammation, such as IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13, and those contributing to cellular inflammation such as IL-1, IL- 2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12.

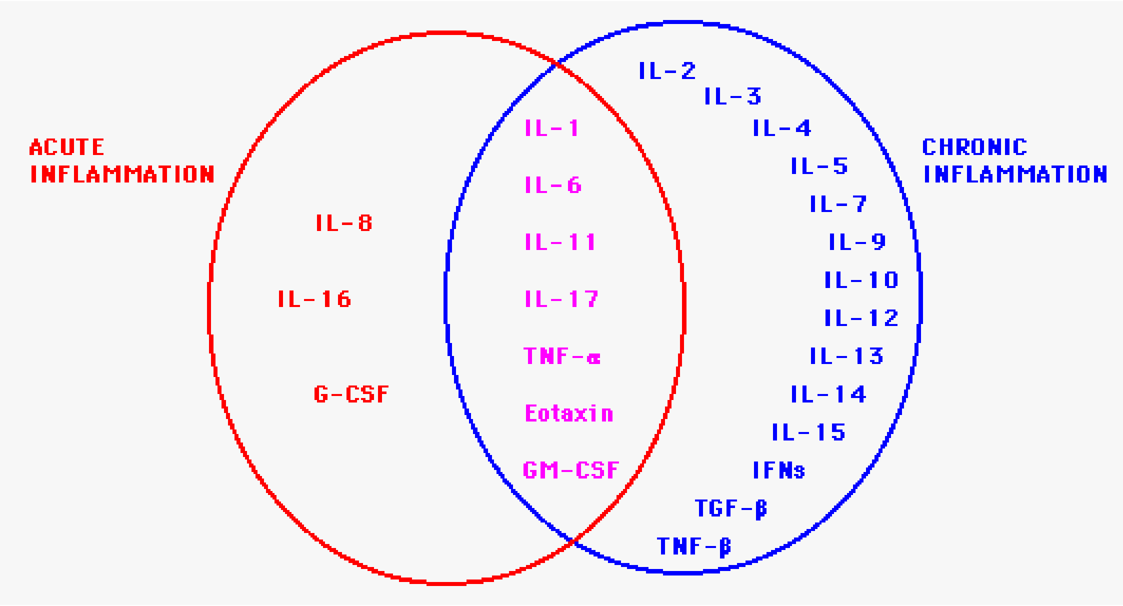

Fig.6;Interleukins involved in acute and chronic inflammatory responses.

Inflammatory diseases are becoming increasingly prevalent worldwide (Rudyk *et al*., 2012). Exposure to environmental pollutants such BPA could be one of the risk factors responsible for the development of such diseases (Neo *et al*., 2013).

Previous studies by Abedelhaffeza *et al*., (2017) revealed that IL-18 is a unique pro-inflammatory cytokine involved in numerous aspects of the inflammatory response, which comprises innate to adaptive immune response switching, vascular repair, and steering and activation of the immune cells (Johnson *et al*., 2015). Administration of BPA elicited an inflammatory response, Savastano *et al*., (2015), found an association between BPA levels and chronic inflammation. Also, it was observed that BPA exposure was responsible for a long-term effect on mast cell-mediated production of pro-inflammatory mediators, which indicated the potential effect of BPA on mast cell dysregulation, and subsequent stimulation of chronic pulmonary inflammation (O’Brien *et al*., 2014). The heighted inflammatory response upon exposure to BPA could be mediated through its ability to activate extracellular rregulated protein kinases (ERK) and nuclear factor _B (NF- _B) signaling cascades with a subsequent increased expression of pro-inflammatory cytokines (Liu *et al*., 2014). In addition, the enhanced oxidative stress observed with BPA exposure may activate NF- _B mediated transcription of pro-inflammatory mediators (Teppala *et al*., 2012).

Significant correlations were found between BPA and inflammatory markers, study have report the evidence of the association between BPA plasma levels of interleukins, as markers of inflammation in rats. Previous studies investigated the association between BPA and altered cytokine markers (Shankar and Teppala 2011; Wang *et al*., 2012). Yang *et al*., (2016) showed that higher BPA levels caused an increase macrophage-related gene and other inflammatory genes, increased systemic and adipose inflammation and higher plasma levels of inflammation factors (Teppala *et al*., 2012).

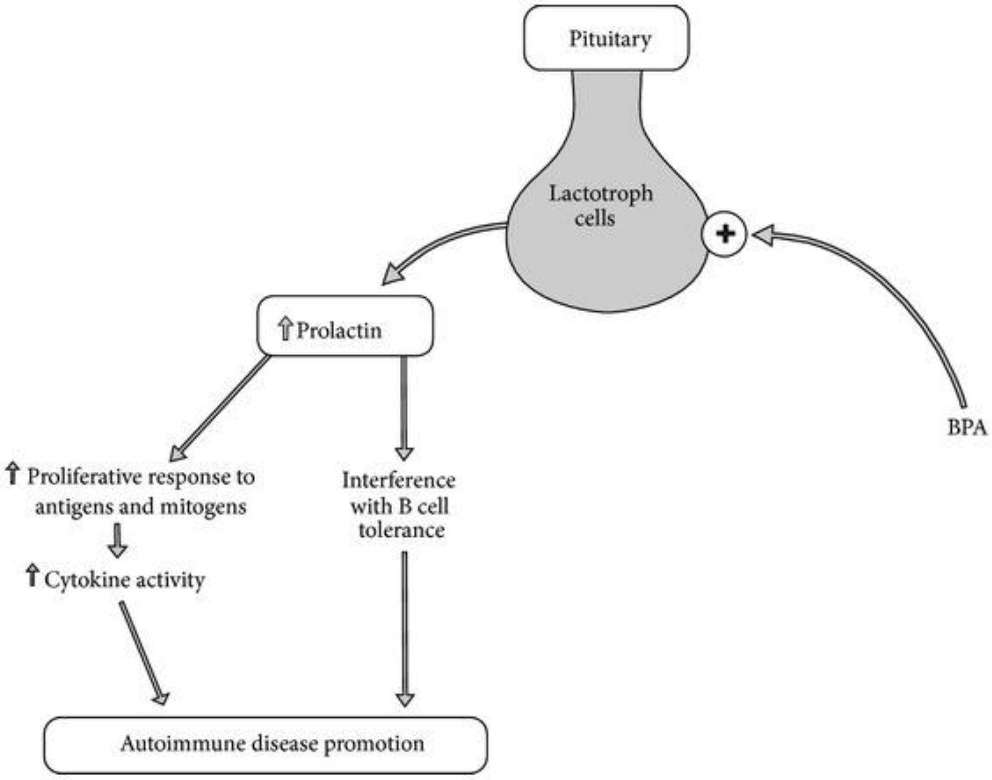

Fig.7; Illustration of bisphenol A activation of hyperprolactinemia and increase immunostimulatory responses (Datis Kharrazian (2014).

Many of these immune-stimulating responses that perpetuate chronic inflammation and immunity may also be potentiated by the estrogenic activity of BPA (Rogers *et al*., 2013). BPA stimulates cell proliferation and induced expression of estrogen responsiveness. BPA treatment in mice induced splenocyte proliferation, a shift of cytokine profiles, and hyperstimulation of cellular immunity (Youn *et al*., 2002). BPA has multiple estrogenic mechanisms in promoting abnormal immune responses and inducing abnormal immune signaling via its disruptive impact on estrogen receptor signaling, aryl hydrocarbon receptor signaling (fig 8), and abnormal signaling of peroxisome proliferator-activated nuclear receptors (Rogers *et al*., 2013). These BPA estrogenic impacts on virtually all the major cells of the immune system and critical signaling pathways may be one way in which BPA promotes pathogenesis of immunity (Ballak *et al*., 2014; Wernstedt Asterholm *et al*., 2014). Several studies have investigated the association of BPA with inflammation (Yang *et al*., 2009; Savastano *et al*., 2015).

Yang *et al*., (2016) study showed that circulating levels of inflammation factors were increased in response to BPA exposure and inflammation factors were also increased. Bodin *et al*., (2015) reveal that for macrophages, the percentage of apoptotic cells was increased, suggesting increased sensitivity to cytokine-induced apoptosis in macrophages.

BPA exposure did induce systemic alterations in the immune system, seen as altered cytokine responses. BPA affects macrophage numbers and functionality. A study by Khanna and co-authors (Khanna *et al*., 2010), reporting increased inflammation and reduced clearance of apoptotic cells. Exposure to low dose BPA activates mRNA expressions of AhR that potentiate immunity with allergic and immune diseases (Takahashi 2010).

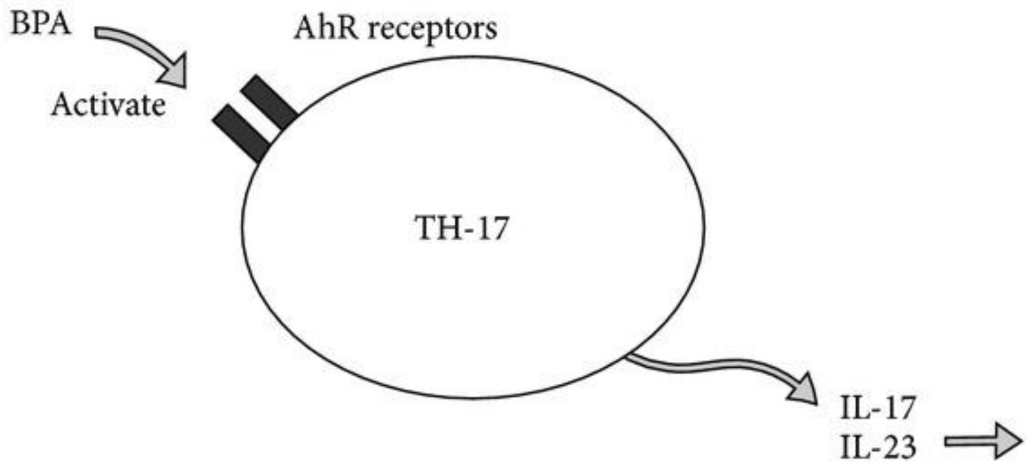

Fig.8; Illustration of bisphenol A activation of immunity by inducing mRNA expression on aryl hydrocarbon receptors on TH-17 cells (Datis Kharrazian (2014).

Fischer *et al*., (2016) revealed that BPA exposure in utero significantly alters the expression of members of the interleukin 1 gene family, and interleukin 2 gene family as well as immune response gene. In utero exposure of BPA resulted in significant changes to inflammatory modulators within mammary tissue (Rao *et al*., 2014). Dysregulation of inflammatory cytokines, both pro-inflammatory and anti-inflammatory, leads to a microenvironment that may promote disordered cell growth through the immune response that targets cancer cells (Fischer *et al*., 2016). Yazdani *et al*., (2016), showed the up-regulation il-1beta and il-1beta genes. This may indicate the possible role of BPA-associated oxidative stress in induction of inflammatory response in this macrophage-like cell type.

Moreover, BPA exposure leads to hemocyte immune dysfunction, potentially increasing its role in induced immunity through immune dysregulation. BPA injected into mussels leads to significant lysosomal membrane destabilization and a dramatic decrease in phosphorylation of the stress-activated p38 mitogen-activated protein kinases (MAPKs) and CREB-like transcription factor (cAMP-responsive element-binding protein) in mussels (Canesi *et al*., 2005). BPA may be a potentially important modulator of immune responses.

Additionally, BPA exposure induced upregulation of Th-1 responses (Yoshino *et al*., 2014). The impact of BPA on naïve immune systems suggests that BPA can augment Th-1 reactions (fig. 9). Specifically BPA increased antigen-specific interferon gamma production leading to exaggerated

T cell activation and polar Th-1 and Th-2 shifts (Goto *et al*., 2007). BPA exposure promotes cytokine inflammatory shifts associated with potential immune development. BPA produced significant shifts of lymphocytes. These responses indicated that BPA has the potential to induce Th-1 polar shifts of transcription factor, exaggerating cellular immune responses that lead to exaggerated Th-1 immune response (Weaver *et al*., 2005). BPA exposure has the ability to exert disruptive effects on macrophages by binding to estrogen receptors and leading to alteration of nitric oxide production (Byun *et al*., 2005).

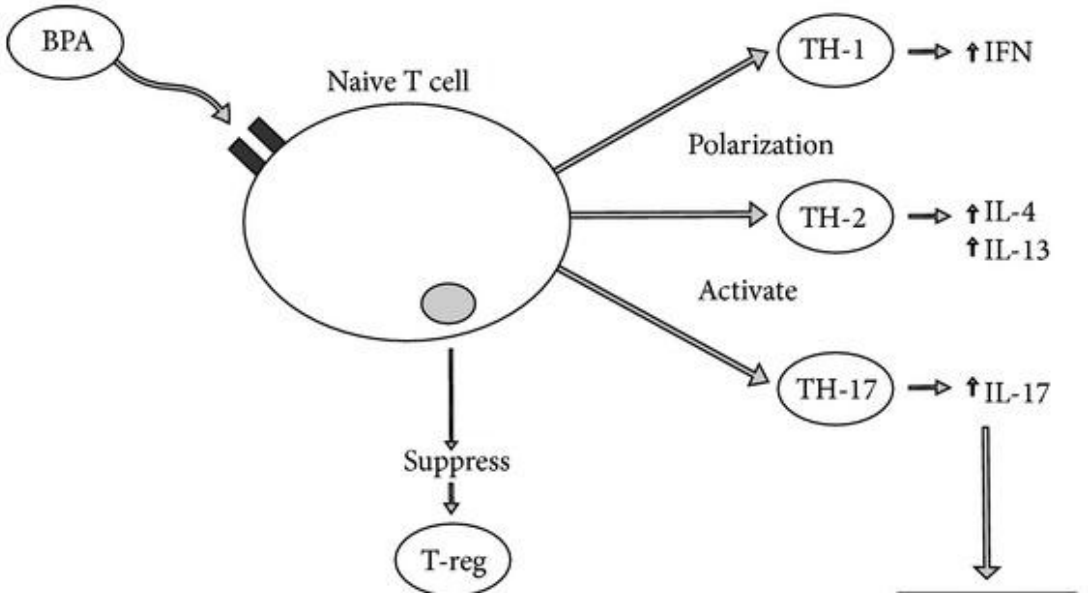

Fig.9; Illustration of bisphenol A induction T cell shifts (Datis Kharrazian (2014).

Increased immunoglobulin reactivity from BPA raise concerns about immune hyperactivity associated such as allergies (Smits 2012). BPA activates immunoglobulins that promotes inflammatory and anti-inflammatory activities. Recent research has shown promot of IgE production, promotes inflammatory responses and the development of immune upregulation. Specifically, exposure to BPA was shown in fig. 10, to increase interleukin production in $CD4^+$ T cells and antigen-specific IgE levels (Lee *et al*., 2003). These immune responses have the ability to potentiate allergies (Altrichter *et al.*, 2011). It was suggested that BPA exacerbates preexisting immune diseases and that continued exposure to BPA may potentiate the incidence and severity of the immune diseases (Yurino *et al.*, 2004).

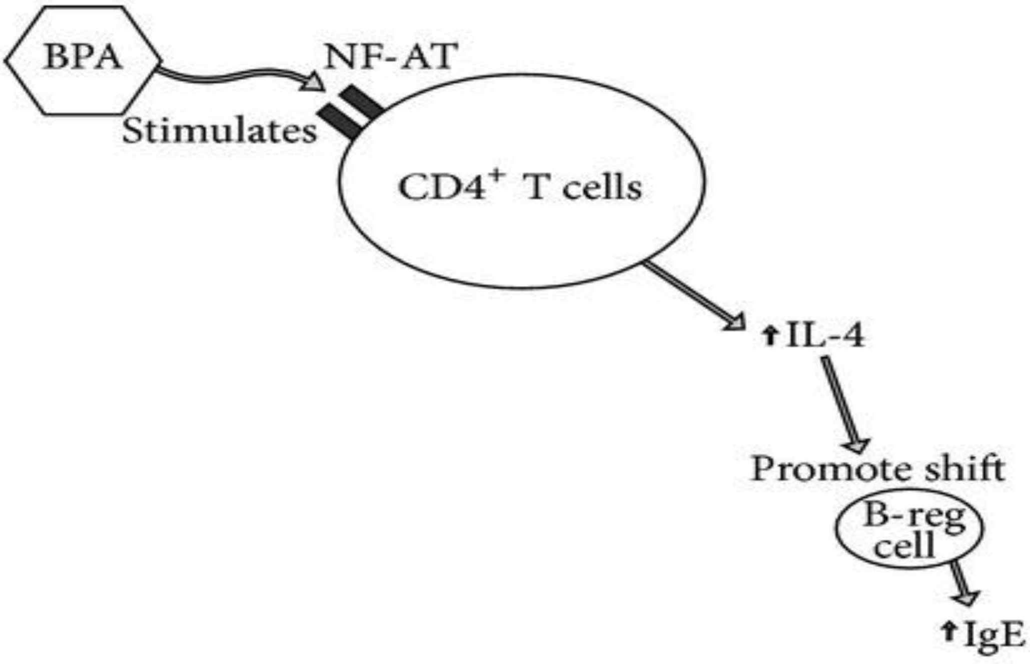

Fig.10; Illustration of bisphenol A impact on immunoglobulin-promoted immunity (Datis Kharrazian (2014).

2.9 EFFECTS OF BISPHENOL A ON INFLAMMATORY RESPONSES

Chitra *et al.*, (2013), showed that the levels of H_2O_2 increased significantly in the treated rats as compared with the corresponding group of control animals, following oral administration of Bisphenol A for 45 days. Sravani, *et al.*, (2016) also revealed an increase H_2O_2. Chen and Schopfer (2012) reported that BPA showed its toxicity by increasing H_2O_2. Kabuto *et al.*, (2014) observed that BPA injection induced over production of H_2O_2 in mouse organs and H_2O_2 is readily converted to hydroxyl radicals. Sravani *et al.*, (2016) showed excess production of H_2O_2 radicals which can only be effectively detoxified by increased glutathione peroxidase activity. Measurable levels of myeloperoxidase found in human samples, circulating myeloperoxidase levels were low (Chettimada *et al.*, 2012). Previous studies in rats (Kunitomo *et al.*, 2008) and mice (Kumar *et al.*, 2014) were able to show a low measure of myeloperoxidase.

2.10 BISPHENOL A AND OXIDATIVE STRESS MARKERS

It has been shown that many environmental contaminants can induce oxidative stress, according with this; Chou *et al.*, (2011), showed that BPA is able to decrease the activity of antioxidant enzymes in the liver. Higher doses of BPA also provoked an antioxidant activity (Invernizzi 2013). Rashid *et al*., (2009), showed an oxidative stress which is augmented by the dietary iron deficiency. BPA can cause oxidative stress by disturbing the redox status in cells (Hasselberg *et al.*, 2004). SOD protects tissues from oxidative stress and damage by catalyzing the conversion of to H_2O_2, a more stable ROS (Hassan *et al.*, 2012). Therefore, the damage at the cellular level by oxidants is attenuated by antioxidant enzyme such as SOD, GSHPx, GSP, CAT and GR (Koc *et al.*, 2005).

A growing body of evidence shows higher BPA concentrations were associated with increased abnormal liver function tests (Oguazu *et al.,* 2015; Lee *et al.,* 2013). BPA has the ability to generate reactive oxygen species (ROS) and reduce antioxidant reserves and enzymes that are critical for hepatic phase I and II biotransformation, including glutathione, superoxide dismutase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, and catalase activity (Hassan *et al.,* 2012). Similarly, others demonstrated that BPA generates ROS that causes oxidative damage in organs and tissues such as the brain, reproductive tract, and kidney of rats (Korkmaz *et al.,* 2010, Aydogan *et al.,* 2010). Catalase and GSHPx catalyze dismutation of the superoxide anion into hydrogen peroxide (H_2O_2) which then convert hydrogen peroxide to water, providing protection against reactive oxygen species (Sayed-Ahmed *et al.,* 2010).

Peroxiredoxins are a ubiquitous family of antioxidant enzymes that also control cytokine-induced peroxide levels and thereby mediate signal transduction in mammalian cells Rhee *et al.,* 2005). The physiological importance of peroxiredoxins is illustrated by their relative abundance, with the most abundant peroxiredoxin 2. Peroxiredoxins have been described as a sensor of peroxide (Burgoyne *et al.,* 2012a; Chae *et al.,* 2012). It is possible that peroxiredoxin-1 becomes more important in regulating PKG dimerization when peroxide levels are elevated above the concentrations present under basal aerobic conditions (Burke-Wolin and Wolin 1999). Interestingly, early observations of peroxiredoxins forming disulfide complexes with other proteins when cardiac muscle is exposed to oxidant conditions (Burgoyne *et al.,* 2012b) have evolved into recent evidence that peroxiredoxin-1 potentially promotes signaling-associated thiol oxidations by forming transient disulfides with the regulated protein (Jarvis *et al.,* 2012). Peroxiredoxin-1 could be hypothesized to have a role in directly promoting PKG dimerization once it has been oxidized by peroxide. The observation that peroxide oxidizes NADPH suggests that NADPH oxidation could be a factor in peroxide promoting PKG dimerization. This could occur through peroxiredoxin-thioredoxin-thioredoxin reductase and glutathione peroxidase-glutathione reductase pathways of peroxide metabolism consuming NADPH (Rudyk *et al.,* 2012).

Systems controlling protein thiol redox in the cytosolic region such as thioredoxin-1 (Trx-1) and glutaredoxin could be hypothesized to have a role in promoting PKG1α dimerization under circumstances in which cytosolic NADPH oxidizes because thioredoxin reductase-1 and glutathione reductase utilize NADPH to maintain Trx-1 and glutathione in their reduced forms (Chae *et al.,* 2012; Neo *et al.,* 2010). Evidence has recently been reported, based on the actions of an inhibitor of thioredoxin reductase, that thioredoxin and thioredoxin reductase function to redox cycle the dimerized form of PKG1α back to the reduced monomeric form of PKG (Burgoyne *et al.,* 2012b; Rudyk *et al.,* 2012). Since thioredoxin reductase-1 utilizes NADPH to maintain Trx-1 in its reduced state (Forman *et al.,* 2004).

BPA increased ROS production as assessed by the measurement of the end product of lipid peroxidation. It was reported that BPA increases the generation of reactive oxygen species (ROS) and induced hepatic damage and mitochondrial dysfunction (Moon *et al.,* 2012; Asahi *et al*., 2010). The MDA concentration increases with the time (Nandi *et al.,* 2005). Studies showed increased TBARS levels in the brain (Korkmaz *et al.,* 2010), testes, and kidneys (Aydogan *et al.,* 2010). Increased lipid peroxidation may indicate an increased oxygen free radical generation. BPA induces ROS production and significantly compromises mitochondrial function (Aboul Ezz *et al.,* (2015). It has been reported that BPA exposure results in augmentation of oxidative stress, with increased levels of lipid peroxidation and reactive oxygen species and depletion of the antioxidant defense system (Eid *et al.,* 2015).

Also, Levels of lipid hydroperoxides and 8-iso-prostaglandin, the major biochemical markers of ROS generation, have been shown to be elevated in the plasma and pericardial fluid and are also positively correlated to its severity (Prysyazhna *et al.,* 2012). Lipid hydroperoxide is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation and excessive damage of cellular macromolecules (protein, lipids, and nucleic acids), it has been shown to be a major contributor to the toxicity of contaminants (Fenichel *et al*., 2012). The increase in Lipid hydroperoxide reported may be the result of increased production of free radicals and/or a decrease in antioxidant status. The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage (Veiga-lopez *et al*., 2015).

Glutathione provides a first line of defence against (ROS), as it can scavenge free radicals and reduce H_2O_2 ((Burgoyne *et al.,* 2012). GSH acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as glutathione peroxidase and glutathione reductase. The reduction of GSH shows the failure of primary antioxidant system to act against free radicals (Huang *et al.,* 2012). The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage, because glutathione peroxidase have been considered the primary scavengers of H_2O_2 (Veiga-lopez *et al*., 2015); However, in absence of adequate glutathione peroxidase activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and may contribute to the oxidative stress of BPA. Decreased GSH concentration indicates an increased generation of ROS, which cause lipid peroxidation in the liver Hassan *et al.*, (2012). Also, GSH is consumed during the conversion of hydrogen peroxide into hydrogen oxide. The peroxide is readily converted to the hydroxyl radical which may be involved in the observed decrease in GSH levels as GSH itself is also a general hydroxy-radical scavenger (Nandi *et al.,* 2005). Therefore, exposure to BPA causes oxidative stress by disturbing the balance between ROS and antioxidant defenses system in liver. GST protects cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes (Sharma *et al.*, 2004). Glutathione-S-transferase catalyses the conjugation of GSH with several compounds produced *in vivo* during oxidative stress. The consumption of GSH during the generation of glutathione-S-conjugates by GSTs thus lowering the level of total intracellular glutathione after prolonged treatment.

Endogenous Antioxidants BPA induces reactive oxygen specis (ROS) production and significantly compromises mitochondrial function CAT activity increased the toxic effect of the free radicals formed from the BPA effect. Eid *et al.*, (2015) also demonstrated that BPA caused a decrease in the activities of antioxidant enzymes.

Nitric oxide (NO) is a highly diffusible free radical. It was demonstrated that, NO reacts with superoxide ($O2_{\bullet}^{-}$) to form peroxynitrite ($ONOO^{-}$) a highly reactive free radical, therefore, NO causes increased nitrosative stress (Grattagliano *et al*., 1999). Moreover, NO is a potent oxidant and nitrating agent is capable of attacking and modifying proteins, lipids, and DNA as well as depleting antioxidant defenses (Sayed-Ahmed *et al*., 2010). It may be postulated that the activation in NO levels induced by BPA administration may lead to the reduction in the rate and force of cardiac contractions (Pant *et al.,* (2011). BPA induced a significant increase in oxidative stress, which is accompanied by marked alterations in TAC (Fridovich, 2007). Because BPA caused induction of free radicals in the hepatic tissue, in consequence, it leads to disruption in the antioxidant defense system.

With regard to nitrotyrosine, oxidized tyrosine moieties have been used to study pathways involved in oxidative stress after BPA exposure (Mitra *et al.,* 2014). The circulating nitrotyrosine levels in both maternal and cord milieu found in study, where umbilical cord levels of nitrotyrosine were found to be higher than in the term maternal samples (Weber *et al.,* 2014). There is a positive correlation between maternal nitrotyrosine levels with cord nitrotyrosine levels (Weber *et al.,* 2014) hence, it was hypothesized that maternal oxidative stress, specifically nitrotyrosine, could be used as a predictor of fetal oxidative stress (Stewart *et al.,* 2005; Cambonie *et al.,* 2007).

BPA causes Elevated nitrotyrosine during pregnancy were found in (Sulkowski *et al.,* 2012), perinatal asphyxia (Badham *et al.,* 2010), and chronic hypoxia (Escorbar *et al.,* 2013). Although the association between BPA and nitrotyrosine in the human study does not prove causality, it points to a potential link. An increase in nitrotyrosine was evident in both maternal and fetal sheep plasma, suggesting a direct response BPA exposure. Importantly, the systemic nitrosative stress evident postnatally in the high prenatal BPA-treated group demonstrates that prenatal BPA exposure can disrupt oxidative stress pathways in postnatal life. The finding that prenatal BPA increased nitrotyrosine in adipose tissue of adult sheep and rats points to tissue-specific programming effects as well (Nishimura *et al.,* 2014).

2.11 BIOLOGICAL MONITORING OF BISPHENOL A

BPA has been a subject for several biomonitoring studies among the general population. It can be measured either from urine and blood in the form of free, conjugated or total (free and conjugated) BPA. The German Federal Environment Agency has recently set a reference value of 7 µg/l for 20–29-year old adults (UBA 2012). Children usually have higher BPA levels than adolescents who in turn have higher levels than adults (Rezg *et al.,* 2014). Mourad and Khadrawy (2012) reported increased urinary BPA (total BPA) levels in epoxy resin sprayers. He *et al.,* (2009) studied BPA exposure in Chinese workers in epoxy resin and BPA manufacturing facilities by air monitoring and by measuring urinary BPA levels. BPA was detected in 96 % of the air samples and the median concentration was 6.67µg/m^3.

In non-occupationally exposed Chinese males, median urinary BPA levels of 1.43 µg/g creatinine have been reported by the same research group (He *et al.*, 2009). Balakrishnan *et al.*, (2010) have estimated the concentration of BPA in urine corresponding to the tolerable daily intake set by EFSA (0.05 mg/kg) on the basis of available data on BPA toxicokinetics after oral exposure.

BPA has been in use commercially for over 50 years, and workers producing this compound and its products (e.g., epoxy resins) have been exposed to time-weighted average air levels to about 10 mg/m^3 over decades. From this, it has been found that high exposures to BPA are irritating to the eye and respiratory tract, and may cause skin lesions and photosensitization of the skin (Carwile and Michels 2011). BPA has the potential to cause serious damage to the eyes. Slight and transient nasal tract epithelial damage was observed in (Xiaoqian and Hong-Sheng 2014). BPA appears to have a respiratory irritation potential.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The materials used include:

3.1.1. EQUIPMENT

Autoanalysers

i. Hitachi 911 Autoanalyser, Boehringer Mannheim GmbH, Mannheim, FGR.
ii. Lx 20 pro Autoanalyser, Beckman Coulter, Woerden, Netherland.
iii. DxC 600 Autoanalyser, Beckman Woulter, Woerden, Netherland.
iv. Elecsys 1010 Autoanalyser (immunoassay system), RocheRoche Diagnostics, a member of the Roche Group, Bielefeld, Germany.
v. Au 480 chemistry Analyser, Beckman Coulter, Woerden, Netherland.
vi. Chemwell chemical Analyzer, Manufacturer: Roche Hitachi, GMI.

Centrifuge – CENTRIFUGE 800D, Techmel and Techmel, USA.

3.1.2. CHEMICALS

All the chemicals used were of analytical grade and were manufactured by Sigma Aldrich, St. Louis, USA, except where otherwise stated. The chemicals include;

i. BPA
ii. Sodium chloride
iii. Formalin
iv. Chloroform

3.1.3. REAGENTS

All the reagents used were already prepared and commercially obtained as kits and are here listed in Appendix XI.

3.1.4. ANIMALS

Male and female albino rats of 5 weeks old were obtained from the animal house, Faculty of Veterinary Medicine, University of Nigeria, Nsukka and used for the study.

3.2 METHODS

3.2.1. PREPARATION OF STOCK SOLUTION OF BISPHENOL A

The stock was prepared by dissolving 10g of Bisphenol A in 2ml of ethanol, the solution was then made up to 500ml with distilled water.

Stock solution was serially diluted to obtain required concentration using the formular:

Required vol x require conc = original vol x original conc

3.2.2. DETERMINATION OF ACUTE TOXICITY (LD_{50})OF BISPHENOL A IN RATS

The LD_{50} of orally and subcutaneous administered BPA in male and female rats were determined using three (3) different methods shown below.

(A) LORKE'S METHOD (Lorke, 1983)

The two phase method of Lorke’s was adopted in this study. However the method was modified to increase the number of groups as well as the number of animals in each group.

ORAL TOXICITY

Phase 1: Lorke’s methods employed the use of nine (9) animals divided into three groups of three (3) animals each. For this study, we used fifty animals divided into five groups of ten animals each for male and female rats respectively. Each group of the animals was orally administered different doses (1000, 2000, 3000, 4000, 5000)mg/kg body weight) of the test substance in solution, using intubation canuli. The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored.

Phase 2: The phase 2 of Lorke’s methods employed the use of three (3) animals divided into three groups of one (1) animal each. For this study, we used fourty (40) animals divided into four groups of ten (10) animals and thirty (30) animals divided into three (3) groups of ten (10) animals each for female and male rats respectively. For the female rats, each group of the animals was orally administered different doses (1100, 1150, 1200, 1250)mg/kg body weight) of the test substance, and the male rats for each group of the animals was orally administered different doses (1300, 1600, 1900)mg/kg body weight) of the test substance using intubation canuli.The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored.

Then the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

SUBCUTANEOUS TOXICITY

The same modification of the Lorke’s method for oral toxicity was applicable here except for the route of administration and dose of BPA used were different

Phase 1: The animals were divided into six groups of ten (10) animals each for the female and male rats. Each group of animals was administered different doses (340, 360, 380, 400, 420, 440 mg/kg body weight) of test substance. The animals were placed under observation for 24 and their behavior and mortality rate were monitored.

Phase 2: The animals were distributed into four groups of ten (10) animals each for the female and male rats. The female animals were administered different doses (35, 70, 105, 140 mg/kg body weight) of test substance and the male rats were administered different doses (100, 145, 190, 135 mg/kg body weight) of test substance then observed for 24 hours for behavior as well as mortality. Then the LD_{50} was calculated by the formula stated previously.

(B) KARBER'S METHOD (Turner, 1965)

The method of Karber's was adopted in this study. However the method was modified to increase the number of groups as well as the number of animals in each group.

ORAL TOXICITY

The Karber's method involves the administration of different doses of test substance to various groups, which has five animals each. The control group was administered with the vehicle in which the test substance was dissolved that is water. However, from the second group onward received different doses of the test substance. The animals in each group received specific doses, while increment in dose progresses from group to group. Depending on the outcome of the first procedure, a decision is made as to whether further procedure is necessary

For this study, we used sixty (60) animals divided into six groups of ten (10) animals each for male and female rats respectively. Each group of the animals was orally administered different doses (control,1000, 2000, 3000, 4000, 5000)mg/kg body weight) of the test substance, while the control was administered 0.001% of ethanol in distilled water, using intubation canuli.The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored.. Following the outcome of the first procedure, a decision was made as to further procedure is necessary

Observations was made between 2000-3000 mg/kg body weight and 2000-4000 mg/kg body weight for female and male rats respectively, Then sixty (60) animals divided into six groups of ten (10) animals each for female and male rats respectively. Each group of the female animals was orally administered different doses (CONTROL, 2000, 2200, 2400, 2800, 3000)mg/kg body weight of the test substance, and each group of the male animals was orally administered different doses (CONTROL, 2000, 2500, 3000, 3500, 4000)mg/kg body weight of the test substance while the control were administered 0.01% of ethanol in distilled water, using intubation canuli.The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored.

Further observations was made for the male rats only, which lead to the decision of the third phase for the male rats. Again, sixty (60) animals divided into six groups of ten (10) animals each for male rats. Each group of the male animals ws orally administered different doses of BPA solution (CONTROL, 2600, 2700, 2800, 2900, 3000) mg/kg body weight). The control group was administered with the vehicle in which the test substance was dissolved that is 1ml of ethanol in 100ml of water. The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored.

Afterwards, the LD_{50} was calculated using the arithmetical method of Karber, which was as follow:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

Where, LD_{50} = Median lethal dose

LD_{100} = Least dose required to kill 100%

a = Dose difference

b = Mean mortality

n = Group population.

SUBCUTANEOUS TOXICITY

The same modification of the Karbers method for oral toxicity was applicable here except for the route of administration and dose of BPA used were different

This method involved the administration of different doses of BPA solution (CONTROL, 100, 200, 300, 400, 500) to six groups, which has ten (10) animals each for the female and male rats respectively. The control group was administered with the vehicle in which the test substance was dissolved that is Iml of ethanol in 100ml of water. Then following the observation a second phase experiment was conducted for both the female and male rats category.

Observation was made between 100-200 and 200-300 for female and male rats respectively, Then sixty (60) animals divided into five groups of ten (10) animals each for female and male rats respectively. Each group of the female animals was orally administered different doses (CONTROL, 100, 120, 140, 160, 180)mg/kg body weight of the test substance and each groups of the male animals were orally administered different doses (CONTROL, 200, 220, 340, 360, 280)mg/kg body weight of the test substance,The control group was administered with the vehicle in which the test substance was dissolved that is Iml of ethanol in 100ml of water. The animals were placed under observation for 24 hour and their behavior and mortality rate were monitored. Afterwards, the LD_{50} was calculated using the arithmetical method of Karber, stated previously.

(C) UP AND DOWN METHOD (Bruce, 1985)

This method involved a stepwise administering the test substance to the experimental animals, and observing which dose concentration was able to kill half of the population samples within 24hrs. Depending on the outcome of the first procedure, a decision is made as to whether further procedure is necessary.

ORAL TOXICITY

In the first phase, the male and female rats of 5 treatment groups each (G-I to G-V), orally received different graded doses of Bisphenol A solution as follows:G-I= 5000 mg/kg, G-II=4000 mg/kg, G-III=3000 mg/kg, G-IV=2000 mg/kg, G-V=1000 mg/kg

For the female rats, observation was between 2000mg/kg and 3000mg/kg doses. This led to the second phase of the test, and four test group were used G-VI=2200 mg/kg, G-VII=2400 mg/kg, G-VIII=2600 mg/kg G-XIX=2800mg/kg), another observation was made at 2200mg/kg. Hence, third phase was considered using 2040mg/kg, 2080mg/kg, 2120mg/kg, 2160mg/kg, 2200mg/kg, orally for female.

For the male rats observations were made between 2000 and 4000mg/kg, this led to another set of experiment that is the second phase, and five groups were used each receiving 2000mg/kg, 2500mg/kg, 3000mg/kg, 3500mg/kgand 4000mg/kg. Further observation was made between 2500mg/kg and 3000mg/kg, then third phase was considered with four groups as followed 2600mg/kg, 2700mg/kg, 2800mg/kg and 2900mg/kg.

SUBCUTANEOUS TOXICITY

The same process as in the oral LD_{50} determination was employed

For subcutaneous administration, In the first phase, the male and female rats of 5 treatment groups each (G-I to G-V), orally received different graded doses of Bisphenol A (G-I= 500 mg/kg, G-II=400 mg/kg, G-III=300 mg/kg, G-IV=200 mg/kg, G-V=100 mg/kg,

For the female rats, observation was between 100mg and 200mg doses. This led to the second phase of the test, and four test group were used G-VI=180 mg/kg, G-VII=160 mg/kg, G-VIII=140 mg/kg G-XIX=120mg/kg) orally for female.

For the male rats observations were made between 200 mg/kg and 300 mg/kg and five groups were used; each receiving 220mg/kg, 240mg/kg, 260mg/kg, 280mg/kg and 300mg/kg orally for male.

3.2.3. ANIMAL EXPERIMENT

The experimental albino rats were randomly selected and marked for easy identification and kept in their cages at the animal house of Gregory University Uturu (GUU), Abia State for 7 days prior to dosing to allow for acclimation to the environmental condition at room temperature. Commercial rat pellets and water were provided *ad libitum*. The Organization For Economic Co-Operation and Development (OECD) (2012) guideline for animal care was employed throughout the study.

The animals were randomly divided into twelve groups of 10 rats each, eleven (11) of these groups (test groups) received graded doses of BPA, while one (1) group served as the control group that received the vehicle and water.

The eleven test groups of experimental animals received graded doses of Bisphenol A in solution. The experimental animals were orally administered 0.5ml of BPA solution contaning 0.05mg, 0.1mg, 0.2mg, 0.3mg, 0.4mg, 0.5mg, 0.6mg, 0.7mg, 0.8mg, 0.9mg, 1mg of BPA/Kg body weight respectively, daily for three (3) months.While the control received 0.5ml of 0.004 ethanol in water solution.

The blood samples of all the animals (control and test groups) were collected at the beginning of the experiment before the administration of the test substance. Afterward,blood samples were collected weekly for three (3) month to monitor the effect of BPA on the desired parameters. The animals' were weighed weekly.

The animals were fasted prior to the substance administration, but allowed free access to drinking water. The test substance was administered daily in a single dose to the animals in groups by oral gavage using an intubation cannular. After administration of the test substance, food was withheld further for 3-4 hours before feeding resumed.

At the end of every week, 2.5ml of whole blood samples were collected from the animals tail using capillary action, the whole blood was allowed to clot, centrifuged and the serum collected was used for the biochemical assays.

3.2.4 BIOCHEMICAL ASSAYS

3.2.4.1 SEX HORMONE ASSAY

a) ESTRADIOL/ESTROGEN (E2) ASSAY (Tietz, 1995)

Principle

The E2 EIA is based on the Principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells were incubated with 25 µl E2 blank, controls, samples, 100 µl Estradiol-HRP Conjugate Reagent and 50 µl rabbit anti-Estradiol reagent at room temperature (18-25ºC) for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the concentration is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample.

Procedure

The desired number of coated wells was placed in the holder. Twenty five (25) µl of reference reagents (blank), specimens and controls were dispensed into appropriate wells. Then 100 µl of estradiol-HRP conjugate reagent was added into each well. This was followed by 50 µl of rabbit anti-estradiol (E2) reagent to each well. The mixture was thoroughly mixed for 30 seconds and incubated for 90 minutes at 25ºC. The microwells were rinsed and flicked 5 times with distilled water. Then, 100 µl of TMB reagent were dispensed into each well and gently mixed for 10 seconds. The mixture was incubated for 20 minutes at temperature 25ºC. The reaction stopped by adding 100 µl of Stop Solution to each well. The final mixture was gently mixed for another 30 seconds. The concentrations were measured at 450 nm within 15 minutes.

b) ESTRONE (E3) ASSAY (Folan, 1988)

Principle

The Principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in blank, control and human samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The intensity of the colour formed is inversely proportional to the concentration of estrone in the sample.

Procedure

The required number of microwell strips was placed in the holder. Then 50 µl of each blanks (control 1 and 2), sample control and specimen sample were Pipetted into the corresponding labelled wells. Another 100 µl of the conjugate working solution were pipetted into each well. And incubated on a plate shaker of 200 rpm for 1 hour at 25ºC. The plate wells were washed 3 times with wash buffer, 300 µl/well for each wash and the plate firmly tapped against absorbent paper to ensure that it is dry. Then 150 µL of TMB substrate were pipetted into each well. And incubated on a plate shaker for 15 minutes at 25ºC temperature. Finally, 50 µl of stopping solution were dispenced into each well. The concentrations were read at 450 nm within 20 minutes.

c) ESTRIOL (E1) ASSAY (Speroff, 1983)

Principle

The estriol ELISA kit is a competitive immunoassay for the quantitative determination of free estriol in biological fluids.The kit for the quantitative measurement of estriol uses a polyclonal antibody to estriol to bind, in a competitive manner, estriol in the sample which has estriol covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added.

After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of estriol in samples.

Procedure

The required number of microwell strips was placed in the holder. Then 100 µl of each blank (Reference Reagent), control and specimen sample were pipetted into the corresponding labelled wells. Then 50µl of blue conjugate was pipetted into each well, except the blank wells. Another 50 µL of yellow Antibody was pipetted into each well, except the Blank wells. And the resultant mixture was incubated on a plate shaker for 2 hours at 500 rpm at 25ºC. The contents of the wells were emptied and washed by adding 400 µL of wash solution to every well. The washing process was repeated 2 more times for a total of 3 washes. After the final wash, the wells were aspirated, and firmly tapped on a lint paper towel to remove any remaining wash buffer. Five (5) µL of the blue conjugate were added to the wells. Then, 200 µl of the pNpp Substrate solution was added to every well and incubate for 1 hour at 25ºC. Finally, 50µl of stop solution was added to every well. The plate reader was blanked against the blank wells, and concentration was read at 405nm.

d) PROLACTIN ASSAY (Tietz, 1995)

Principle

The prolactin quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

Procedure

The desired number of coated wells was placed in the holder. Then, 50 µl of blank (Reference Reagent), specimens, and controls were dispensed into appropriate wells. And 100 µl of enzyme conjugate reagent was added into each well. The mixture was gently mixed for 10 seconds. And incubated for 45 minutes at 25ºC. The incubation mixture was removed by flicking plate contents. The flicking and rinsing of the microtiter wells was done 5 times with distilled water. The wells were striked sharply onto absorbent paper towels to remove residual water droplets. Then, 100 µl TMB reagent was dispensed into each well, and was gently mixed for 10 seconds. And incubated in the dark for 20 minutes at 25ºC. The reaction was stopped by addition of 100µl of stop solution to each well. The final mixture was gently mixed for another 30 seconds. The concentration was measured at 450nm within 15 minutes.

e) PROGESTERONE ASSAY (Kim, 1974)

Principle

This kit is based on the Principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti- progesterone.

In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl progesterone blank, controls, samples, 100 µl progesterone-HRP conjugate reagent and 50 µl rabbit anti-progesterone reagent at room temperature (18-25°C) for 90 minutes.

During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the sample, or quality control serum for a fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of progesterone in the specimen increases. Unbound progesterone peroxidase conjugate is then removed and the wells washed.

Next, a solution of TMB reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color.

The color development is stopped with the addition of stop solution, and the concentration is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled progesterone in the sample.

Procedure

The desired number of coated wells was placed in the holder. Twenty five (25)µl of blank (control 1 and 2), specimens, and controls were pipetted into appropriate wells. Then, 100µl of working progesterone-HRP conjugate reagent was dispensed into each well. And 50µl of rabbit anti-progesterone reagent as added to each well, and the mixture was thoroughly mixed for 30 seconds. Then the plate and its contents were incubated for 90 minutes at 25°C. This was followed by rinsing and flicking the microtiter wells 5 times with distilled water. Then 100µl of TMB reagent was dispensed into each well and gently mixed for 10 seconds. The mixture was incubated at

25°C for 20 minutes. The reaction was stopped by adding 100µl of stop solution to each well, and gently mixed for another 30 seconds. The concentrations were measured at 450nm within 15 minutes.

f) TESTOSTERONE ASSAY (Hall, 1988)

Principle

The Principle of the following enzyme immunoassay test follows the typical competitive binding schematic.

Competition occurs between an unlabeled antigen and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The concentration is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of testosterone in the sample.

Procedure

The required number of microwell strips was placed in the holder and 50µl of each calibrator, control and specimen sample were pipetted into correspondingly labeled wells. Then, 100µl of the conjugate working solution was pipetted into each well. And the mixture was incubated on a plate shaker at 200 rpm for 1 hour at 25°C. The wells were washed 3 times with 300 µl of diluted wash buffer per well, and tapped firmly against absorbent paper to ensure that it is dry. Another 150µl of TMB substrate was pipetted into each well. And incubated on a plate shaker for 15 minutes at 25°C. The reaction was stopped by pipetting 50µl of stop solution into each well. The concentration was measured at 450nm within 20 minutes.

g) LEUTEINIZING HORMONE (LH) ASSAY (Tietz, 1995)

Principle

The Principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for LH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of LH is conjugated to horse radish peroxidase (HRP). LH from the sample and blank are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution.

The concentration is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample.

Procedure

The required number of microwells strips was placed in the holder and 25 µl of each calibrator, control and specimen sample were dispenced into correspondingly labelled wells. Then, 100 µl of assay buffer was pipetted into each well and incubated for 30 minutes at 25°C. The wells were washed 3 times with 300 µl of diluted wash buffer per well and tapped firmly against absorbent paper to ensure that it is dry. Another 100 µl of the conjugate working solution was pipetted into each well and incubated for 30 minutes at 25°C. The plate wells were washed again in the same manner as stated earlier. Then, 100 µl of TMB substrate was dispenced into each well, and incubated for 20 minutes at 25°C. Finally, 50 µl of stop solution was pipetted into each well. The concentration was measured at 450 nm within 20 minutes after addition of the stop solution.

h) FOLLICLE STIMULLATING HORMONE (FSH) ASSAY (Gore-Langton and Armstrong, 1988)

Principle

The Principle of the following enzyme immunoassay test follows a typical two-step capture or „sandwich" type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for FSH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of FSH is conjugated to horse radish peroxidase (HRP). FSH from the sample and blank are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The concentration is measured on a microtiter plate reader. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of FSH in the sample.

Procedure

The required number of microwell strips was placed in the holder and 25 µl of each calibrator, control and specimen sample were pipetted into correspondingly labeled wells. Then, 100 µl of assay buffer was added into each well, and incubated for 30 minutes at 25°C. The wells were washed 3 times with 300 µl of diluted wash buffer per well and tapped firmly against absorbent paper to ensure that it is dry. Again, 100 µl of the conjugate working solution was added into each well, and incubated on for 30 minutes at 25°C. The washing of the wells was repeated again in the same manner. Another 100 µl of TMB substrate was pipetted into each well and incubated on a plate shaker for 20 minutes at 25°C. Then, 50 µl of stop solution was added into each well to stop the reaction. The concentrations were read at 450 nm within 20 minutes after addition of the stop solution.

i) ANDROSTENEDIONE ASSAY (Kim, 1974)

Principle

Androstenedione *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of androstenedione in serum and plasma. A 96-well plate has been precoated with anti-androstenedione IgG.

Samples and the androstenedione-HRP conjugate are added to the wells, where androstenedione in the sample competes with the added androstenedione-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of stop solution which stops the color development and produces a color change from blue to yellow.The intensity of signal is inversely proportional to the amount of androstenedione in the sample and the intensity is measured at 450 nm.

Procedure

The required number of microwell strips was placed in the holder. Then, 25 µl of blank (Reagent), control and sample were added into their respective wells. Also, 200 µl androstenedione-HRP conjugate was added to each well except for the blank well. The wells were covered with the foil and incubate for 1 hour at 37°C. When incubation has been completed, the foil was removed and the content of the wells were aspirated and washed three times with 300 µl washing Solution. Another 100 µl TMB substrate solution was added into all wells, and incubated for 15 minutes at 25°C in the dark. The reaction was stopped by the addition of 100µl stop solution into the wells and the microplate was shaked gently. The concentration was measure at 450 nm against blank within 5 minutes.

3.2.4.2. THYROID HORMONE ASSAY

a) THYROID STIMULATING HORMONE (TSH) ASSAY (Witherspoon and Shuler,1984)

Principle

The TSH ELISA test is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization. A goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, changing the color to yellow. The concentration of TSH is directly proportional to the color intensity of the test sample. Concentration is measured spectrophotometrically at 450 nm.

Procedure

The desired number of coated wells were placed in the holder. Then, 100 µl of blank, specimens, and controls were dispensed into appropriate wells. After which 100 µl of enzyme conjugate reagent was added into each well. The mixtures were thoroughly mixed for 30 seconds. And incubated at 25°C for 60 minutes. The incubation mixture was removed by flicking plate contents. The microtiter wells were rinsed and flicked 5 times with distilled water and sharply striked onto absorbent paper towels to remove all residual water droplets. Another 100 µl of TMB reagent was dispensed into each well. The mixture was gently mixed for 10 seconds and incubated at 25°C for 20 minutes. The reaction was stopped by adding 100 µl of stop solution to each well and mixed for 30 seconds. The concentrations were read at 450 nm within 15 minutes.

b) THYROXINE (T4) ASSAY (Abuid *et al*., 1974)

Principle

To measure T4 by competitive immunoassay techniques, a sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody. In this T4 EIA, antibody to T4 is coated on a solid phase (microtiter well). A measured amount of patient serum and a constant amount of T4 labeled with horseradish peroxidase are added. During incubation, T4 in the patient sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody. After 60-minute incubation at room temperature, the solid phase is washed with water to remove unbound-labeled T4. A solution of tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCI, and the resulting yellow color is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of T4 in the sample.

Procedure

The desired numbers of coated wells were placed in the holder and 25µL of blank, specimens, and controls was pipetted into appropriate wells. Then 100 µL of working conjugate reagent was added into each well and was thoroughly mixed for 30 seconds. The mixture was incubated at 25°C for 60 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter wells rinsed and flicked 5 times with distilled water and the wells were striked sharply onto absorbent paper towels to remove all residual water droplets. Then 100µL of TMB reagent was dispenced into each well and was mixed gently for 5 seconds. Again, the mixture was incubated at 25°C, in the dark, for 20 minutes. Stop solution (100µL) was added to each well to stop the reaction. The content of the wells were gently mixed for 30 seconds. The concentration was measured at 450nm within 15 minutes.

c) TRIIODOTHYROXINE(T3) ASSAY (Abuid *et al*., 1974)

Principle

Competitive Enzyme Immunoassay – Analog Method for Free T3

The essential reagents required for a solid phase enzyme immunoassay include immobilized T3 antibody, enzyme-T3 conjugate and native free T3 antigen. The enzyme-T3 conjugate should have no measurable binding to serum proteins especially TBG and albumin. The method achieves this goal.

Upon mixing immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{c.w.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{c.w.} + {}^{Enz}AgAb_{c.w.}$$

Ab c.w. = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Free Antigen (Variable Quantity)
EnzAg = Enzyme-antigen Conjugate (Constant Quantity)
AgAb c.w. = Antigen-Antibody Complex
EnzAg Ab c.w. = Enzyme-antigen Conjugate -Antibody Complex
Ka = Rate Constant of Association
k-a = Rate Constant of Disassociation
K = ka / k-a = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme concentration in the antibody-bound fraction is inversely proportional to the native free antigen concentration.

Procedure

The desired numbers of coated wells were placed in the holder. Then, 50 µl of the appropriate serum reference, control and specimen was pipetted into the assigned well. Again. 100µl of fT3-enzyme reagent solution was added to all wells. The microplate was swirled gently for 30 seconds to mix; it was then covered and incubated 60 minutes at 25°C. The content of the microplate was discarded by aspiration. Then 300µl of wash buffer was added to each wells and aspirated. The wash process was repeated two additional times for a total of three washes. Also, 100µl of working substrate solution was added to all wells and incubated for 15 minutes at 25°C. Finally, 50 µl of stop solution was added to each well and mixed for 20 seconds. The concentrations in each well were measured at 450nm.

3.2.4.3. INTERLUKINS (IL) ASSAY (Chan and Perlstein, 1987).

We assayed for interleukins 1- 33 except IL- 19, 20, 24, 28, and 29 because of the inability to get the test kits.

Principle

IL *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL in serum, plasma, buffered solutions and cell culture medium.

A monoclonal antibody specific for the particular IL has been coated onto the wells of the microtiter strips provided. Samples, including blank of known IL concentrations, control specimens or unknowns were pipetted into these wells. During the first incubation, the blank or samples and a biotinylated monoclonal antibody specific for the particular IL are simultaneously incubated. After washing, the enzyme Streptavidin- HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL present in the samples.

Procedure

The number of microwell strips required was placed in the holder. The microwell strips was washed twice with exactly 400 µl Wash Buffer prior to the analysis. The wells were thoroughly aspirated of its contents between washes. The Wash Buffer was allowed to sit in the wells for 15 seconds before aspiration. After the last wash step, wells were emptied and tapped on absorbent paper towel to remove excess Wash Buffer. The microwell strips were used immediately after washing. Exactly 100 µl of Sample Diluent was added to all blank wells. Another, 100 µl of prepared Reference Reagent was pipetted into the well. The contents of wells were mixed by repeated aspiration and ejection, and 100 µl of the mixture were transfered to another wells B1 and B2, respectively. Another, 100 µl of sample diluent was added to the blank wells. Exactly, 50 µl of sample diluent was added to the sample wells. Also, 50 µl of each sample was added to the sample wells. Then 50 µl of biotin-conjugate was added to all wells. Covered with an adhesive film and incubated at 25°C in the dark over night. After the incubation, adhesive film was removed and the wells emptied. Then the microwell strips was washed 6 times according to step two. Then 100 µl of diluted streptavidin-HRP to all wells. Covered with an adhesive film and incubated at 25°C for 1 hour on a microplate shaker set at 400 rpm in the dark. After the incubation, the adhesive film was removed and the wells emptied. The microwell strips was washed 6 times according to step two.

At the amplification stage, 100 µl of amplification solution I was added to all wells. Covered with an adhesive film and incubated at 25°C for 15 minutes on a microplate shaker set at 400 rpm in the dark. After the incubation, the adhesive film was removed and the wells emptied. The microwell strips was washed 6 times according to step two. Another, 100 µl of amplification solution II was added to all wells. Covered with an adhesive film and incubated at 25°C for 30 minutes, on a microplate shaker set at 400 rpm in the dark. After the incubation, the adhesive film was removed and the wells emptied. The microwell strips was washed 6 times according to step two. Again, another 100 µl of TMB substrate solution was pipetted to all wells and incubated at 25°C for 20 min. The enzyme reaction was stopped by pipetting 100 µl of stop solution into each well. The plate reader was blanked using the blank wells and concentration of both the samples and the control was determined at 450 nm as the primary wave length.

3.2.4.4. INFLAMMATORY BIOMARKERS ASSAY

a) HYDROGEN PEROXIDASE AND HYDROGEN PEROXIDE ASSAY (Halliwell and Gutteridge, 1999)

Principle

The Hydrogen Peroxide/Peroxidase Assay Kit is a sensitive quantitative colorimetric assay for hydrogen peroxide or peroxidase. In the presence of peroxidase, the probe reacts with H_2O_2 in a 1:1 stoichiometry to produce a bright pink colored product. This product can be easily read by a standard colorimetric microplate reader with a filter in the 540nm range. Concentration values are proportional to the H_2O_2 or peroxidase levels within the samples.

Procedure

I. Hydrogen Peroxide

The number of microwell strips required was placed in the holder. Then, 50 µL of each sample of H_2O_2 blank, control and unknown were added into an individual microtiter plate well. Another, 50 µL of hydrogen peroxide working solution was dispenced to each well. The well contents was mixed thoroughly and incubated for 30 minutes at 25°C in the dark. The concentration was measured at 540nm.

II. Peroxidase

The number of microwell strips required was placed in the holder. Then, 50 µL of each sample of HRP blank, control and unknown were added into an individual microtiter plate well. Another 50 µL of peroxidase working solution was pipetted to each well. The well contents was mixed thoroughly and incubated for 30 minutes at 25°C in the dark. The concentration was measured at 540nm.

b) MYELOPEROXIDASE (MPO) ASSAY (Klein, 1982)

Principle

The Myeloperoxidase Kit is a quantitative assay for measuring the myeloperoxidase concentration within a sample. The MPO enzyme catalyzes the reaction of hydrogen peroxide (H_2O_2) with chloride ions to create hypochlorous acid (HOCl), which rapidly reacts with taurine to produce a stable taurine chloramine product. This step readily neutralizes the HOCl, which would otherwise accumulate and inactivate MPO. A catalase-containing stop solution is added to stop MPO catalysis by eliminating hydrogen peroxide. Finally, taurine chloramine reacts with the yellow TNB chromogen probe, with a decrease in color indicating higher MPO concentration. Concentration is measured at 405 nm. The rate of chromophore reduction is proportional to the concentration of myeloperoxidase within the sample.

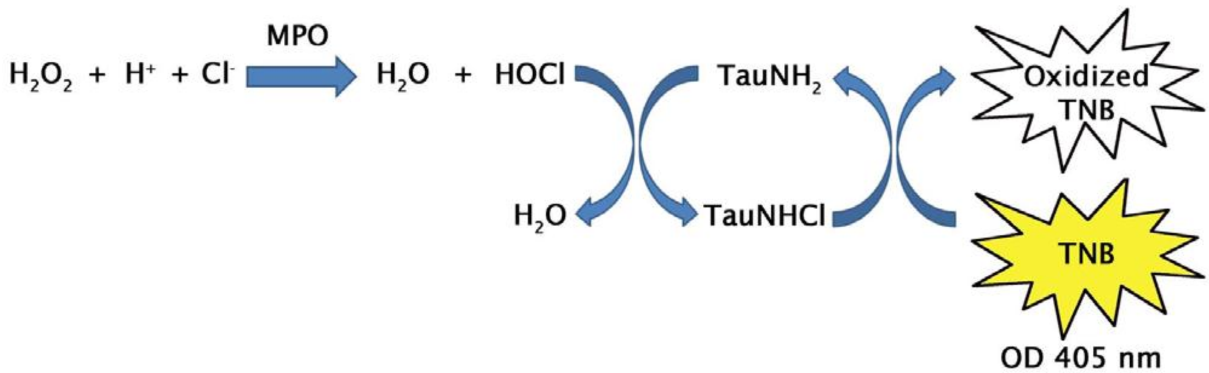

Fig. 11; Assay Principle for Myeloperoxidase

Procedure

The number of microwell strips required was placed in the holder. Then, 25 µL of each unknown sample and control was added to a 96-well plate. Another 25 µL of the 1 mM hydrogen peroxide solution was added to each well, mixed thoroughly on a horizontal shaker, the plate covered and incubated at 25°C for 30 minutes in the dark. The stop solution was vortexed and 50 µL of the vortexed stop solution was added to each sample well, mixed briefly and incubated at 25°C for 15 minutes. Then 50 µL of the 1 mM chromogen working solution was added to each well to which stop solution was added in step 4, and mixed briefly. The plate was covered and incubated at 25°C for 15 minutes in the dark. The concentration was measured using 405nm as the primary wave length.

3.2.4.5 OXIDATIVE STRESS MARKER ASSAY

a) ENDOGENOUS ANTIOXIDANTS ASSAY

i) SUPERIOXIDE DISMUTASE (SOD) ASSAY (Nebot *et al*., 1993)

Principle

Superoxide anions (O2-) are generated by a Xanthine/Xanthine Oxidase (XOD) system, and then detected with a chromagen solution. However, in the presence of SOD, these superoxide anion concentrations are reduced, yielding less colorimetric signal.

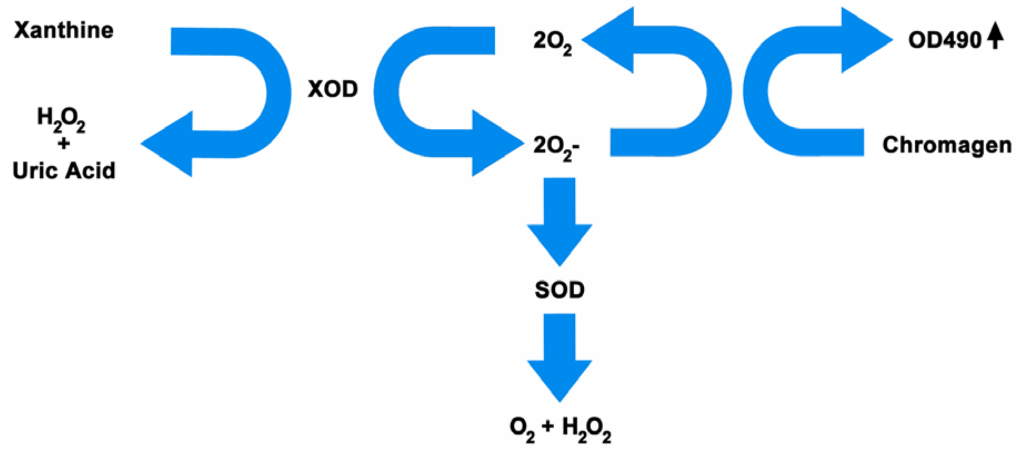

Fig12; Assay Principle for superoxide dismutase

Procedure

The samples was prepare including a blank in a 96-well microtiter plate according to the below table.

TABLE 12; Procedure for SOD Analysis

Component	Blank	Sample
SOD Sample	0 µL	15 µL
Inhibitor	0 µL	15 µL
Xanthine Solution	5 µL	5 µL
Chromagen Solution	5 µL	5 µL
10X SOD Assay Buffer	10 µL	10 µL
DI Water	70 µL	20 µL
Total	90 µL	90 µL

Finally, 10 µL of pre-diluted xanthine oxidase solution was added to each well, mixed well and incubated for 1 hour at 37ºC. The concentration was read at 490 nm.

ii) CATALASE ASSAY (Aebi, 1973)

Principle

Catalase Assay involves two reactions. The first reaction is the catalase induced decomposition of hydrogen peroxide H_2O_2 into water and oxygen. The rate of disintegration of hydrogen peroxide into water and oxygen is proportional to the concentration of catalase. A catalase-containing sample can be incubated in a known amount of hydrogen peroxide. The reaction proceeds for exactly one minute, at which time the catalase is quenched with sodium azide. The remaining hydrogen peroxide in the reaction mixture facilitates the coupling reaction of DHBS and AAP in conjunction with an HRP catalyst. The quinoneimine dye coupling product is measured at 520nm, which correlates to the amount of hydrogen peroxide remaining in the reaction mixture.

Reaction 1: $$2\ H_2O_2 \xrightarrow{\text{CATALASE}} 2\ H_2O + O_2$$

Reaction 2: $$2\ H_2O_2\ \text{(Left over)} + \text{DHBS} + \text{AAP} \xrightarrow{\text{HRP}} \text{Quinoneimine Dye}$$

Fig 13; Catalase Assay Principle

Procedure

The number of microwell strips required was placed in the holder. Then, 20 µL of the diluted catalase blank and unknown samples was added to a 96-well microtiter plate, 50 µL of the hydrogen peroxide working solution (12mM) was added to each well, then it was mixed thoroughly and incubated for 1 minute. The reaction was stopped by adding 50 µL of the catalase quencher into each well and mixed thoroughly. Another 5 µL of each reaction well was transfered to a fresh well, and 250 µL of the chromogenic working solution was added to each well and incubated for 60 minutes with vigorous mixing. The concentration was measured at 520 nm.

iv) NITRIC OXIDE ASSAY (Stuehr and Marletta, 1985)

Principle

Nitric Oxide (Nitrite/Nitrate) Assay Kit is a simple, colorimetric assay that quantitatively measures NO in various samples by NO^{2-}/NO^{3-} determination. First, the nitrate (NO^{3-}) in the sample is converted to nitrite (NO^{2-}) by nitrate reductase enzyme. Next, total nitrite is detected with griess reagents as a colored azo dye product (concentration 540 nm).

Principle

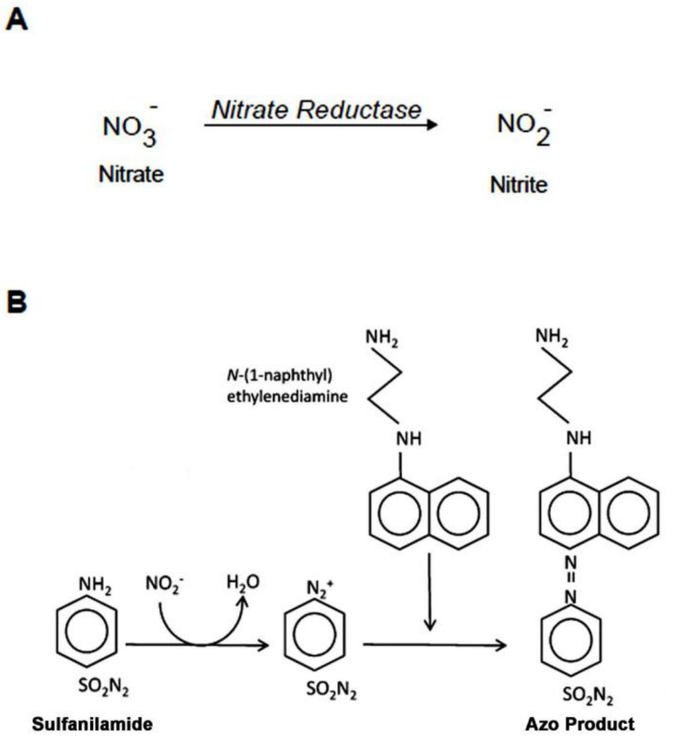

Fig 14; Conversion of nitrate to nitrite by nitrate reductase (A), followed by the Griess Reaction (B).

Procedure

The number of microwell strips required was placed in the holder set. Then, 50 μL of the nitrite blank, samples, and blanks was added to the wells. Another 50 μL of PBS was added to each well, then 50 μL of griess reagent A was also added to each well. Immediately 50 μL of griess reagent B was added to each well and incubated the plate at 25°C for 10 minutes. The concentration was measured at 540 nm.

v) NITROTYROSINE ASSAY (Ischiropoulos *et al*., 1992)

Principle

The nitrotyrosine quantitation kit is a competitive ELISA. The unknown protein nitrotyrosine sample or nitrated BSA blank are first added to a nitrated BSA preabsorbed EIA plate. After a brief incubation, an anti-nitrotyrosine antibody is added, followed by an HRP conjugated secondary antibody. The protein nitrotyrosine content in unknown sample is determined by comparing with a predetermined nitrated BSA blank.

Procedure

The number of microwell strips required was placed in the holder set. Then, 50 μL of unknown protein sample, nitrated BSA blank were added to the wells of the EIA plate and incubate at 25°C for 10 minutes on an orbital shaker. Then 50 μL of the diluted anti-nitrotyrosine antibody was added to each well, and incubated again at 25°C for 1 hour on an orbital shaker. The microwell strips was washed 3 times with 250 μL wash buffer per well with thorough aspirated between each wash. After the last wash, the wells were emptied and tapped on absorbent paper towel to remove excess wash buffer. Then 100 µL of the diluted secondary antibody-enzyme conjugate was dispenced into all wells and incubated at 25°C for 1 hour on an orbital shaker. The microwell strips were washed 3 times according to step 2 above. Substrate solution was kept to warm at 25°C, 100 µL of the warmed substrate solution was added to each well and incubated at 25°C on an orbital shaker for 30 minutes. The enzyme reaction was stopped by adding 100 µL of stop solution into each well. The concentration of each microwell was measured at 450 nm.

vi) PROTEIN CARBONYL ASSAY (Cadenas *et al*., 1977)

Principle

BSA reference reagent or protein samples (10µg/mL) are adsorbed onto a 96-well plate for 2 hrs at 37ºC. The protein carbonyls present in the sample are derivatized to DNP hydrazone and probed with an anti-DNP antibody, followed by an HRP conjugated secondary antibody. The protein carbonyl content in unknown sample is determined by comparing with predetermined reduced and oxidized BSA reference reagent.

Procedure

The number of microwell strips required was placed in the holder. Each 10 µg/mL sample and BSA blank were be assayed in the well. Then, 100 µL of 10 µg/mL protein samples were added to the 96-well protein binding plate and incubated at 37ºC for 2 hours. The wells were washed 3 times with 250 µL PBS per well. After the last wash, the wells were emptied and tapped on absorbent paper towel to remove excess wash solution. Another 100 µL of the DNPH working solution was added and incubated for 45 minutes at 25°C in the dark. The wells were washed with 250 µL of PBS/Ethanol (1:1, v/v) and incubated on an orbital shaker for 5 minutes. The washing of the plate wells was repeated for 5 times, aspirating between each. After the last wash, the wells were emptied and tapped on absorbent paper towel to remove excess wash solution. Afterwards, it was washed 2 times with 250 µL of PBS. Then 200 µL of blocking solution was added to each well and incubated for 2 hours at 25°C on an orbital shaker. The plate wells were washed 3 times with 250 µL of wash buffer with thorough aspiration between each wash. After the last wash, the wells were emptied and tapped on absorbent pape towel to remove excess wash buffer. Again, 100 µL of the diluted anti-DNP antibody was added to all wells and incubated for 1 hour at 25°C on an orbital shaker. The strip wells were wshed 3 times according to step 5 above. Another 100 µL of the diluted HRP conjugated secondary antibody was added to all wells and incubated for 1 hour at 25°C on an orbital shaker. The strip wells were washed 5 times as earlier described. The substrate solution was brought to 25°C. 100 µL of the warmed substrate solution was added to each well and incubate at room temperature on an orbital shaker for 30 minutes. The enzyme reaction was stopped by adding 100 µL of stop solution to each well. The concentration of each well was measured on a plate reader using 450 nm.

b) LIPID PEROXIDATION ASSAY

i) MALONDIALDEHYDE (MDA) ASSAY (Janero, 1990).

Principle

The free MDA present in the sample reacts with Thiobarbituric Acid (TBA) to generate a MDA-TBA adduct, which can be easily quantified colorimetrically (532 nm). This assay can detect as low as 1 nmol/well MDA in colorimetric assay.

Procedure
The number of microwell strips required was placed in the holder. Then, 600 µL of TBA reagent was dispenced into each well containing 200 µL blank (Reference Reagent) and 200 µL sample and incubated at 95°C for 60 minutes. The plate was allowed to cool to 25°C in an ice bath for 10 minutes. And 200 µL of the supernatant was teken and placed into a new set 96-well microplate for analysis. The concentration was measure immediately on a microplate reader at 532 nm.

ii) LIPID HYDROPEROXIDE ASSAY (Yamamoto *et al*., 1987).
Principle
This kit measures the hydroperoxides directly utilizing the redox reactions with ferrous ions. Hydroperoxides are highly unstable and react readily with ferrous ions to produce ferric ions.
The resulting ferric ions are detected using thiocyanate ion as the chromogen.

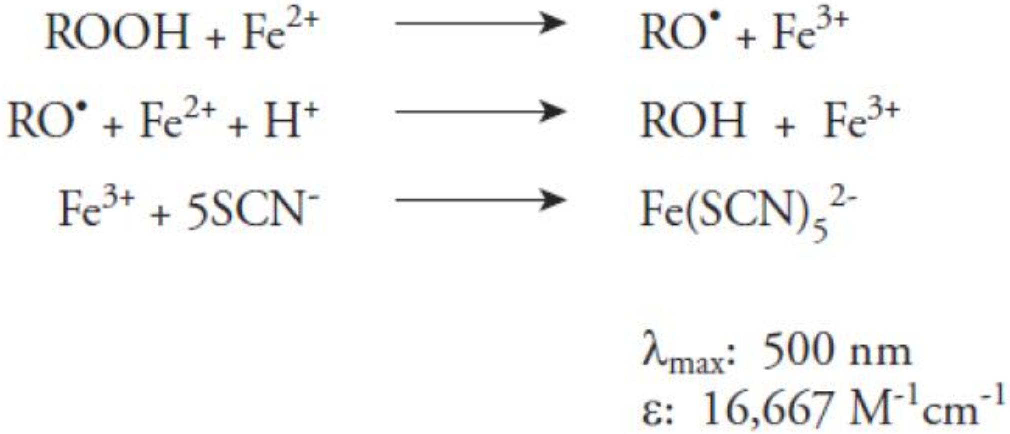

Fig 15. Assay scheme - Reduction/Oxidation Reactions

Since this method relies on the measurement of ferric ions generated during the reaction, ferric ions present in the sample are a potential source of error. Also, many biological samples contain hydrogen peroxide which readily reacts with ferrous ions to give an over-estimation of lipid hydroperoxides. These problems are easily circumvented by performing the assay in chloroform.
An easy to use, quantitative extraction method was developed to extract lipid hydroperoxides into chloroform and the extract is directly used in the assay. This Procedure eliminates any interference caused by hydrogen peroxide or endogenous ferric ions in the sample and provides a sensitive and reliable assay for lipid peroxidation.

Procedure
The number of microwell plate required was set up in the holder. Then, 500 µl of the chloroform extract of each sample was added to appropriately labeled wells. Aliquot 450 µl of chloroform-methanol solvent mixture was added to the sample test tubes. And 50 µl of the freshly prepared chromogen was added to each assay wells; the wells were closed tightly with polypropylene caps and mixed well by vortexing. The assay wells were kept at 25°C for five minutes. The concentration of each tube was measured at 500 nm using a plate reader.

iii) 8-ISOPROSTANE ASSAY (Morrow, 2005)

Principle

The Direct 8-isoprostane ELISA kit is a competitive immunoassay for the quantitative determination of 8-iso-Prostaglandin in biological fluids. The kit uses a polyclonal antibody to 8-isoprostane to bind, in a competitive manner, the 8-isoprostane in the sample or an alkaline phosphatase molecule which has 8-isoprostane covalently attached to it. After a simultaneous incubation at either room temperature or 4°C, the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of 8-isoprostane in samples.

Procedure

The number of microwell Plate required was set up in the holder. Then 200 ml of sample dilution buffer was added into the blank wells and 100 ml of sample dilution buffer into control wells. 100 ml of each of the samples was added into the appropriate wells. 100 ml of the HRP conjugate was dispenced in the all wells except the blank and control wells. The resultant mixture was incubated at 25°C for two hours. The plate was washed three times with 400 ml of wash buffer. After the last of the three wash cycles, the inverted plate was pat dry onto absorbent paper towels. Also 200 ml of the TMB substrate was added to all of the wells and incubated at 25°C for 30 minutes. Then 50 ml of 2N sulfuric acid was pipetted into all of the wells. The concentration was measured at 450 nm.

vi) HEXANOYLLYSINE (HEL) ASSAY (Kato *et al*., 1999).

Principle

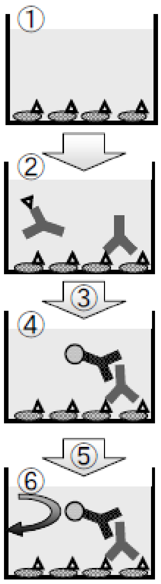

Fig. 16; Hexanoyllysine assay principle

Procedure

The required number microtiter plate precoated with hexanoy-Lys adduct (HEL) was set up on the holder. Then, 50 µL of sample were added into each well. For the Blank well, 100 µL of washing solution was used. Then 50 µL of primary antibody was added to all the wells except blank well. The microtiter plate was sealed tightly with plate seal, mixed gently by shaking the microtiter plate horizontally and incubated at 7 ˚ C for over night. The plate seal was removed, and the contents of mictotiter plate were poured off by turning the plate upside down. Again 250 µL of washing solution was added to each well, mixed gently by horizontal shaking, and the contents was removed similarly as described earlier. The washing procedure was repeated twice. Then 100 µL of secondary antibody was pipetted to all well. The microtiter plate was sealed with plate seal, mixed gently by shaking the microtiter plate horizontally and incubated at 24˚C for 1 hour. After the incubation, the plate seal was removed, and the plate was washed as mentioned at step 3 for 3 times. Another 100 µL of chromogen solution was added to all well and incubated at 25˚C for 15 minutes in the dark. And 100 µL of stop solution was added to all well, mixed gently, and allowed to stand for 3 minutes, and then the concentration was measured at 450 nm.

v) OXIDIZED LIPID (OxLDL) ASSAY (Esterbauer *et al*., 1991).

Principle

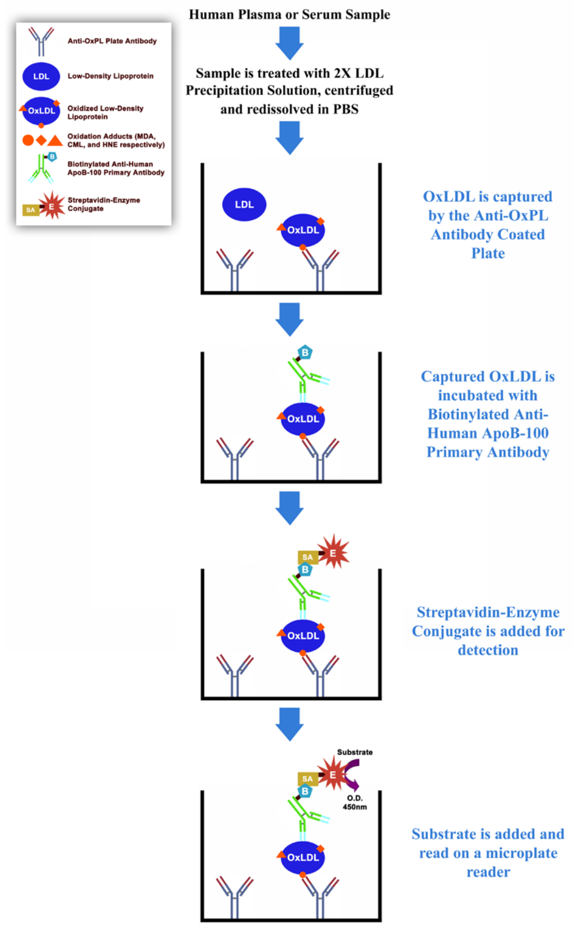

Fig.17; Oxidized LDL assay principle

Procedure

The required number precoated microtiter plate was set up on the holder. Exactly 100 µL of unknown sample was added to the anti-OxPL antibody coated plate. The plate was covered with a plate cover and incubated at 25°C for 2 hours on an orbital shaker. The microwell plate was washed 5 times with 250 µL wash buffer and thoroughly aspirated between each wash. After the last wash, the wells were emptied and tapped on absorbent paper towel to remove excess wash buffer. Then 100 μL of the diluted biotinylated anti- Apo B-100 antibody was added to each well and incubated at 25°C for 1 hour. The microwell plate was washed 5 times according to step 2 above. Another 100 μL of the diluted streptavidin-enzyme conjugate was pipetted into each well and incubated at 25°C for 1 hour on an orbital shaker. The microwell plate was washed 5 times according to step 2 above. Again 100 μL of warmed substrate solution was added to each well and incubated at 25°C on an orbital shaker for 20 minutes. The enzyme reaction was stopped by adding 100 μL of stop solution into each well, and the concentration of each microwell was measure at 450 nm.

c) PEROXIREDOXIN SYSTEM ASSAY (Fujii and Ikeda, 2002)

The peroxidoxine system comprise peroxiredoxin-1(PRX-1) to peroxiredoxin-6 (PRX-6)

Principle

This assay is common and employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for the particular PRDX has been pre-coated onto a microplate. Blank and samples were pipetted into the wells and any PRDX present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for PRDX is added to the wells. After washing, avidin conjugated horseradish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PRDX bound in the initial step. The color development is stopped and the intensity of the color is measured.

Procedure

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C before use. Exactly 100µl of controls and sample were pipetted into each well, the plate was covered with the adhesive strip and incubated for 2 hours at 37°C. The liquid of each well was removed by decanting. Another 100µl of biotin-antibody was added to each well, the plate was covered with a new adhesive strip and incubated for 1 hour at 37°C. Each well was aspirated and washed 3 times by filling each well with wash buffer (200µl) using a multi-channel pipette, and let it stand for 2 minutes. After the last wash, any remaining wash buffer was removed by aspiration. The plate was inverted and blotted against clean absorbent paper towels. Then 100µl of HRP-avidin was added to each well, the plate was covered with a new adhesive strip and incubated for 1 hour at 37°C. The aspiration and wash process was repeated for five times. Another 90µl of TMB substrate was added to each well and incubated for 30 minutes at 37°C in the dark. The reaction was stopped by adding 50µl of stop solution to each well, and the plate was gently tapped to ensure thorough mixing. The concentrations were determined at 450 nm.

d) GLUTATHIONE SYSTEM ASSAY

i) GLUTATHIONE REDUCTASE ASSAY (Carlberg and Mannervik, 1985)

Principle

The Glutathione Reductase Assay Kit is a quantitative assay for measuring the glutathione reductase concentration within a sample. Glutathione Reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. Subsequently, the chromogen reacts with the thiol group of GSH to produce a colored compound that absorbs at 405 nm. The intensity of chromophore production is proportional to the concentration of glutathione reductase within the sample.

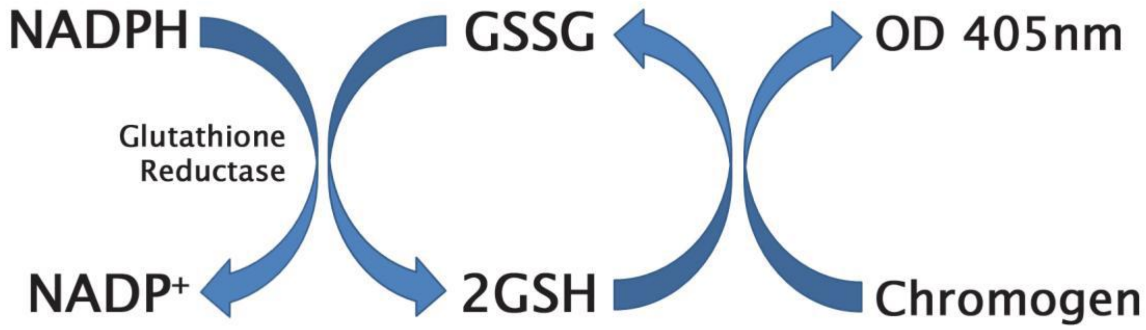

Fig.18. Glutathione reductase Assay Principle

Procedure

In a 96-well plate set up on the holder, 25 µL of the NADPH solutions was added to each well to be tested. 100 µL of the controls and samples was added, 50 µL of the chromogen was added to each wells and mixed briefly. Then 25 µL of the glutathione disulfide (GSSG) solution was then addeded and the plate contents were mixed briefly. Immediately the concentration was measured at 405 nm.

ii) GLUTATHIONE PEROXIDASE ASSAY (Mannervik, 1985)

Principle

Glutathione Peroxidase Assay Kit (Colorimetric), GPx reduces cumene hydroperoxide while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease of NADPH (easily measured at 340 nm) is proportional to GPx concentration.

The glutathione peroxidase (GPx) family of enzymes plays an important role in the protection of organisms from oxidative damage. GPx converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water. Several isozymes have been found in different cellular locations and with different substrate specificity. Low levels of GPx have been correlated with free radical related disorders.

Procedure

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C prior to use. A 40 µL of reaction mix was pipetted to sample, positive control and reagent control wells, mixed and incubated at 25°C for 15 minutes to deplete all GSSG in the samples. GSH solution 2 was added. Then 10 µL cumene hydroperoxide solutions were added to start the glutathione peroxidase (GPx) reaction and the contents were mixed well. The concentration was measure at 340 nm. The mixture was incubated at 25ºC for 5 min in the dark.

The final concentration was measured 340 nm.

iii) TOTAL GLUTATHIONE, OXIDISED GLUTATHIONE AND REDUCED GLUTATHIONE ASSAY (Tietze, 1969)

Principle

Samples and diluted blank are added to wells of a 96-well plate.

Reaction mix is added to the wells to initiate the reaction.

The plate is transferred to a plate reader and concentration readings are taken at 405 nm.

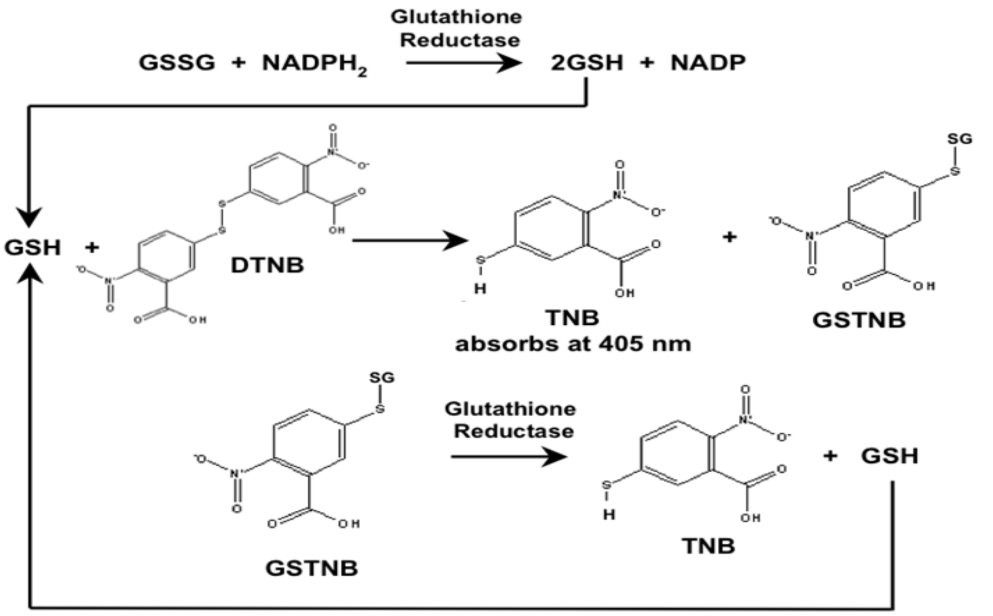

Fig. 19; Total Glutathione Assay Principle

Procedure

Total Glutahione Assay

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C prior to use. A 50 µl of assay buffer as pipetted into all the wells of the microtiter plate. 50 µl of the 4 µm GSSG was added to the wells and mixed well by pipeting the solution up and down ten times. Again 50 µl of the mixture from wells were transfered to new wells and mix well. Another 50 µl of experimental samples was added to the wells. Using a multichannel pipetor, 150 µl of freshly prepared reaction mix was added to each well. Immediately the concentration was measured and recorded at 405 nm.

Oxidized Glutathione Assay

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C prior to use. And 1 µL of 2M 4-vinylpyridine per 50 µL of sample and 4 µM GSSG were dispenced in to the wells and incubated for one hour at 25°C. Diluted 4-vinylpyridine-treated GSSG and dilute 4-vinylpyridine-treated experimental samples were added. Immediately the concentration was measured and recorded at 405 nm.

e) THIOREDOXIN SYSTEM ASSAY

i) THIOREDOXINE ASSAY (Holmgren and Bjornstedt, 1995)

Principle

The test Principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Thioredoxin (Trx). Samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Thioredoxin (Trx). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Thioredoxin (Trx), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm.

Procedure

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C prior to use. A 100µl sample was added to each well and incubated 1 hour at 37°C, the wells were aspirated, 100µl prepared detection reagent A was added and again incubated for 1 hour at 37°C. After the incubation, the wells were aspirated and washed 3 times and 100µl prepared detection reagent B was added and incubated 30 minutes at 37°C, afterward, the wells were aspirated and washed 5 times. Then 90µl substrate solution was added and incubated for 25 minutes at 37°C and 50µl stop solution was added. The concentrations were measured at 450nm immediately.

ii) THIOREDOXINE REDUCTASE ASSAY (Holmgren and Bjornstedt, 1995)

Principle

Thioredoxin reductase (TrxR) is a ubiquitous enzyme which is involved in many cellular processes such as cell growth, p53 activity, and protection against oxidation stress, etc. The mammalian TrxR reduces thioredoxins as well as non-disulfide substrates such as selenite, lipoic acids, lipid hydroperoxides, and hydrogen peroxide.

Thioredoxin Reductase (TrxR) Assay Kit provides a convenient colorimetric assay for detecting TrxR concentration in various samples. In the assay, TrxR catalyzes the reduction of 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (TNB^{2-}), which generates a strong yellow color ($\lambda max = 412$ nm). Since in crude biological samples other enzymes, such as glutathione reductase and glutathione peroxidase, can also reduce DTNB, therefore, TrxR specific inhibitor is utilized to determine TrxR specific activity.

Two assays are performed: the first measurement is of the total DTNB reduction by the sample, and the second one is the DTNB reduction by the sample in the presence of the TrxR specific inhibitor. The difference between the two results is the DTNB reduction by TrxR.

Procedure

The required number precoated microtiter plate was set up on the holder and the temperature of all the reagents and samples were brought to 37°C prior to use. A 10 µL of the TNB, sample and control was pipetted into 96-well plate. 40 µL reaction mix was added to each well, 30 µL assay buffer, 8 µL DTNB solution and 2 µL NADPH were added to each well respectively and the plate was mixed well. Concentration was measured at 412nm then the mixture was incubated at 25°C for 20 min in the dark and the concentrations were measured again at 412nm.

3.3 STATISTICAL ANALYSIS

The data obtained from each set of study were subjected to statistical analysis using the IBM Statistics software, version 20. Each attribute were subjected to ANOVA analysis, post hoc analysis was carried out using turkey, and $P \leq 0.05$ was considered significant (see appendix I - X)

CHAPTER FOUR

RESULTS

4.1 RESULT OF ACUTE TOXICITY (LD_{50})

LORKE'S METHOD

From the experiment the following results were obtained. LD_{50} was calculated by substituting for the values in each instance using the formular as stated earlier.

TABLE 13: Result obtained from Lorkes method for LD_{50} determination.

Route of Administration	Sex	D_0	D_{100}	LD_{50} (mg/kg)
ORAL	Male	1600	5000	2828.427
	Female	1150	4000	2144.76
SUBCUTANEOUS	Male	150	400	244.949
	Female	70	380	163.095

KARBER'S METHOD

From the experiment the following results were obtained. LD_{50} was calculated by substituting for the values in each instance using the formular stated earlier.

TABLE 14: result obtained from Karbers method for LD_{50} determination.

Route of Administration	Sex	D_{100}	a			b			n			LD_{50}(mg/kg)
ORAL	Male	5000	1000	500	100	8	9	5.5	6	6	6	2825.00
	Female	4000	1000	200	-	9.5	7.5	-	6	6	-	2166.67
SUBCUTANEOUS	Male	400	100	20		9	3.5		6	6		235.417
	Female	380	100	20		11.5	5.5		6	6		170

UP AND DOWN METHODS

TABLE 15: result obtained from Up and Down method for LD_{50} determination.

ROUTE OF ADMINISTRATION	SEX	LD_{50}
ORAL	FEMALE	2160
	MALE	2800
SUBCUTANEOUS	FEMALE	160
	MALE	240

TABLE 16: Comparism of the LD_{50} obtained using up and down (AT) method, Lorkes method (LM) and Karbers method (KM) equation for LD_{50} determination

Route of Administration	SEX	LD_{50} (Mg/Kg B.W)		
		Lorke's Method	Karber's Method	Up and Down Method
ORAL	FEMALE	2144.76	2166.6	2160
	MALE	2828.43	2825.00	2800
SUBCUTANEOUS	FEMALE	163.10	170.00	160
	MALE	244.949	235.42	240

Other results obtained from the study are presented in the appendix XII to XX and results in bar chart to show profile changes in experimental group within weeks, and a profile of concentration against groups revealing group behavior over time.

4.2 EFFECT OF BISPHENOL A ON BODY WEIGHT OF RATS

Result at wk 0 showed that weight of rats were not evenly distributed, but in course of time a dose dependent weight gain with the highest weight gain recorded by animal administered 50µg/kg see (fig 20a). It is apparent from the group performance profile that in all weeks, group 1 recorded highest body weight increase. The increase was steady with a peak at week 13 (see fig.20b). Although the body weight were initially higher than that of the control, but there was a dose dependent decrease in body weight across the various group as the duration extends, Low doses of BPA (50 – 400µg/kg) show high gain in body weight. As the doses increased the body weight decreases.

The body weight of the rats increased at lower doses with duration, as the rats were maintained on constant feed ad libitum. This increase in body weight of BPA treated rats was significantly higher ($p \leq 0.05$) than that of rats in the control group. It was observed that the lower doses of BPA triggered higher weight gain compared with higher doses. This was despite the fact that those groups on higher doses of BPA consumed more feed. The BPA concentration found to trigger the maximum weight gain was 50µg/kg. This result seems to suggest that BPA is an Obesogen at lower doses.

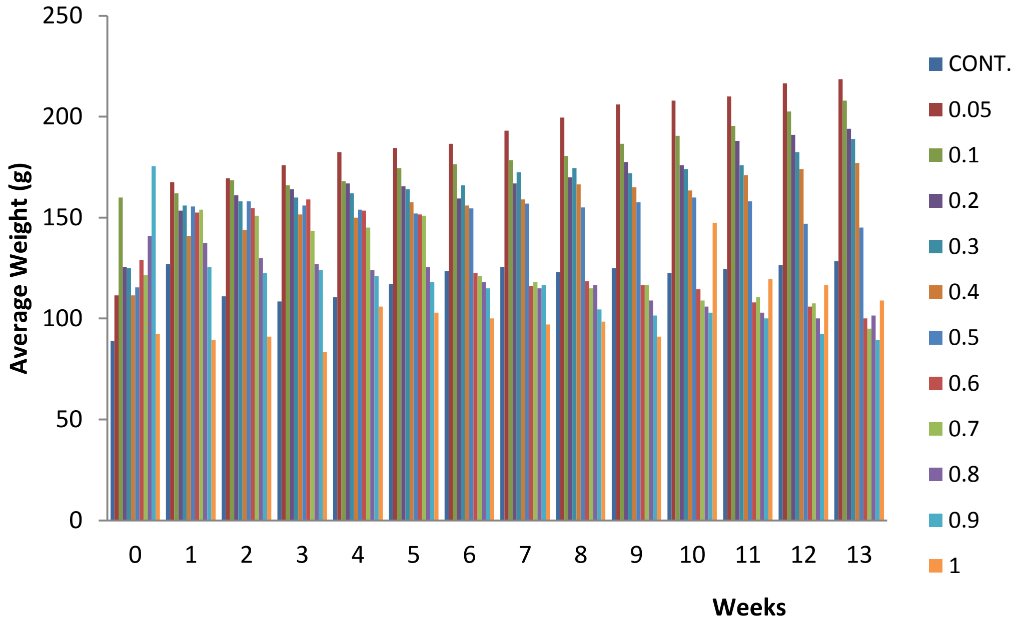

Fig. 20a; Bar chart of change in body weight throughout the 13 weeks duration of the research.

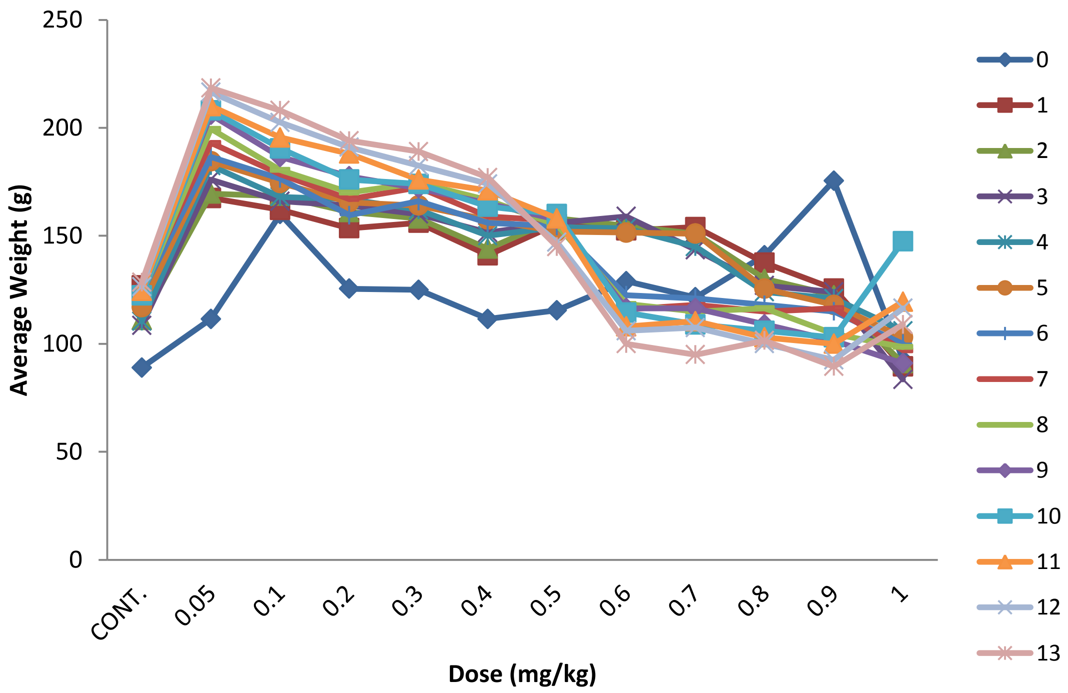

Fig. 20b; Graph of Average weight throughout the 13 weeks duration of the research.

1.3 EFFECT OF BISPHENOL A ON SEX HORMONE

a) EFFECT ON ESTRADIOL (ESTROGEN (E2)

It was observed that BPA induced a dose dependent increase in estrogen concentration, which increased with sustained administration as shown in fig 21a. The higher concentrations of BPA caused a spike in estrogen concentration which was maintained for all the weeks, on the other hand, lower concentration appeared to maintaine aslight but steady increase in estrogen concentrations over time, with sustained administration, which did not differ significantly from the estrogen level in the control group for weeks 1 to 3 for the groups that received 0.05mg/kg to 0.5mg/kg of BPA. Across the weeks, there was significant increase ($p \leq 0.05$) in the serum estrogen level, In each week, within the groups there was a significant increase that was dose dependent especially at groups 0.6mg/kg to 1mg/kg. There was significant increase in estradiol at groups 0.6mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/kg and 1mg/kg at all time interval. The group perfomance profile revealed tht BPA enhanced estrogen production in dose dependent manner. Higher doses of BPA induced higher estrogen production. However, there was noticeable reversion in estrogen profile with time after the BPA dose of 0.8mg/kg group (see fig 21b). there was instant estrogen spike, which steadily increase as time progressed for group 0.9mg/kg and 1mg/kg but the observed increase was reversed as the duration of the experiment progressed.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group that received 1mg BPA per kg body weight showed the highest level of estradiol, followed by 0.9mg/kg. These groups recorded highest value at week 1 and gradually decrease over time; the concentration of estradiol for these groups was lowest at week 13 (fig 21a). Groups 0.6mg/kg and 0.7mg/kg were significantly higher than those of control and week 0, at $p \leq 0.05$. These group recorded the lowest value at week 1, and showed steady increase over time with their highest value obtained at week 13. Between weeks 3 to 13, a significant dose dependent increase was observed for groups 0.05mg/kg, 0.1mg/kg and 0.2 mg/kg at $p \leq 0.05$; which was consistent over time. Groups 0.05mg/kg to 0.7 mg/kg recorded their highest value at week 13 and lowest value for estradiol at week 1. While groups 0.8mg/kg to 1 mg/kg recorded their highest value at week 1 and lowest value at week 13 (fig 21a).

The highest serum concentration of estradiol was recorded in week 13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estradiol levels at week 13 and high estradiol levels at week 1. In fig 21b, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estradiol continued to increase, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 21b).

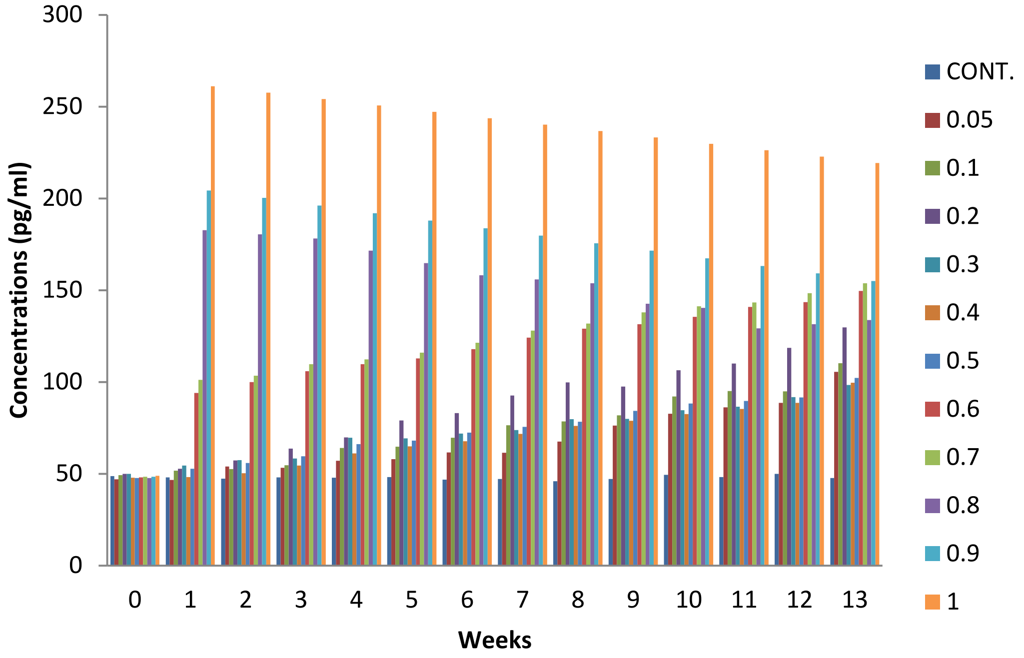

Fig. 21a; Bar chart of concentration against weeks (durations) for change in Estradiol E2 (Estrogen).

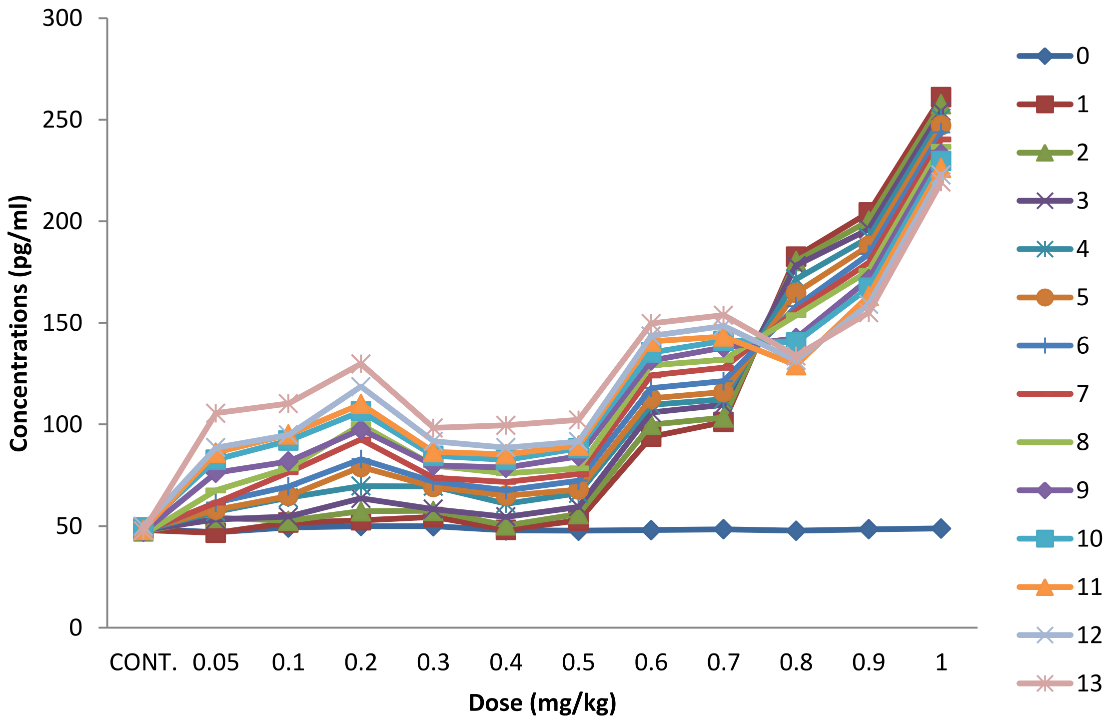

Fig. 21b; Graph of concentration against dose for change in serum Estradiol E2 (Estrogen) levels.

b) EFFECT ON ESTRONE

At the onset of the experiment, it was observed that the estrone serum concentration were increase across the weeks (fig. 22a) . Estrone levels were higher in week 1 for groups 0.8mg/kg to 1mg/kg. There were significant increases in estrone level when compared with the control at $p \leq 0.05$. Groups 0.05mg/kg to 0.5mg/kg showed no signifiant difference at weeks 1 to 3. As the duration of exposure increased, the level of estrone increased correspondingly (fig 22a). At groups 0.8mg/kg to 1mg/kg, there is a turn around with the week 13 having the least increase in estrone level. The group performance profile reveal a dose dependent increase which is more pronounced at higher doses 1mg/kg, it was observed that the estrone serum concentration showed slight increase over time for group 0.05mg/kg to 0.5mg/kg, this was followed by an increase. Again from group 0.8mg/kg, there was decrease in estrone (see fig. 22b). Estrone levels were higher in week 13 for groups 0.05mg/kg to 0.7mg/kg, but the reverse was the case in groups 0.8mg/kg to 1mg/kg.

In all instances, a dose dependent increase was observed for group 0.8mg/kg, 0.9mg/kg and 1mg/kg. The group that received 1mg BPA per kg body weight showed the highest level of estrone, these were followed by 0.9mg/kg. These groups recorded highest value at week 1 and gradually decrease over time; the concentrations of estrone for these groups were lowest at week 13 (fig 22a). Groups 0.6mg/kg and 0.7mg/kg were significantly higher than those of control and week 0, at $p \leq 0.05$. These group recorded the lowest value at week 1, and showed steady increase over time with their highest value obtained at week 13. Between weeks 3 to 13, a significant dose dependent increase was observed for groups 0.05mg/kg, 0.1mg/kg and 0.2mg/kg at $p \leq 0.05$; which was consistent over time. Groups 0.05mg/kg to 0.7mg/kg recorded their highest value at week 13 and lowest value for estrone at week 1. While group 0.8mg/kg to 1mg/kg recorded their highest value at week 1 and lowest value at week 13 (fig 22a).

The highest serum concentration of estrone was recorded in week 13 for groups 0.05mg/kg to 0.7mg/kg; groups 0.8mg/kg to 1mg/kg recorded low estrone levels at week 13 and high estrone levels at week 1. In fig 22b, a time dependent effect was observed, as the duration of exposure to graded doses of BPA continued, the serum concentration of estrone continued to increase, up-to group 0.8mg/kg to 1mg/kg where the reverse effect was obtained with the estradiol level decreasing as the duration of the exposure to BPA extends. Between group 0.7mg/kg and 0.8mg/kg was the point of reversal of the trend (fig 22b).

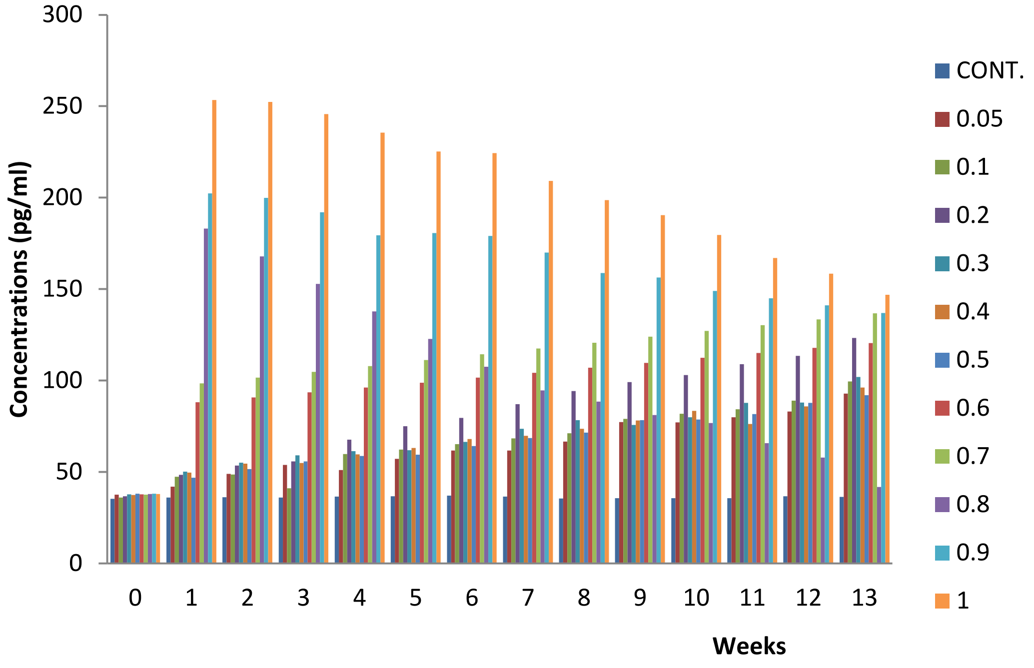

Fig. 22a; Bar chart of concentration against weeks (durations) for change in serum estrone level.

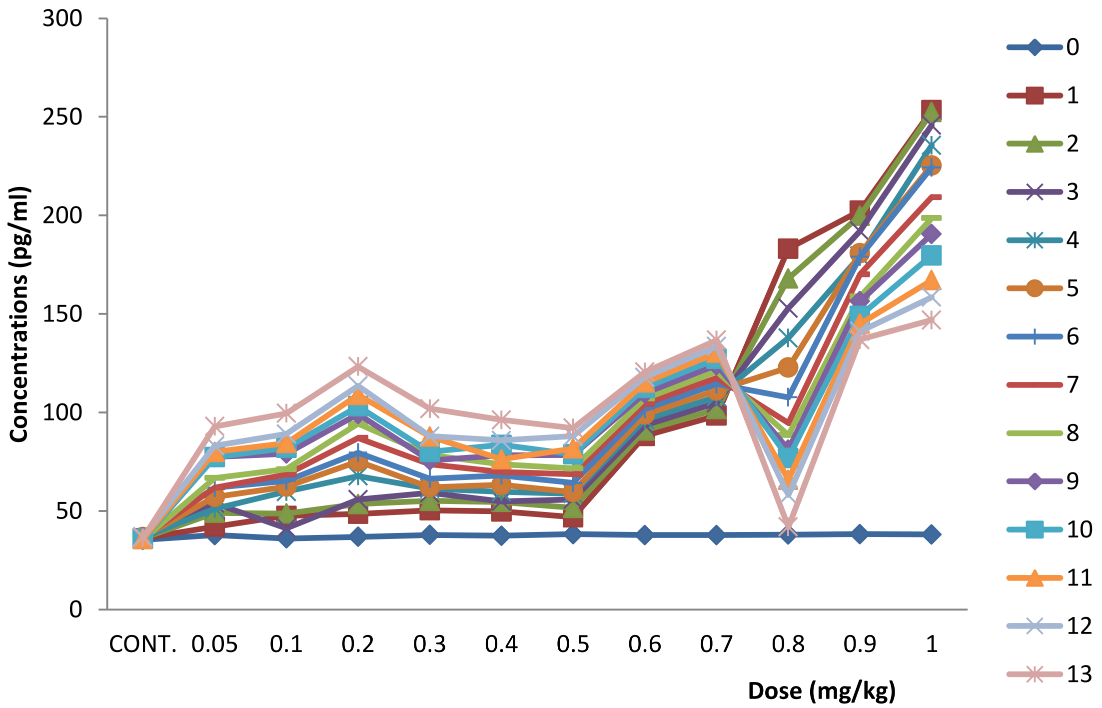

Fig. 22b; Graph of concentration against dose for change in serum estrone level.

c) EFFECT ON ESTRIOL

There is a significant decrease in serum estriol level when compared with the control at $p \leq 0.05$(see fig. 23a). As the estriol descreases from group 0.05mg/kg to 0.3mg/kg, at group 0.4mg/kg, there was a peak; group 0.8mg/kg showed the lowest level of estriol (fig.23b). As the estriol concentrations descreases from group 1(0.05mg/kg) to 4(0.3mg/kg), at group 5(0.4mg/kg), there was an increase (fig.23b); group 0.8mg/kg showed the lowest levels of estriol; the week effect observed was mild, as it appeared to be superimposed on each other (fig. 23b). The trend observed for estradiol and estrone levels were almost similar. But the trend observed for estriol was different, in that there was a drastic and significant ($p \leq 0.05$) decrease in the estriol level in all the treated groups. The BPA group 0.4mg/kg, gave an increase (peak) effect in the estriol which is still significantly lower than that of the control. There is an instant decrease in serum estriol level of all the treated rats within the first week(fig 23a). This decrese which was significant ($p \leq 0.05$) was sustained virtually consistently at the same level throughout the thirteen (13) weeks of the study (fig 23b). The decrease in estriol level appear to be dose dependent except at groups 0.4mg/kg (400µg/kg) and 0.7mg/kg (700µg/kg). The result suggests that the reduction in serum estriol level is influenced by dose of BPA administered but not by time.

In fig. 23a, it was observed that following the administration of BPA, a pattern of behaviour was established by the groups throughout the duration of the experiment. There was a dose dependent decrease in estriol levels from doses 0.05mg/kg to 0.3mg/kg, then an increase by 0.4mg/kg, which decrease at 0.5mg/kg to 0.6mg/kg, another increase was observed at group 0.7mg/kg, this was followed by a decrease at group 0.8mg/kg from which dose dependent increase in estriol through 0.9mg/kg to 1mg/kg (fig 23a). The test dose groups showed a time dependent effect which was mild. All the weeks peaks at test dose 0.4mg/kg group (fig. 23b).

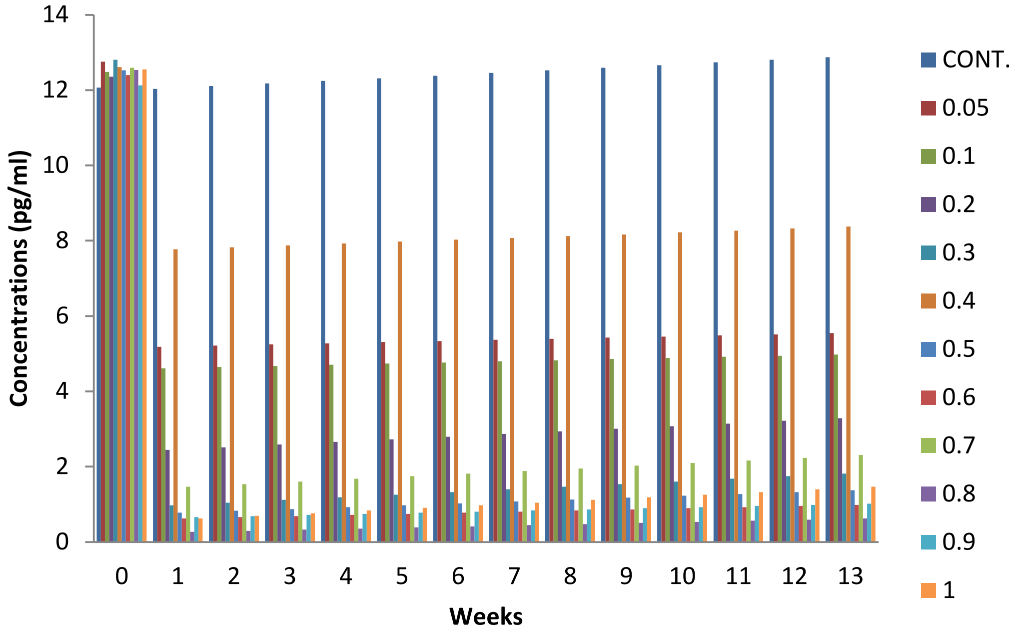

Fig. 23a; Bar chart of concentration against weeks (durations) for serum estriol concentration.

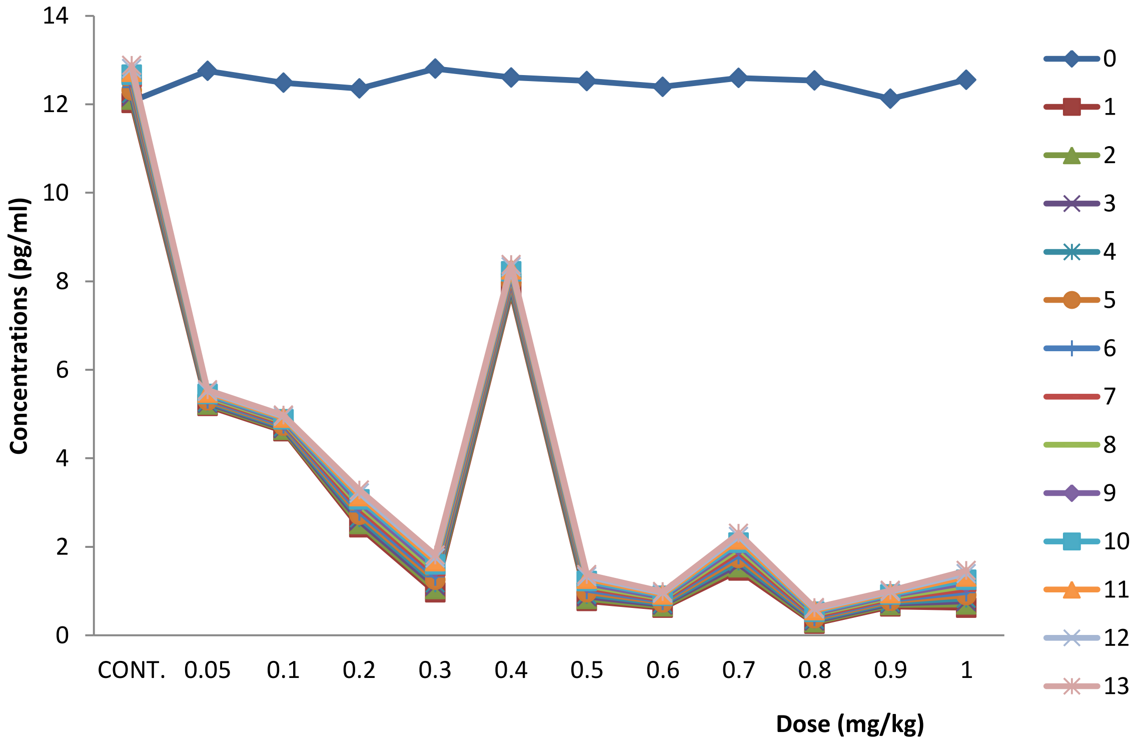

Fig. 23b; Graph of concentration against dose for serum estriol.

d) EFFECT ON PROLACTIN

There was a significant increase in the prolactin level when compared with the control at $p \leq 0.05$. Groups 0.2mg/kg, 0.4mg/kg to 0.6mg/kg and 0.8mg/kg to 1mg/kg showed consistent increase in prolactin level over time (fig 24a). Group 0.05mg/kg and 0.1mg/kg showed no statistical significant difference in the levels of prolactin weeks 7 to 9 period of exposure (fig.24b). Group 0.3mg/kg showed an initial increase at the on set of the experiment (week 1) and decreases through the weeks to a level of 0.06±0.008 at week 6 even below that of the control (fig.24a and 24b) then began to increase to its peak at 1mg/kg. An increase in prolactin was demostrated throughout the weeks of administration (fig 24a), while the change in prolactin level for groups 0.8mg/kg and 1mg/kg tends to be constant throughout the duration of the study, other groups showed manifestingly steady increase in the course of time with group 6(0.5mg/kg) revealing the best time dependent incremental profile (see fig.24b). Prolactin increases induced by group 1 and 2 are not statistically significant at weeks 7, 8 and 9.

From fig.24a, it was observed that after the administration of BPA at weeks 1 and 2, the effect shown by all the dose group followed the same pattern. There was an increase from 0.05mg/kg to 0.1mg/kg; a decrease at 0.2mg/kg group, an increase at 0.3mg/kg, a decrease at 0.4mg/kg, an increase at 0.5mg/kg, another decrease at 0.6mg/kg, an increase through 0.7mg/kg to 0.8mg/kg, another decrease at 0.9mg/kg and finally an increase at 1mg/kg. At week 3, it was observed that there was an increase at 0.05mg/kg to 0.1mg/kg, that decrease at 0.2mg/kg, then increases through 0.3mg/kg to 0.5mg/kg, and decrease through 0.6mg/kg to 0.7mg/kg, increase at 0.8mg/kg, decreased at 0.9mg/kg and increase to peak at 1mg/kg (fig.24a). At week 4, an increase was observed at 0.05mg/kg to 0.1mg/kg, that decreas at 0.3mg/kg, increase through 0.4mg/kg to 0.5mg/kg, another drop through 0.6mg/kg to 0.7mg/kg, that increase at 0.8mg/kg, drop again at 0.9mg/kg and finally peaks at 1mg/kg (fig. 24a).

Weeks 5 to 8, showed high prolactin level at 0.05mg/kg dosage that decrease at 0.1mg/kg and spiked high at 0.2mg/kg. This was followed by a decrease at 0.3mg/kg with its lowest value at week 6 (also see fig. 24b); then a spike increase of prolactin level was observed at 0.4mg/kg through to 0.5mg/kg, which decrease through 0.6mg/kg to 0.7mg/kg and rise again at 0.8mg/kg, dropped at 0.9mg/kg and increase at 1mg/kg (fig 24a).

Weeks 9 to 13, showed a dose dependent increase in serum prolactin concentration after the administration of BPA from 0.05mg/kg to 0.2mg/kg group that decreased at 0.3mg/kg and increase through 0.4mg/kg to 0.5mg/kg; it then decreased again through 0.6mg/kg to 0.7mg/kg and another dose dependent increase through 0.8mg/kg to 1mg/kg (fig. 24a)

The doses 0.8mg/kg and 1mg/kg of BPA showed no time dependent effect (fig. 24b). The group that received 0.5mg/kg body weight of BPA, showed a clear and pronounced time dependent effect (fig. 24b). Other dose groups showed time effect that is not time dependent; week 1 exhibited the maximum effect for the dose group 0.05mg/kg and 0.1mg/kg while week 6 showed the least effect for dose group 0.3mg/kg and 0.7mg/kg (fig.24b).

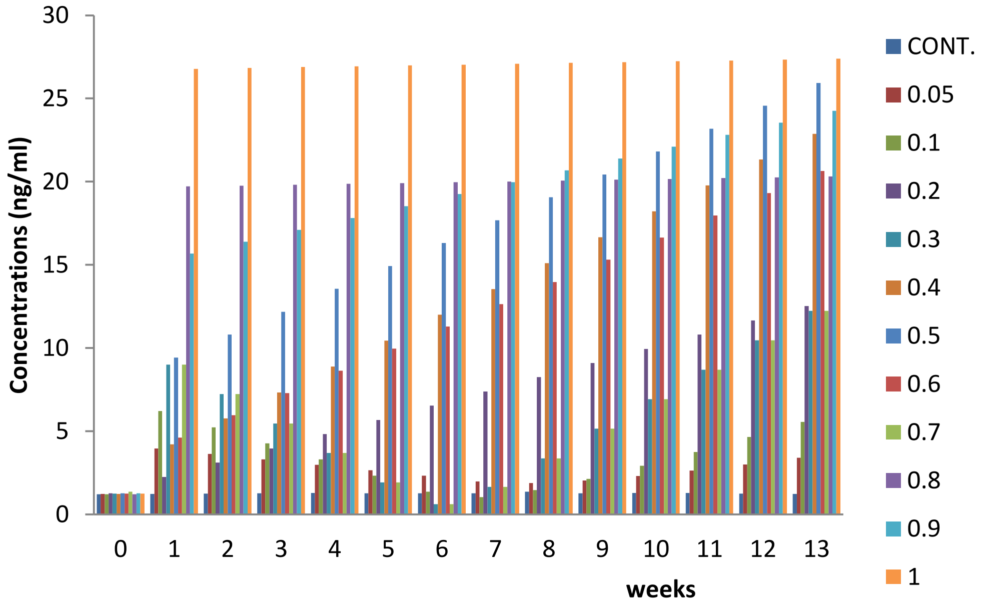

Fig. 24a; Bar chart of concentration against weeks (durations) for serum prolactin concentration.

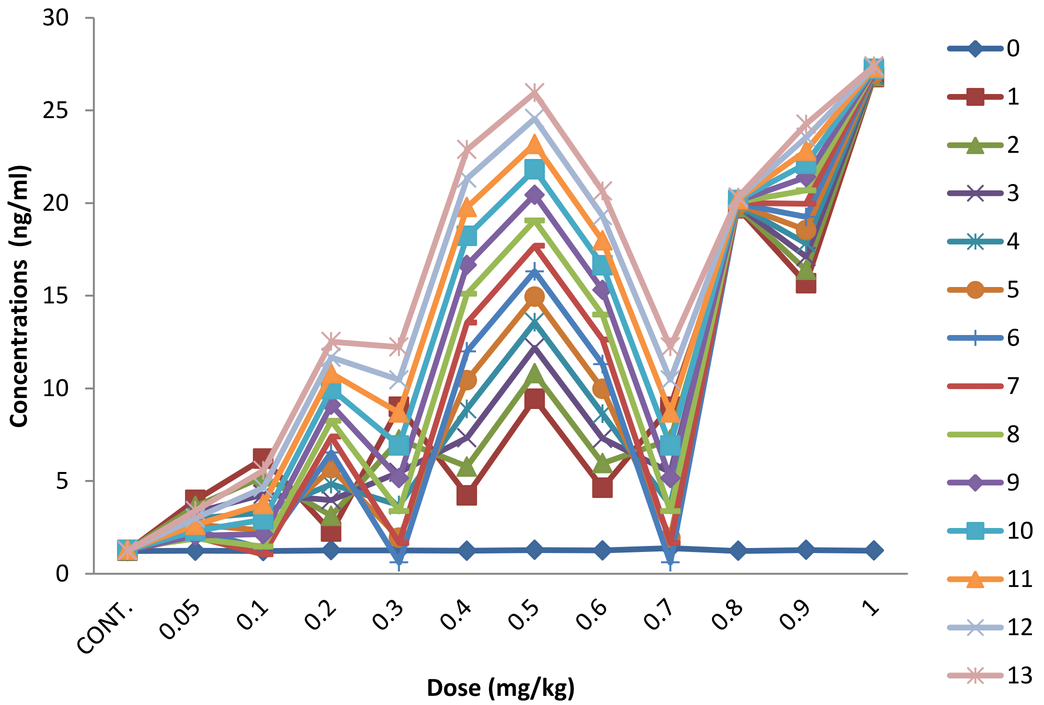

Fig. 24b; Graph of concentration against dose for serum prolactin.

e) EFFECT ON PROGESTERONE

There was significant dose dependent increase in the progesterone level when compared with the control at $p \leq 0.05$. Only animals administered 1mg/kg (1000µg/kg) showed consistent increase in progesterone levels (fig 25a). At doses 0.4mg/kg, 0.5mg/kg, 0.8mg/kg and 0.9mg/kg, it was observed that there was fluntuation during the various weeks of administration (fig 25b). At other dose range there were marginal increases in progesterone levels which show some dose dependent relationship but bear no sensitivity to time (see fig 25b). Group 0.5mg/kg, 0.8mg/kg and 0.9mg/kg shows no significant decreases at weeks 7 and 8, weeks 1 and 2, and weeks 5, 6 and 7 respectively (fig 25b).

It was observed that from weeks 1 to 13, there were dose dependent increases in progesterone concentrations from 0.05mg/kg to 0.3mg/kg dose (fig.25a). After which there was a decrease in the progesterone levels by 0.4mg/kg dose except at weeks 8 and 10, where its level (0.4mg/kg) was equal and higher than that of 0.3mg/kg group. It was observed for weeks 1 to 3, that there was an increase at 0.5mg/kg, a decrease at 0.6mg/kg, an increase at 0.7mg/kg, decrease at 0.8mg/kg and finally an increase through 0.9mg/kg to 1mg/kg (fig.25a). At weeks 4 to 10, there was a decrease through 0.4mg/kg to 0.5mg/kg, an increase through 0.6mg/kg to 0.7mg/kg, and decrease again through 0.8mg/kg to 0.9mg/kg, finally peaks at 1mg/kg, with group 0.9mg/kg showing it least effect (0.06 ± 0.008) at week 6 (also see fig.25b). At weeks 11 to 13, another dose dependent increase from 0.5mg/kg through to 1mg/kg was observed. Throughout the experiment the group that received 1mg/kg body weight of BPA had the maximum effect effect on progesterone, and the effect was time dependent (fig.25b). The time of exposure does not have effect on the exposure to BPA for the dose group 0.05mg/kg to 0.3mg/kg, 0.6mg/kg and 0.7mg/kg (fig.25b).

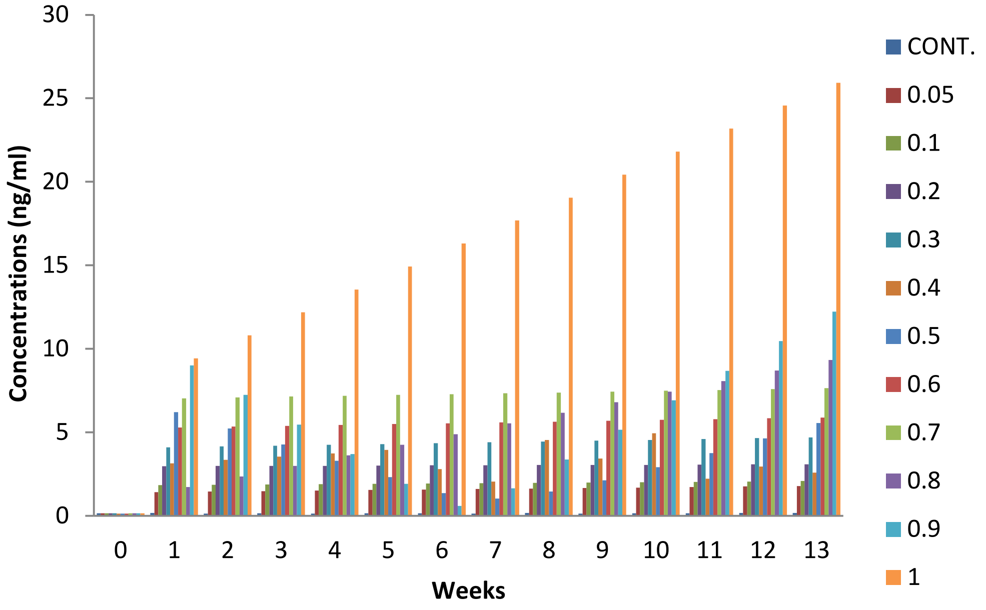

Fig. 25a; Bar chart of concentration against weeks (durations) for serum progesterone concentration.

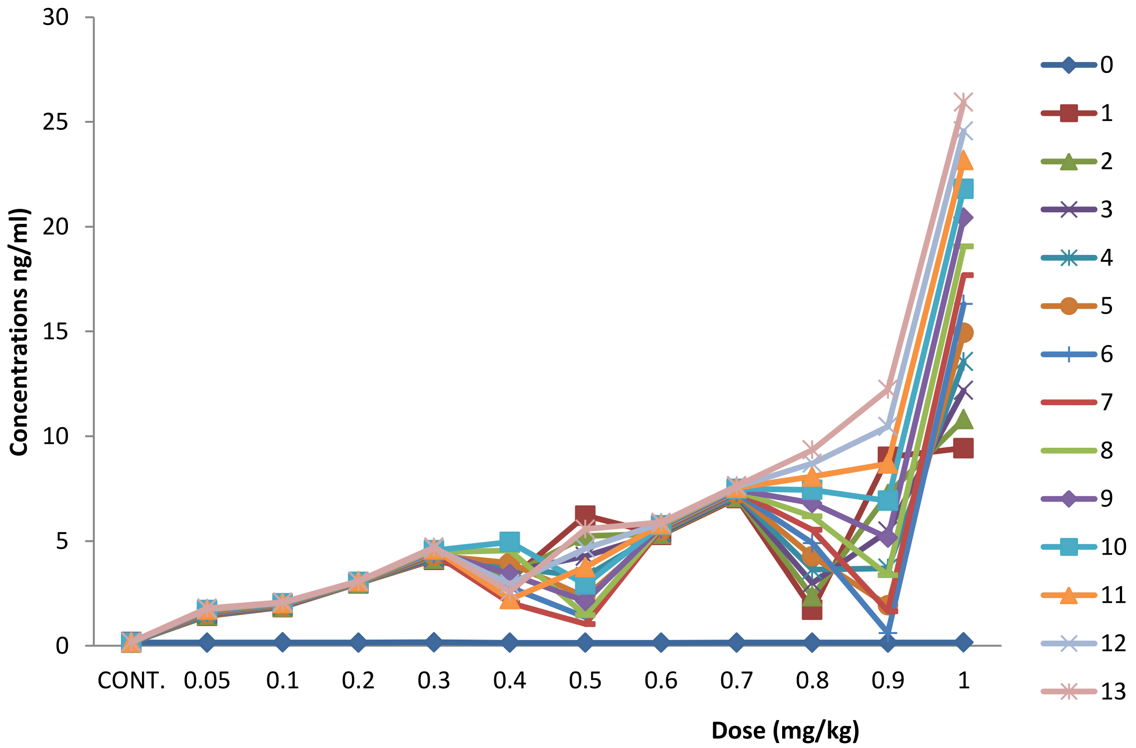

Fig. 25b; Graph of concentration against dose for serum progesterone.

f) EFFECT ON TESTOSTERONE

There were significant increases in the testosterone level of treated rats when compared with the control at $p \leq 0.05$. This increase which was across board, was most pronounced in groups 0.4mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/kg and 1mg/kg (fig.26a). Group 0.05mg/kg showed no statistical difference when compared with the control (also see fig.26b). Testosterone level in the BPA treated groups were observed to show a slight and steady increase with time. A characteristic increase that was time dependent was observed at dose 0.8mg/kg. Except for groups of animal administered 0.8mg/kg (800µg/kg.bw), 0.2mg/kg and 1mg/kg. Increased effect of BPA on testosterone does not show sensitivity to duration of dministration (see fig 26b).

Fig.26a showed a dose dependent effect which initially peaks at dose 0.4mg/kg, then decrease in 0.5mg/kg, again increase to peak at dose 0.7mg/kg, dropped at dose 0.8mg/kg and finally increase through 0.9mg/kg to 1mg/kg (fig.26a). Over time the value obtained for each dose remained within the same range, except for dose 0.2mg/kg and 0.8mg/kg that showed weekly effect, while 0.2mg/kg weekly effect was mild, 0.8mg/kg weekly effect was very pronounced, with week 13 showing the maximum effect (fig.26b).

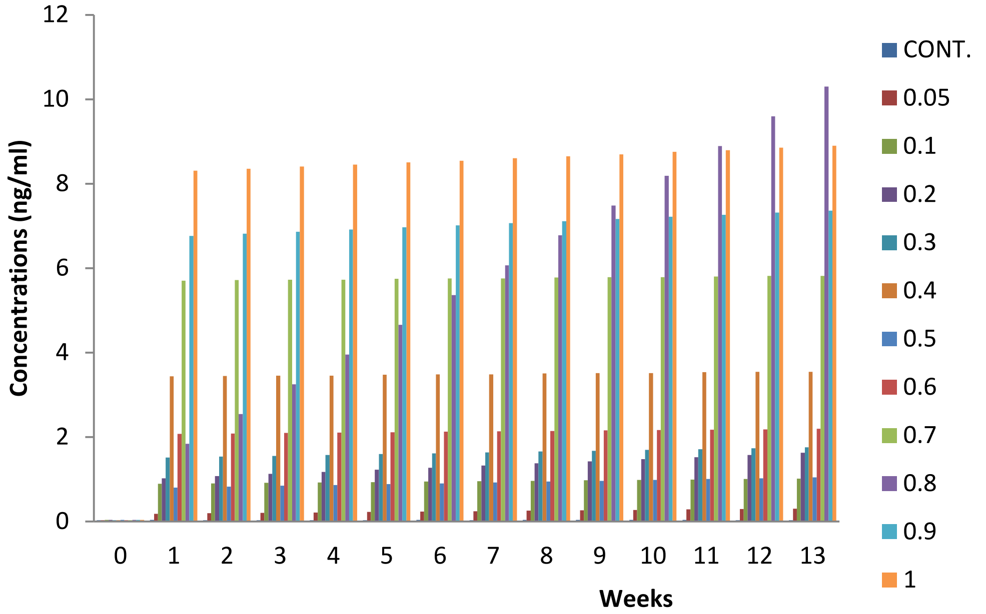

Fig. 26a; Bar chart of concentration against weeks (durations) for serum testosterone concentration.

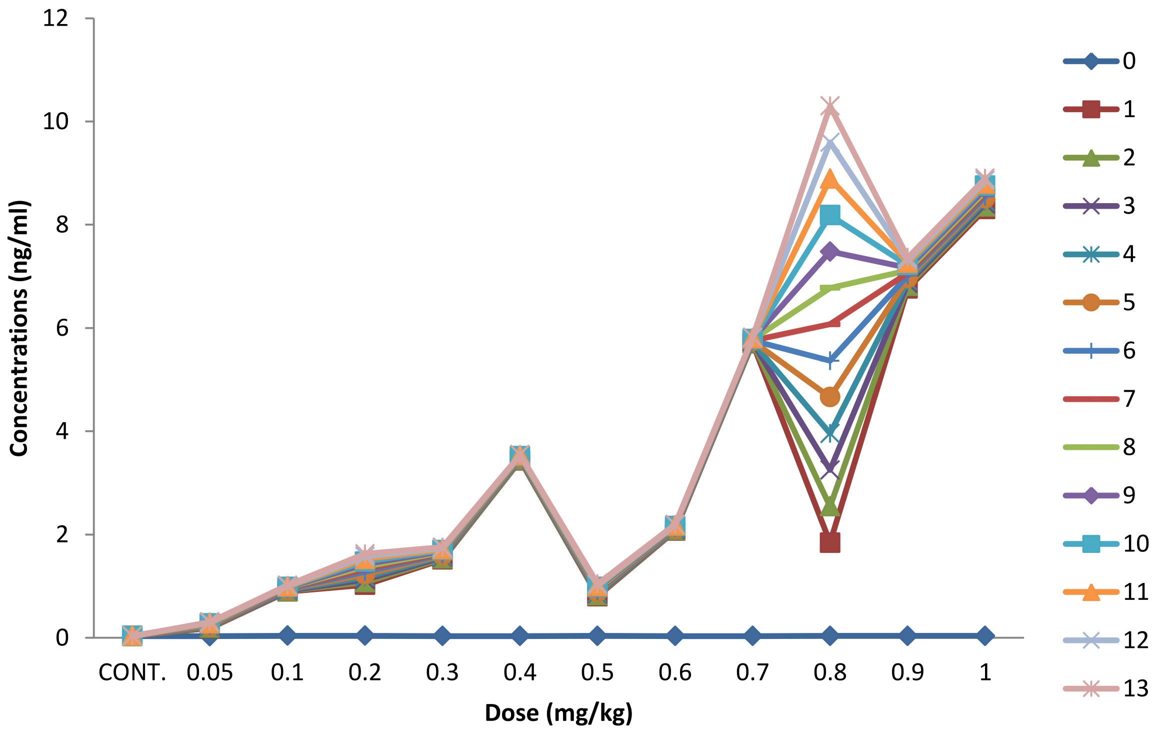

Fig. 26b; Graph of concentration against dose for serum testosterone.

g) EFFECT ON LEUITEINIZING HORMONE

There was significant dose dependent increase in the Luteinizing Hormone level when compared with the control at $p \leq 0.05$. Dose 0.1mg/kg at the first 4 weeks of the experiment showed decreased LH concentration, below the control level. This was followed by a steady increase with time (see fig 27a). The peak effect was observed at dose of 0.6mg/kg, and then a slight decrease which is sharp at group 0.9mg/kg, the maximum effect was observed at group 1mg/kg (see fig 27b). Again, increase at lower doses does not appear to be dose dependent. Doses above 0.05mg/kg showed dose dependent increases in LH, upto 0.6mg/kg, after which there was a decrease up to 0.9mg/kg and then a spiked at dose of 1mg/kg. In all instances, the LH level increased in the course of time, week 13 showed the maximum effect except at dose 0.3mg/kg where is a time dependent decrease from weeks 9 to 13 (see fig. 27b).The BPA groups with high level of LH are group 0.6mg/kg and 1mg/kg. There is a steady increase up to 0.6mg/kg group, then the LH levels began to decrease at group 0.7mg/kg through to group 0.9mg/kg, and then it starts to increase again. Although none of these decreases fall below the control level except for group 0.1mg/kg at week 1 through to week 3. Dose 0.1mg/kg slightly decreased below the control at weeks 1 to 2, while at weeks 3 and 4 it showed no significant in the increase at $p \leq 0.05$ when compared with the control. At week 5, it significantly increased, along side with other dose group at $p \leq 0.05$ when compared with the control.

All the groups showed steady and gradual increase over time (fig.27a). At all point in time, dose 0.05mg/kg increase, then dropped at 0.1 and then there was dose dependent increase which peaks at 0.7mg/kg for weeks 1 and 2, for weeks 3 to 13, the peak was observed at 0.6mg/kg, then from the various peaks established there was dose dependent decrease up to 0.9mg/kg and finally a spike in the concentration of LH for the dose 1mg/kg, with highest concentration of LH been observed at week 13 (373.43±0.005) and the lowest at week 1 for 1mg/kg exposure group (194.93±0.005) in fig.27a.

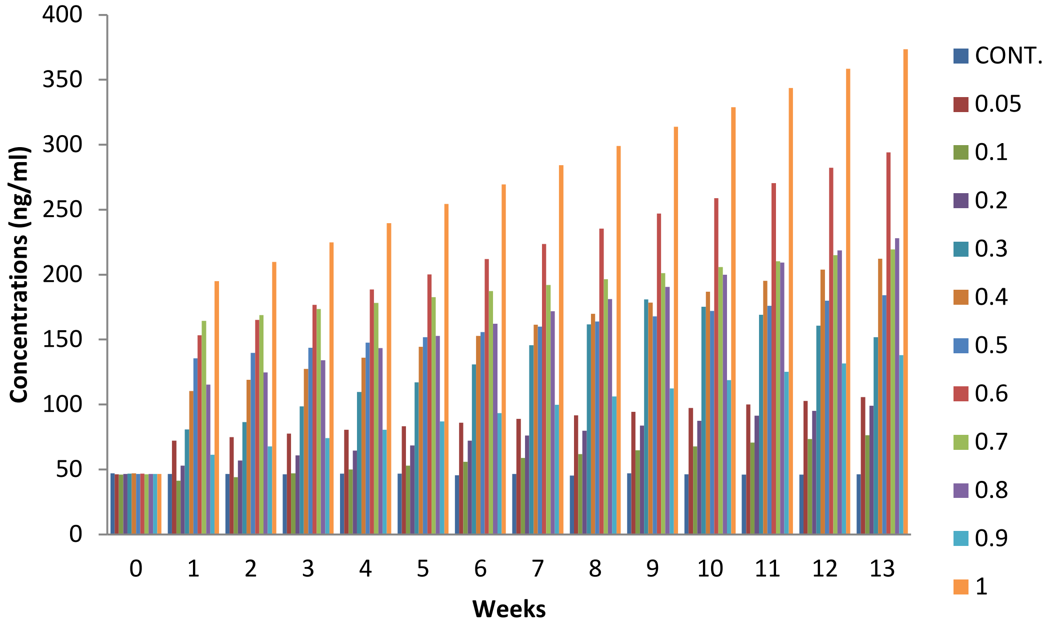

Fig. 27a; Bar chart of concentration against weeks (durations) for serum Luteinizing Hormone.

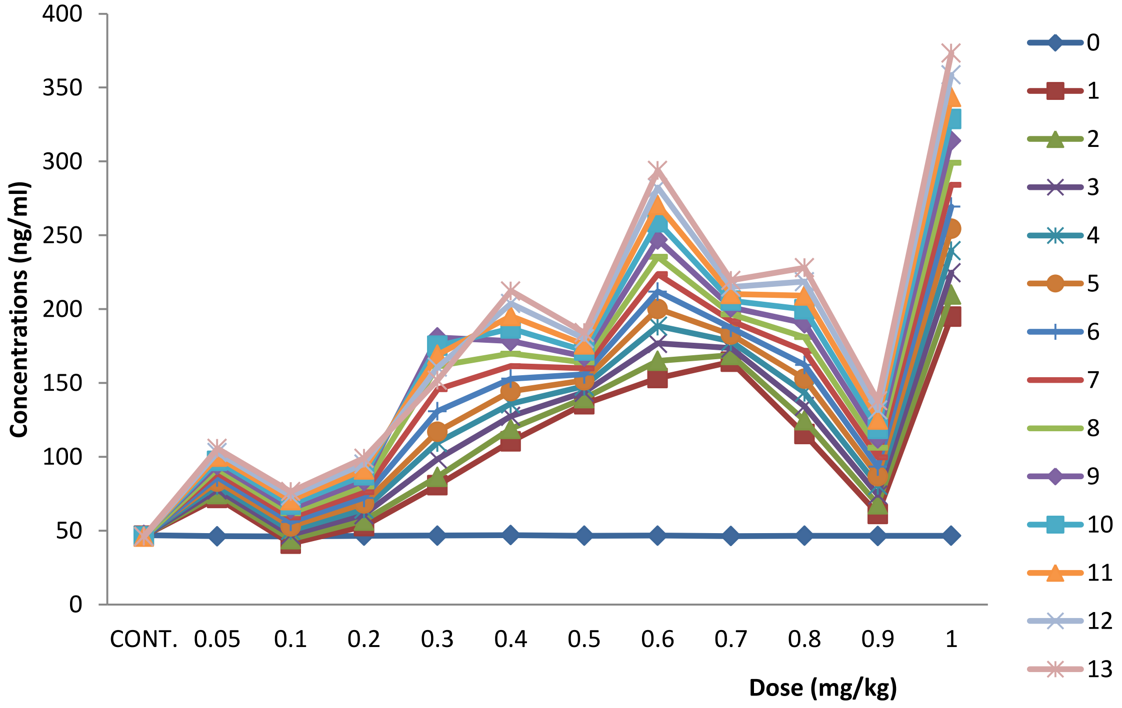

Fig. 27b; Graph of concentration against dose for serum Luteinizing Hormone.

h) EFFECT ON FOLLICLE STIMULATING HORMONE

There was significant decrease in the follicle stimulating hormone level of BPA treated rats, when compared with the control at $p \leq 0.05$ (see fig 28a). Although, the decrease was steady in course of time, no particular pattern was established except at group 7(0.6mg/kg) (see fig 28b). There was a characteristic non-steady and non- dose dependent weekly effect.

At doses of 0.1mg/kg, 0.4mg/kg and 0.6mg/kg there were consistently high concentration of follicle stimulating hormone relative to other dose groups at different times of the experiment. The group exposed to 0.1mg/kg showed high FSH level at weeks 1, 2, 3, 4, 6, 8 and 11; the group exposed to 0.4mg/kg showed high FSH level at weeks 1 and 2, while the 0.6mg/kg exposure group showed high FSH level at weeks 5, 7, 9, 10, 12 and 13. (fig 28a).

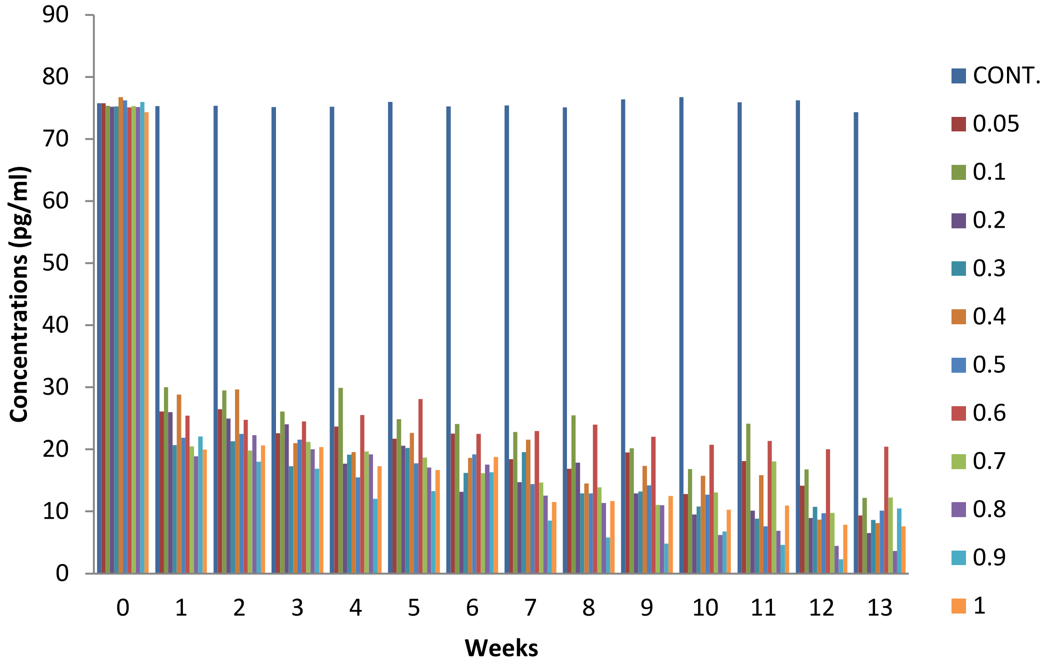

Fig. 28a; Bar chart of concentration against weeks (durations) for follicle stimulating hormone concentration.

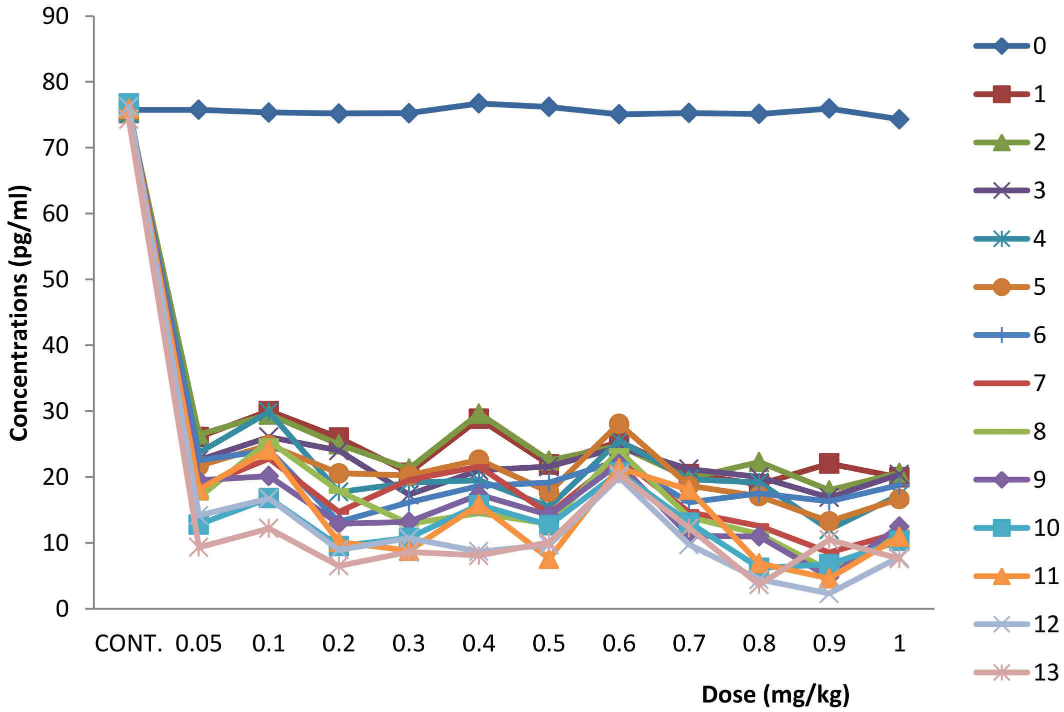

Fig. 28b; Graph of concentration against dose for follicle stimulating hormone concentration.

i) EFFECT ON ANDROSTENEDIONE

There was significant time- dependent increase in the androstenedine level when compared with the control at p≤0.05. The dose concentrations that induce significant increases in androstenedione level were 50 µg/kg(gp1), 100 µg/kg(gp2), 600 µg/kg(gp7), 700 µg/kg(gp8), 800 µg/kg(gp9), 900 µg/kg(gp10) and 1000 µg/kg(gp11). Other doses (200 µg/kg to 500 µg/kg (gp 3-6) triggered a non significant decrease in androstenedione concentrations at weeks 1 to 5, and a non significant increase at weeks 6 to 8. There is no consistency in the effect of the graded doses of BPA on the behaviour of androstenedione (fig 29b). Groups 0.2mg/kg – 0.5mg/kg showed a relatively constant level of adrostenedione. It was observed that, they were not only constant but also decreased below the control for the first 5 weeks of exposure (fig.29b), after which the weeks concentration of androstenedione were above the control level, although, the other weeks that showed higher values were not significant when compared with the control at p≤0.05 except weeks 9 to 13.

The established pattern of response was observed in weeks 1 to 7 such that groups 0.05mg/kg and 0.1mg/kg showed increase in serum androstenedione level which were steady throughout the experiment with concentration of adrostenedione exhibited by group 0.05mg/kg aways higher than those of 0.1mg/kg. Groups 0.2mg/kg to 0.5mg/kg showed dose dependent increases, which were lower than those of the control at weeks 1 to 4, but gradually increases above the control in weeks 5 to 13 (fig.29a). The groups 0.2mg/kg to 0.7mg/kg showed dose dependent increases that peaked at dose 0.7mg/kg for weeks 1 to 7, the observed increases were consistent and steady throughtout the experiment, but at weeks 8 to 13 the concentration of androstenedione dropped below its 0.6mg/kg counterpart, making dose 0.6mg/kg the peak at these weeks (fig.29a). Again, it was observed that the group 0.8mg/kg showed a decrease relative to 0.6mg/kg and 0.7mg/kg groups, there was an increase in the concentration by 0.9mg/kg dose groups, with 1mg/kg dose group showing a decrease in serum level of androstenedione relative to 0.9mg/kg dose groups, although the decrease was not below those of the control (fig.29a). Throughout the study, the entire dosage showed consistent increase in androstenedione over time, the dose group 0.9mg/kg showed the highest level of androstenedione throughout the study (fig.29a). The peak was observed at week 13(318.90±0.003) and (320.67±0.006) for 0.6mg/kg and 0.9mg/kg respectively.

Fig.29b showed a clear time dependent effect with week 1 showing the lowest level for the entire dose groups, as the duration of the study increased, the level of serum androstenedione increased with week 13 showing the maximum effect for all the dose groups.

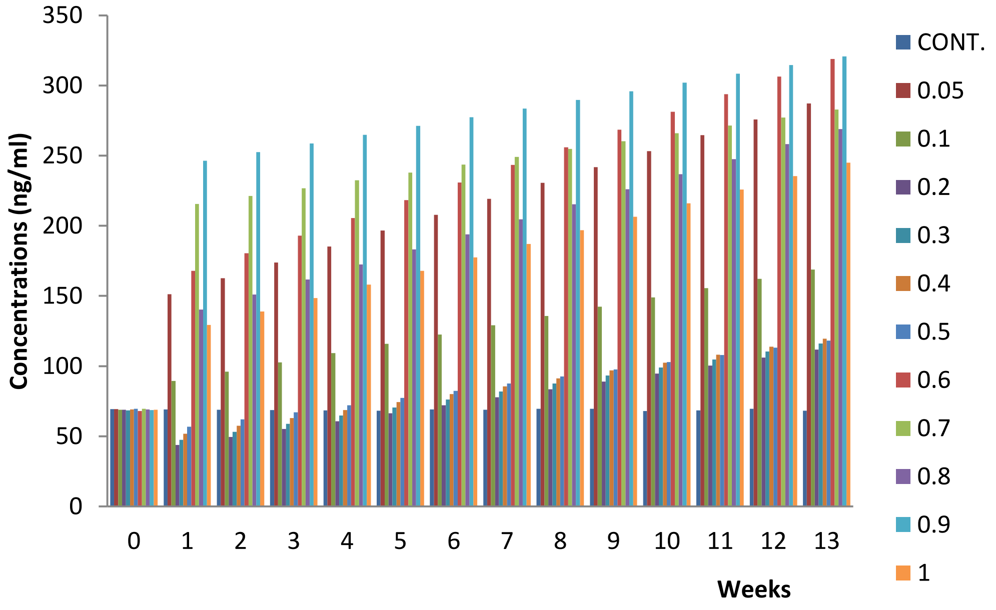

Fig. 29a; Bar chart of concentration against weeks (durations) for androstenedione concentration

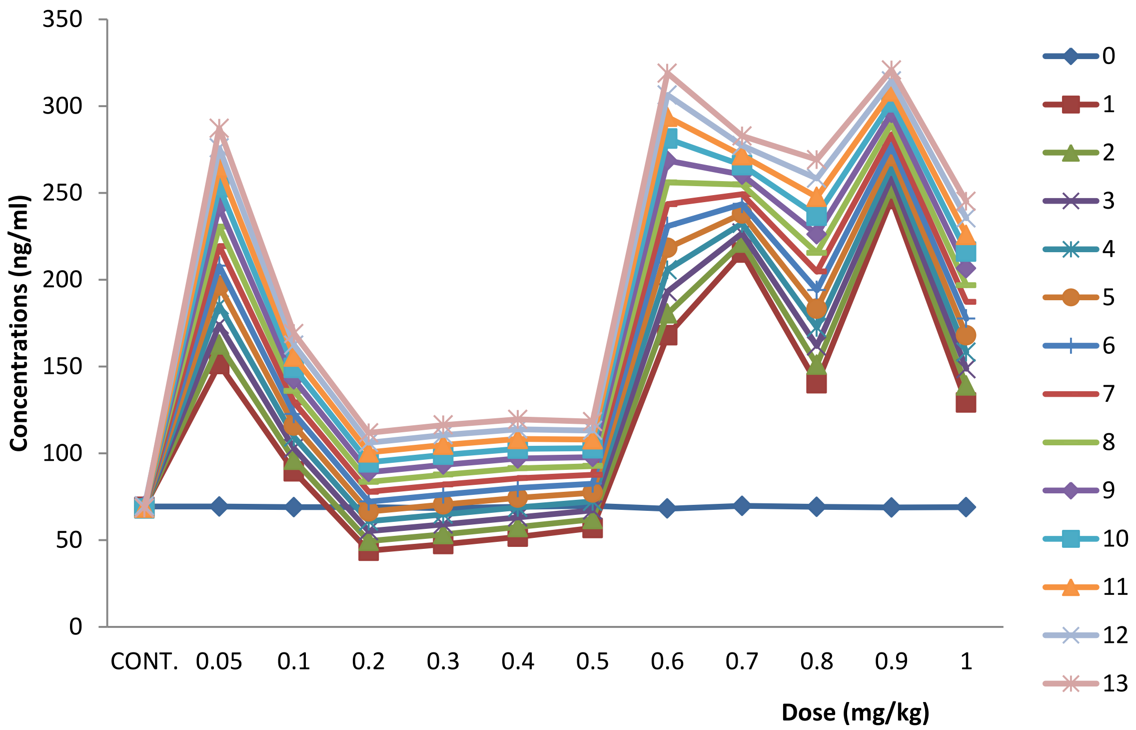

Fig. 29b; Graph of concentration against dose for androstenedione concentration.

1.4 EFFECT OF BISPHENOL A ON THYROID HORMONE

a) EFFECT ON THYROID STIMULATING HORMONE

There was an instant dose dependent increase in the thyroid stimulating hormone level of BPA treated rats when compared with the control at $p \leq 0.05$ except for group 0.05mg/kg that showed a non significant effect throughout the duration of the study. The test group that received 0.1mg/kg BPA showed significant ($p \leq 0.05$) only at weeks 7 to 13. The dose dependent effect was maintained in the course of the study. Only groups 0.1mg/kg and 0.6mg/kg showed sensitivity to the time of study (see fig.30b). A very slight but constant effect was observed for TSH at dose group 0.05mg/kg.

The serum concentration of thyroid stimulating hormone showed a dose dependent increase for the first four (4) weeks of exposure that is weeks 1 to 4 (fig.30a), at weeks 2 to 4 the levels of TSH exhibited by the group that received 0.1mg/kg BPA decreased showing its lowest effect (0.25±0.00) at week 3 (fig.30a). Weeks 5 to 10 showed a characteristic pattern of dose dependent increases that peaked at 0.6mg/kg; and another dose dependent effect from dose group 0.7mg/kg to 1mg/kg as its peak. Weeks 11 to 13 exhibited a different response behaviour in which there was dose dependence from dose group 0.05mg/kg to 0.1mg/kg, 0.2mg/kg to 0.6mg/kg and 0.7mg/kg to 1mg/kg BPA (fig.30a). The group 1mg/kg had the highest effect on serum levels of TSH throughout the study with its maximum effect (51.65±0.006) at week 13 (fig.30a).

The change in the serum concentration of thyroid stimulating hormone in fig.30b showed that at dose 0.05mg/kg, 0.2mg/kg to 0.5mg/kg and 0.7mg/kg to 1mg/kg had no effect on the time of exposure. The effect after the 1mg/kg exposure to BPA was only different at week 13 (fig.30b).

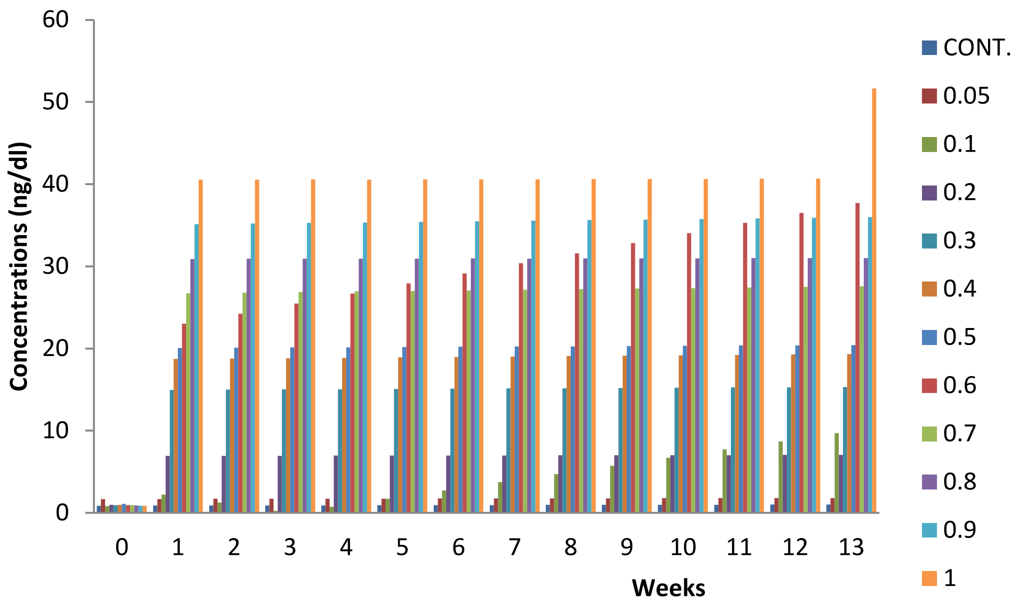

Fig. 30a; Bar chart of concentration against weeks (durations) for thyroid stimulating hormone level.

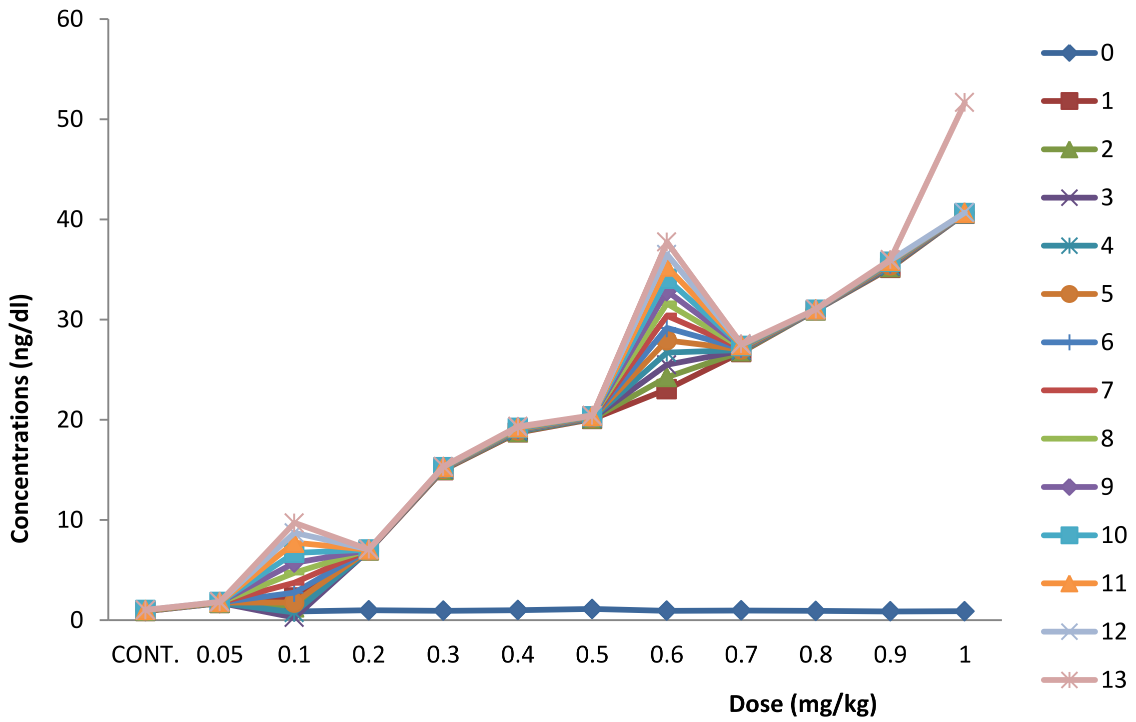

Fig. 30b; Graph of concentration against dose for thyroid stimulating hormone level.

b) EFFECT ON TOTAL THYROXINE (TT4)

There were significant increases in the Total Thyroxine (TT4) level at all groups when compared with the control at $p \leq 0.05$. There seems to be no much difference in the levels of thyroxine at the different point of measurement (see fig. 31a).

Generally, there is a significant increase in total thyroxine levels at $p \leq 0.05$ when compared with the control. Fig 31a showed a consistent pattern of response throughout the duration of the experiment; the test group that received 0.05mg/kg BPA showed high levels of total thyroxine, there is a dose dependent increase from the group 0.1mg/kg to 0.4mg/kg as the peak, the groups that received 0.5mg/kg showed a characteristic decrease relative to those of dose group 0.4mg/kg (fig31a), another dose dependent increase in total thyroxine was exhibited by the treatment groups that received 0.6mg/kg to 1mg/kg BPA except for 0.9mg/kg treatment group that showed a deline relative to those of 0.8mg/kg (fig 31a). In all instances, the highest total thyroxine levels were revealed by the group that were administered 1mg/kg BPA, followed by 0.05mg/kg BPA group. Over the time, the concentration of total thyroxine appeared to be relatively constant (fig.31a, also fig.31b), revealing that response to BPA exposure was not time dependent (fig.31b). The test group that received 0.9mg/kg BPA showed the lowest level of total thyroxine.

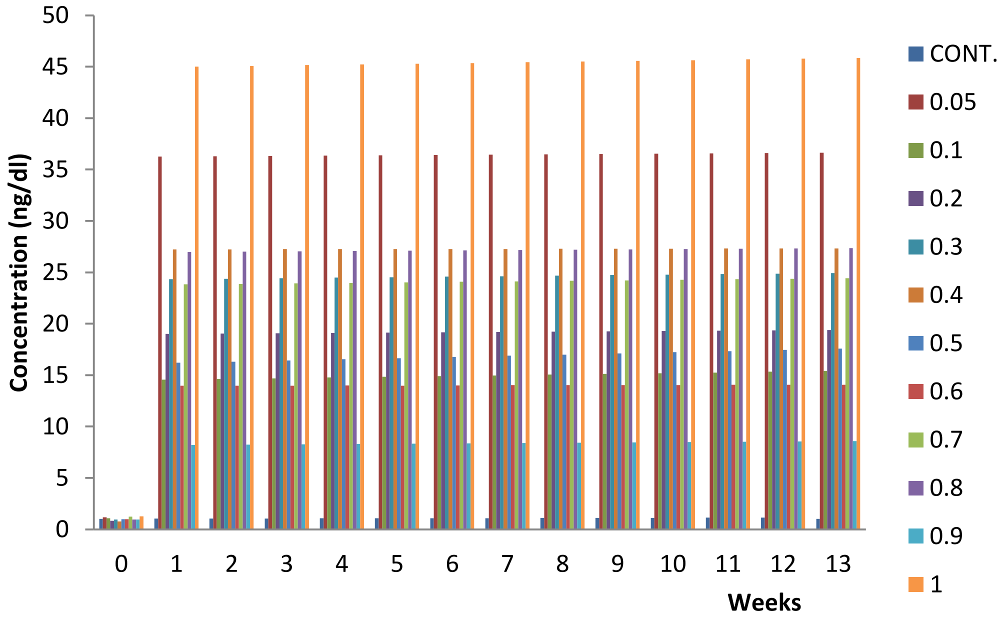

Fig.31a; Bar chart of concentration against weeks (durations) for total Thyroxine (TT4) level.

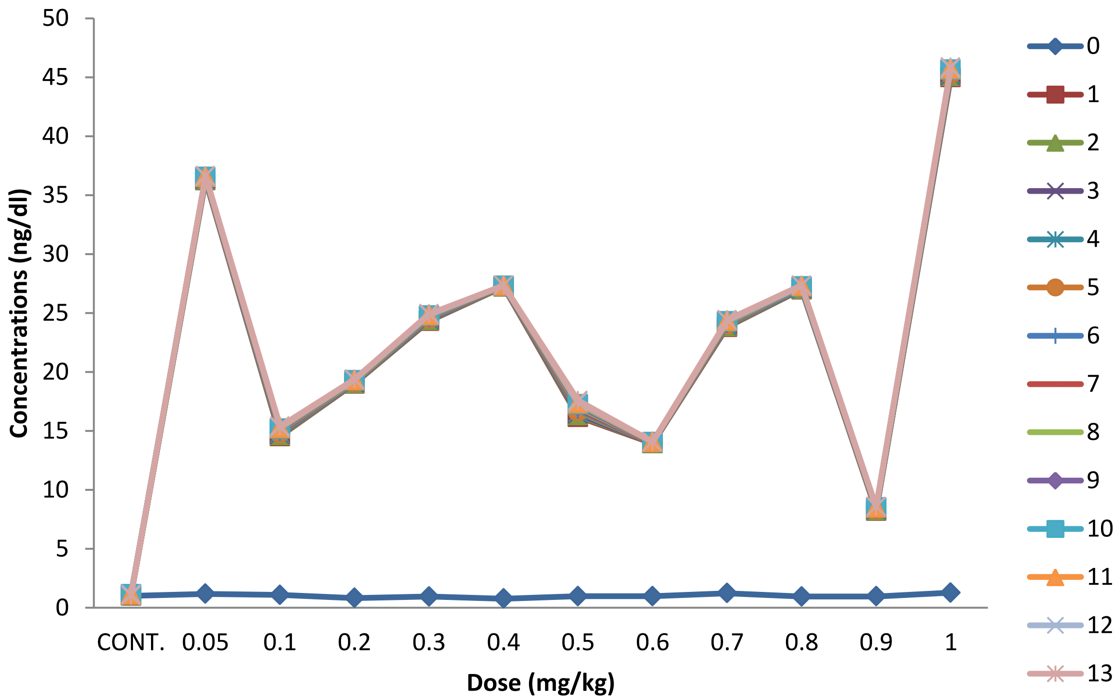

Fig. 31b; Graph of concentration against dose for total Thyroxine (TT4) level.

c) EFFECT ON FREE THYROXINE (FT4)

There were significant increases in the Free Thyroxine (FT4) at all levels of exposure, when compared with the control at $p \leq 0.05$. The concentration of FT4 measured were significant throughout the weeks of exposure. There was a decrease in the serum concentrations of free thyroxine by the test group that received 0.6mg/kg BPA (fig 32b).

Generally, there was significant increase in free thyroxine levels at $p \leq 0.05$ when compared with the control. Fig 32a showed a consistent pattern of response throughout the duration of the experiment; there was dose dependent increase from the dose group that received 0.05mg/kg to 0.4mg/kg as the peak, the groups that received 0.5mg/kg showed a characteristic decrease relative to those of dose group 0.4mg/kg (fig32a), another dose dependent increase in free thyroxine was exhibited by the treatment groups that received 0.6mg/kg to 1mg/kg BPA (fig 32a). In all instances, the highest total thyroxine levels were found in the group that were administered 1mg/kg BPA, followed by 0.9mg/kg BPA group. Over the time, the concentration of total thyroxine appeared to be relatively constant (fig.32a, also fig.32b), and a mild weekly response was exhibited by the only the test group that received 1mg/kg BPA (fig.32b)

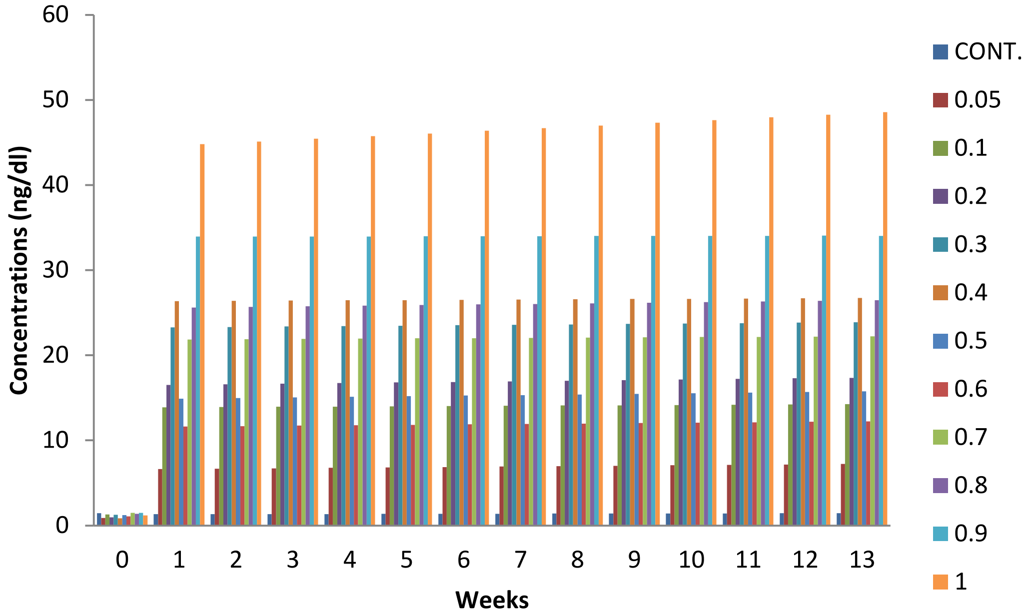

Fig. 32a; Bar chart of concentration against weeks (durations) for Free Thyroxine Ft4 level.

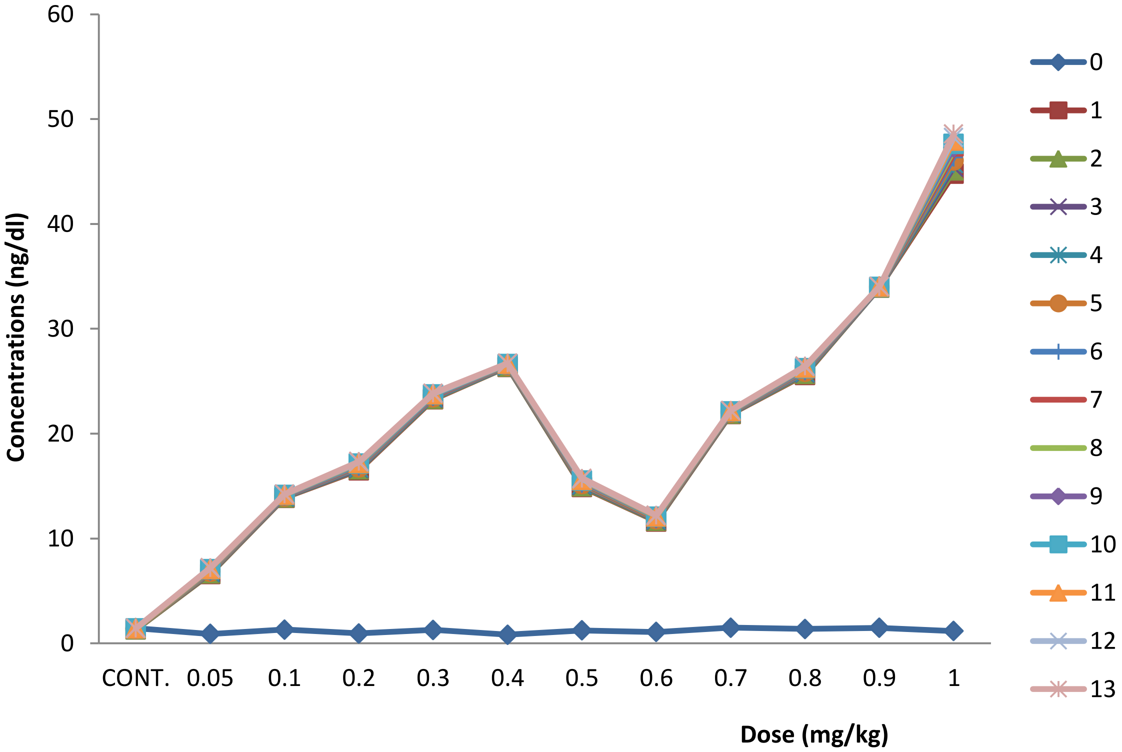

Fig. 32b; Graph of concentration against dose for Free Thyroxine (FT4) level.

d) EFFECT ON CONJUGATED THYROXINE (BT4)

The serum conjugated thyroxine concentration significantly decreased ($p \leq 0.05$) when compared with the control group and week 0. The treatment group that received 1mg/kg BPA showed a steady increase over time with the lowest concentration (0.03±0.004) at week 2 and highest concentration at week 13 (fig.33a). The test groups that were administered 0.05mg/kg, 0.3mg/kg, 0.7mg/kg and 0.9mg/kg showed a relative constant effect over the duration of the study (fig.33a). The control and week 0 values showed that, there was no consistency in the levels of conjugated thyroxine(fig.33a; and fig.33b). Mild weekly responses were observed at test dose 0.1mg/kg, 0.2mg/kg, 0.4mg/kg, 0.5mg/kg, 0.6mg/kg and 0.8mg/kg. Test group 1mg/kg BPA showed a time dependent decrease (33b) with its maximum effect (2.72±0.00) at week 13. The concentration of BT4 measured were significant throughout the weeks of exposure.

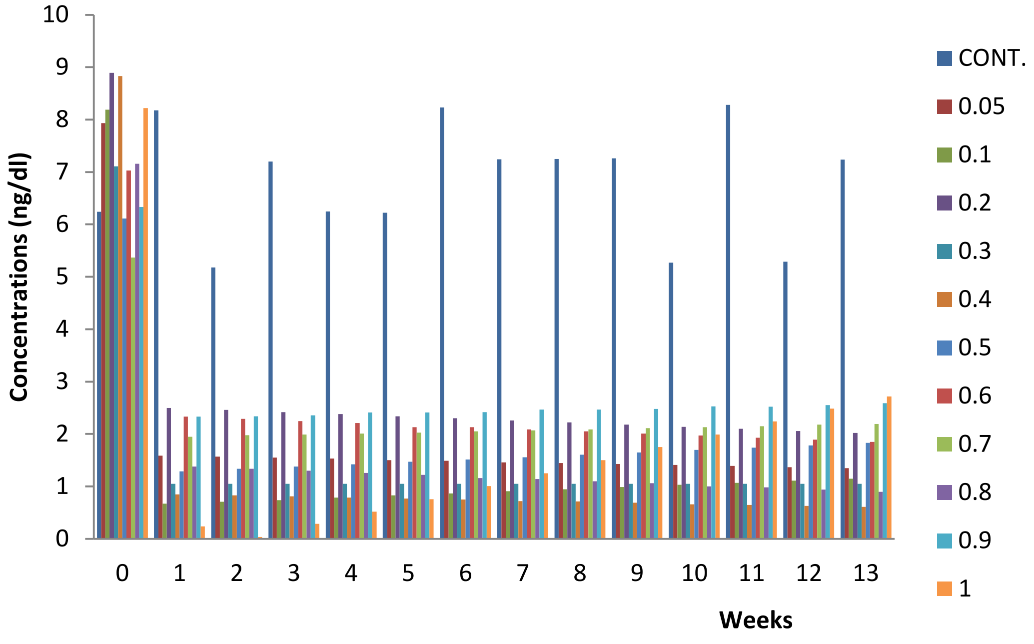

Fig.33a; Bar chart of concentration against weeks (durations) for conjugated thyroxine

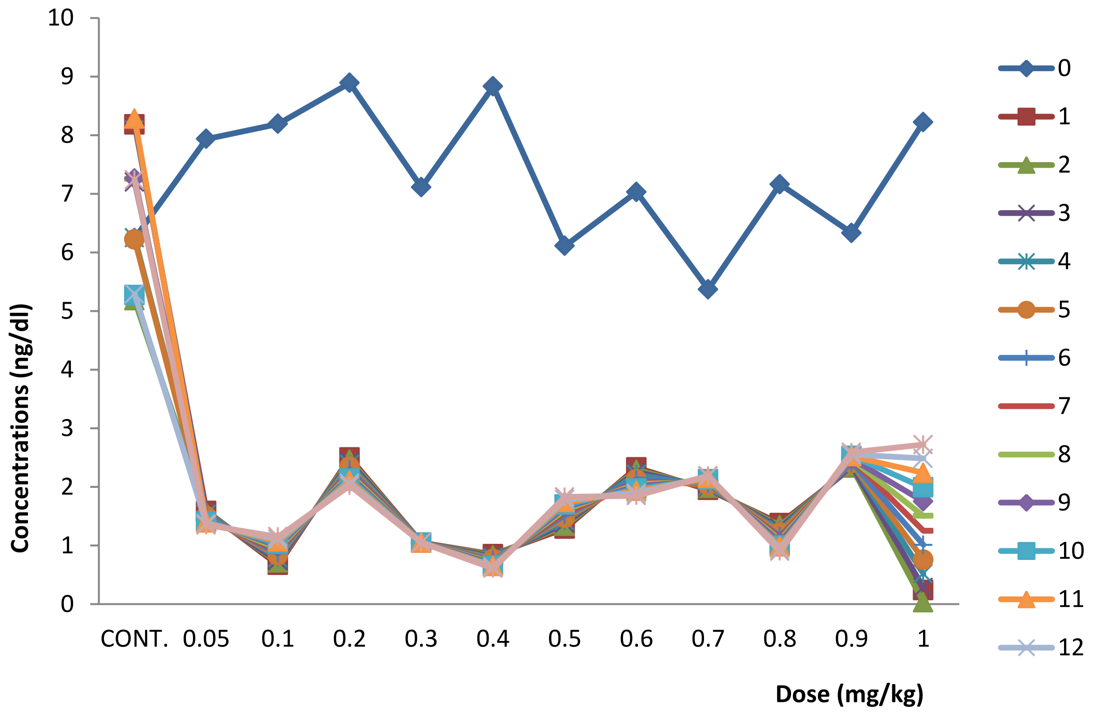

Fig.33b; Graph of concentration against dose for conjugated thyroxine

f) EFFECT ON TOTAL TRIIODOTHYROXINE (T3)

In all the test groups, 0.05mg/kg concentration of total triiodothronine was lower than that of the control (fig.34a and 34b). Other test groups (0.1mg/kg to 1mg/kg BPA) showed significant increases in serum level of total triiodothyronine at $p \leq 0.05$ (fig.34a and 34b). Although at group 8 (0.7mg/kg), there tends to be a decrease which is still significantly higher than that of the control (fig.34b). Group 1(0.05mg/kg BPA) shows a decrease below the control values, the decrease was not significant (fig. 34b).

In all the weeks of exposure, the entire test groups had characteristic dose dependent increase in the serum concentrations of total triiodothyronine, starting from the test group that received 0.05mg/kg BPA to those that received 1mg/kg BPA, except for the groups which received 0.7mg/kg and 0.9mg/kg that showed a relative decrease in total triiodothyronine when compared with their immediated counterparts (fig34a). Throughout the duration of exposure, the test group 1mg/kg had the highest effect on TT_3 concentrations; this was followed by group 0.8mg/kg (fig.34a).

The weekly effect was mild as shown in fig.34b, all the weeks of exposure showed low concentration of TT_3 at test group 0.05mg/kg (fig.34b), this was then followed ny a consistent increase up to the test group 0.6mg/kg, then there was a sharp decrease at test dose 0.7mg/kg, although still higher than those of control and week 0 (fig.34b).

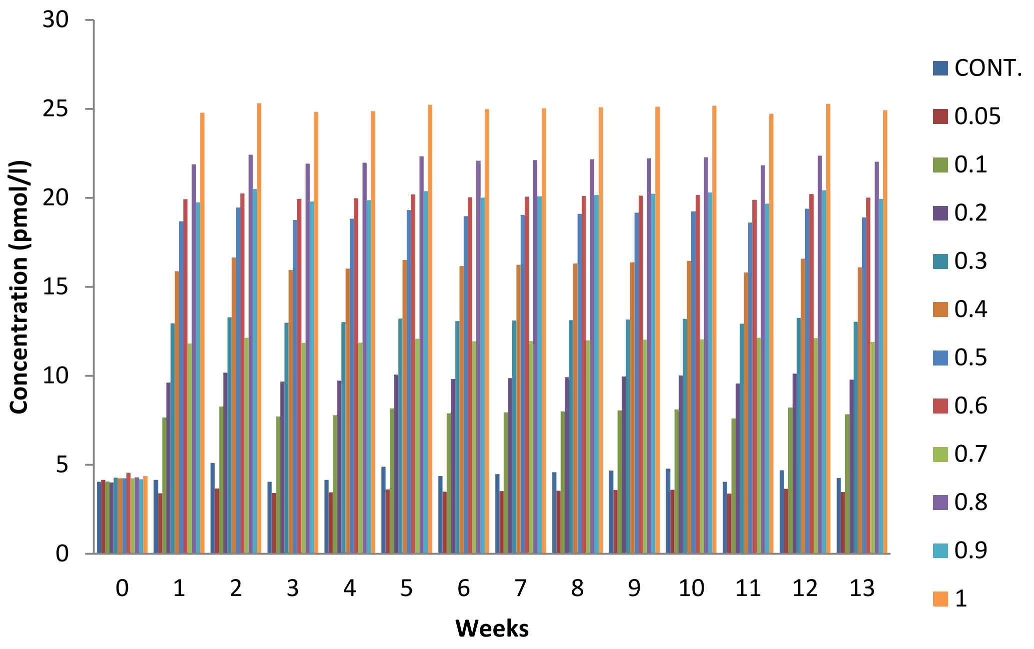

Fig. 34a; Bar chart of concentration against weeks (durations) for total Triiodothyronine (TT3) level.

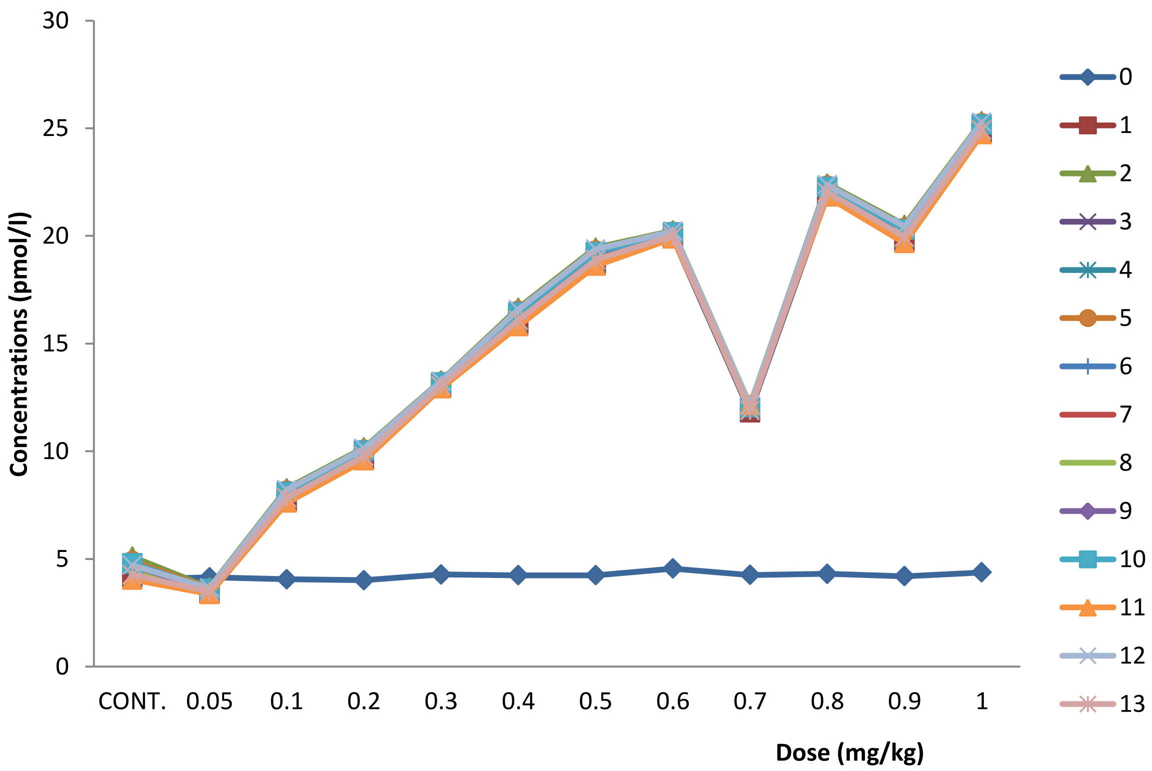

Fig. 34b; Graph of concentration against dose for total Triiodothyronine (TT3) level.

g) EFFECT ON FREE TRIIODOTHYRONINE (FT3)

There was significant increase in the serum levels of Free Triiodothyronine (FT3) of all dose group except for dose group 1 (0.05mg/kg) which showed no significant dercrease at $p \leq 0.05$ when compared with the control. Although there was a rise and fall in the levels at various groups, group 1(0.05mg/kg) showed no significant decrease in FT3 concentration when compared with the control, the effect observed shows a similar pattern to that obtained in TT3 (see fig. 35b and 34b).

It was observed that there was a dose dependent increase shown by the groups that were administered 0.05mg/kg to 0.2mg/kg BPA, 0.3mg/kg to 0.6mg/kg and 0.7mg/kg to 1mg/kg except that in all instance, there was a decreasee shown by the test group that received 0.9mg/kg BPA relative to those of 0.8mg/kg and 1mg/kg BPA (fig.35a). The serum levels of free triiodothyronine appeared relatively constant over time (fig.35a).

Again it was observed that BPA exposure at various doses were insensitive to time (fig.35b). In fig.35b, the observed weekly characteristic revealed that the increase in free triiodothyronine was not time dependent, only very mild effect was observed at test group 0.1mg/kg, 0.2mg/kg and 0.6mg/kg.

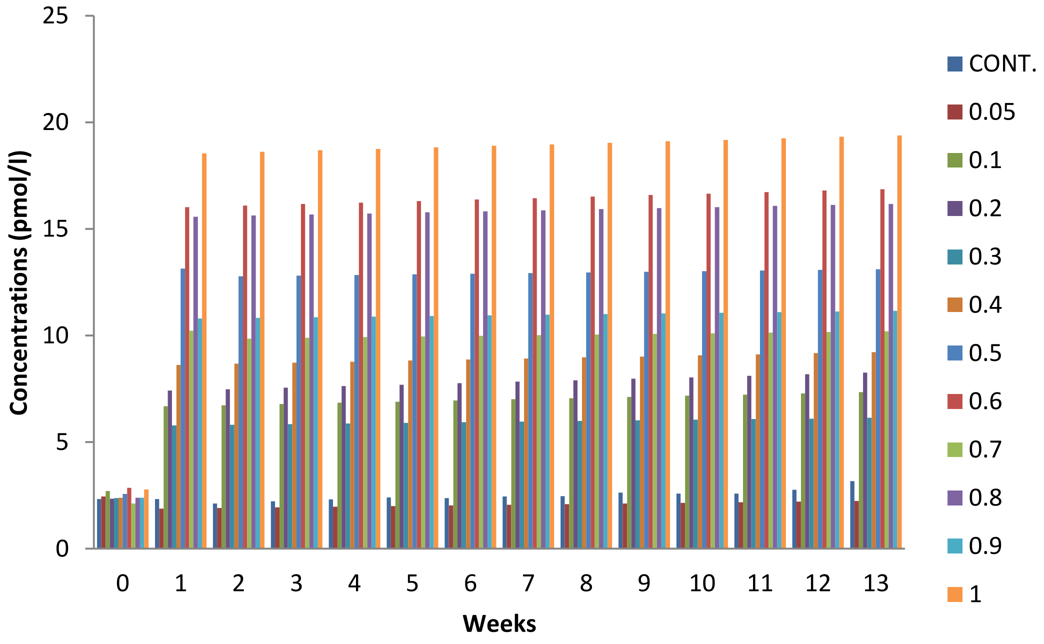

Fig. 35a; Bar chart of concentration against weeks (durations) for Free Triiodothyronine Ft3 level.

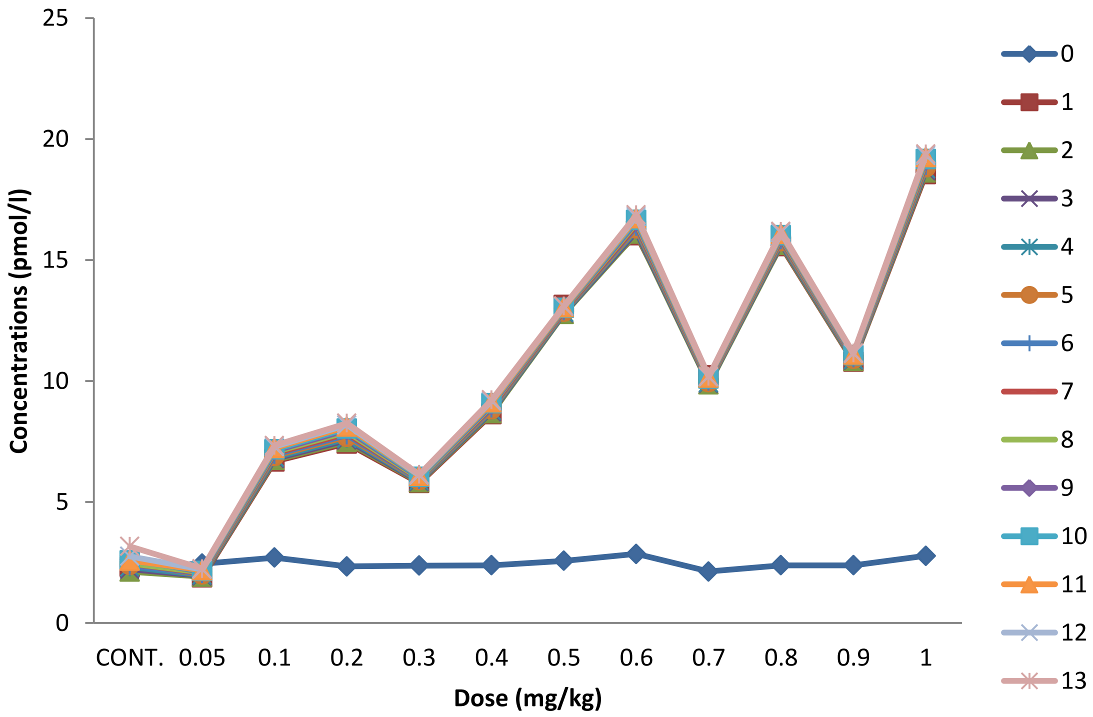

Fig. 35b; Graph of concentration against dose for Free Triiodothyronine (FT3) level.

h) EFFECT ON CONJUGATED TRIIODOTHYRONINE (BT3)

There was a significant instant and drastical decrease in the conjugated Triiodothyronine (BT3) level in all BPA treatment groups, when compared with the control at $p \leq 0.05$. Although there was a increase and decrease in the levels of BT3 at various groups (fig.36a), the effect of BPA administartion on conjugated triiodothyroxine was not sensitive time, and the serum level of conjugated triiodothyronine tends to be relatively constant (see fig. 36b).

Following the exposure to BPA, the groups established a pattern of response throughout the duration of the study. The group that received 0.05mg/kg revealed a relatively high level of conjugated triiodothyronine to those of 0.1mg/kg (fig 36a). A dose dependent increase in conjugated triiodothyronine was shown by those that received 0.1mg/kg to 0.4mg/kg (fig.36a), those of test group 0.5mg/kg to 0.7mg/kg showed a gradual dose dependent decrease in the concentration of conjugated triiodothyronine over time (fig.36a). In all instances, the test group of 0.9mg/kg showed the maximum effect on conjugated triiodothyronine levels.

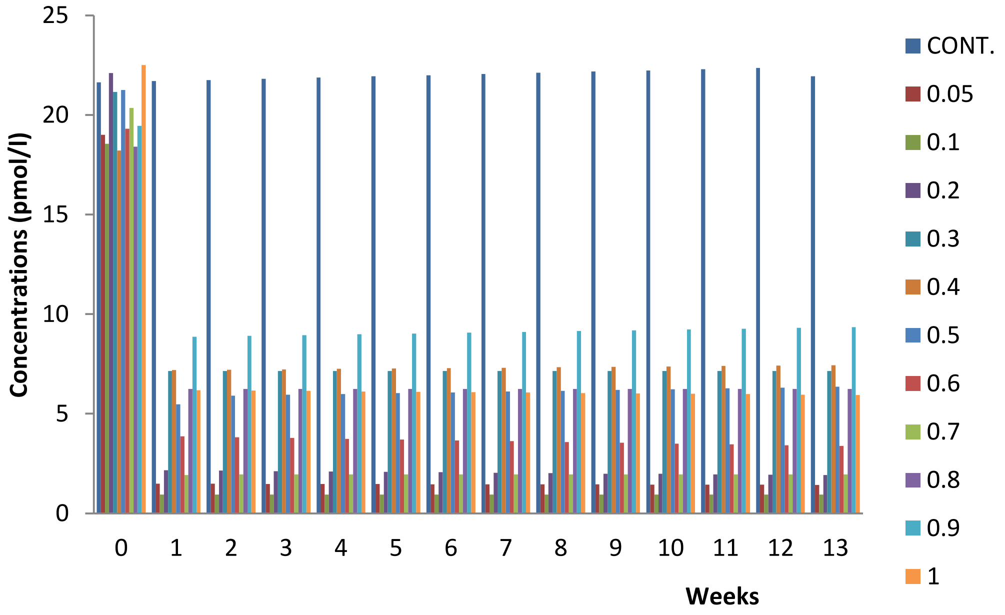

Fig. 36a; Bar chart of concentration against weeks (durations) for conjugated Triiodothyronine (BT3) level.

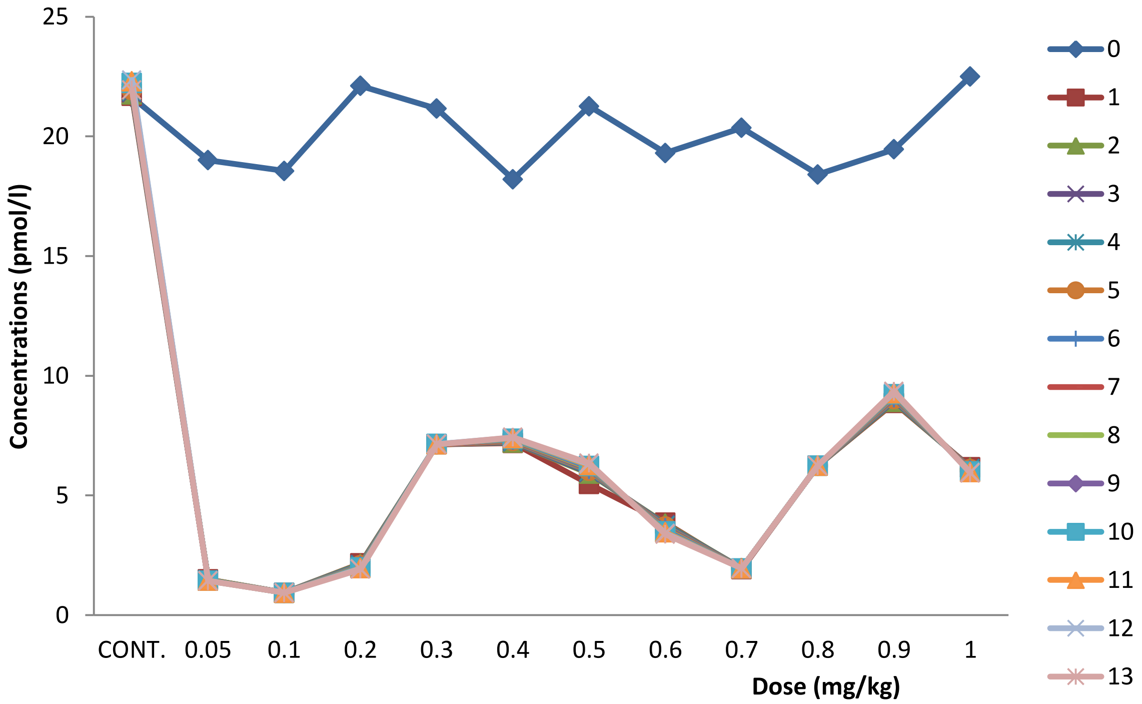

Fig. 36b; Graph of concentration against dose for conjugated Triiodothyronine (BT3) level.

1.5 EFFECT OF BISPHENOL A ON INTERLEUKINS

- **EFFECT ON INTERLEUKINS IL-1**

There was a significant steady increase in the interleukin-1 level when compared with the control at $p \leq 0.05$ (fig 37a).The BPA treatment group maintained a constant steady but gradual increase in IL-1 level as the doses increased with time (see fig 37b).

There was a consistent dose dependent increase in the serum levels of interleukin-1 at weeks 1 to 5, except that at week 2, where the level of interleukin-1 recorded for the test group 0.8mg/kg decreased relatively to those of 0.7mg/kg and 0.9mg/kg (fig 37a). At weeks 3 and 4, a decreased level of interleukin-1was shown by those that received 0.3mg/kg. At week 5, it was observed that the level of interleukin-1 does not confine to the dose dependent manner like those test group that the received 0.3mg/kg and 0.5mg/kg, which showed a decrease instead.

The serum concentration of interleukin-1 during weeks 6 to 13, the entire groups revealed a particular pattern of response in all instances, that is a dose dependent increase by the groups that received 0.05mg/kg to 0.2mg/kg as it peak, another dose dependent increase by test group 0.4mg/kg to 1mg/kg BPA (fig37a). The test group the received 0.3mg/kg showed a decrease in serum concentration of interleukin-1 relative to those of 0.2mg/kg (fig.37a). In all cases, groups 10 and 11 (0.9mg/kg and 1mg/kg) showed the maximum effect on interleukin-1 (fig. 37a and fig.37b) and as the duration of the study increases, the concentration of interleukin-1 also increased except for group 9 (0.8mg/kg) where week 2 value dropped below that of week 1 (fig.37b) and at dose 0.4mg/kg where weeks 6, 7 and 8 values also dropped below week 1, while weeks 9 and 10 below week 2 and week 11 dropped below week 5; and at dose of 0.5mg/kg where weeks 5 to 7 dropped below week 1 (fig.37b). For the groups 0.05mg/kg and 0.1mg/kg, week 9 gave the highest value, followed by weeks 10 and 11. Weeks 12 and 13 appeared between the values for weeks 7 and 8. The maximum effect of BPA administration in interleukin-1was observed at dose 0.9mg/kg and 1mg/kg (fig.37b).

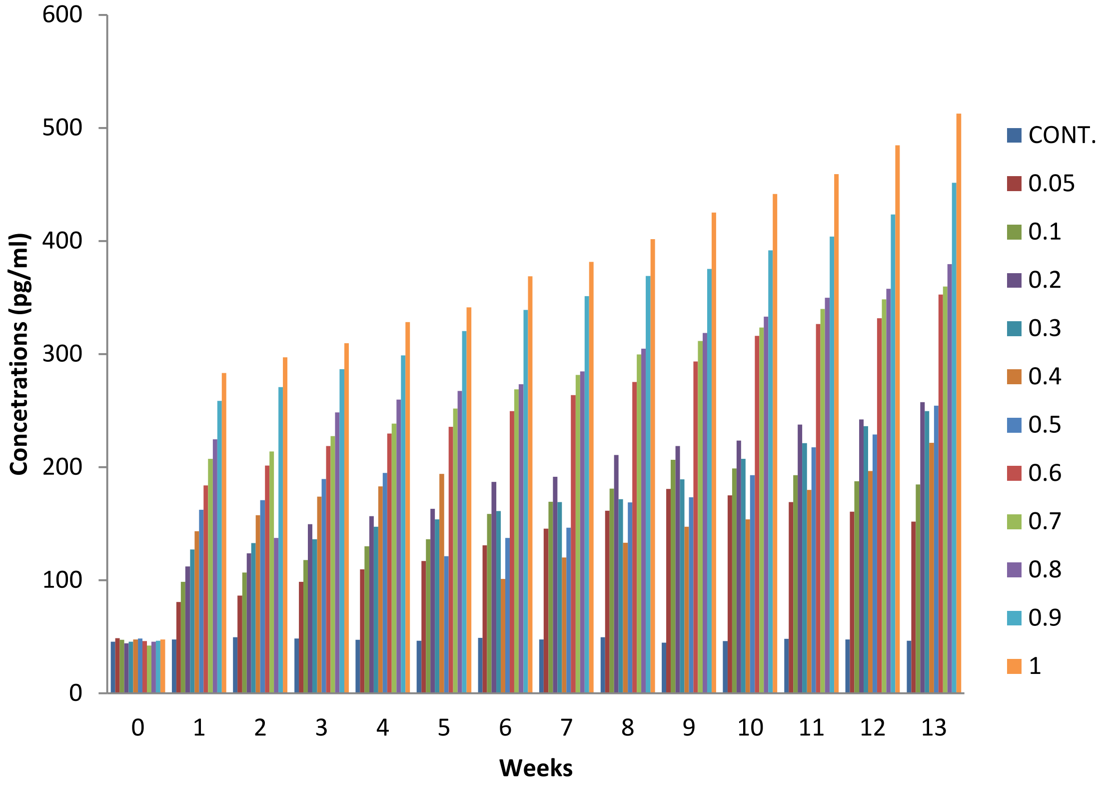

Fig. 37a; Bar chart of concentration against weeks (durations) for interleukin-1 level.

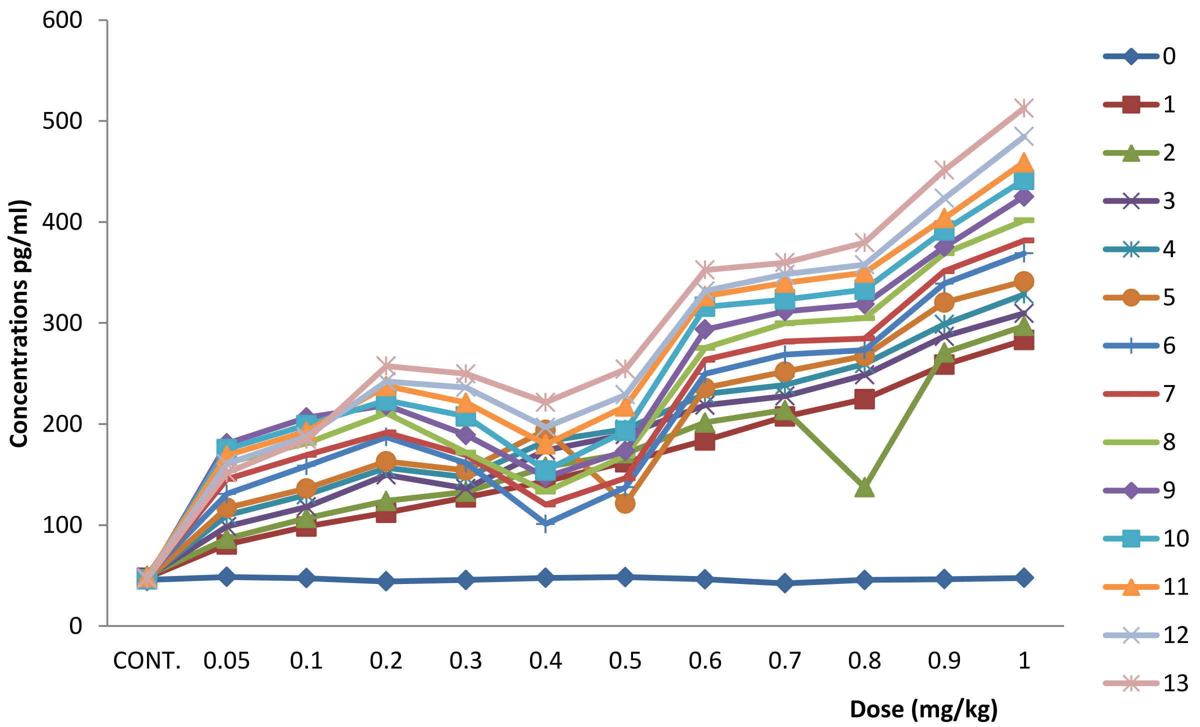

Fig. 37b; Graph of concentration against dose for interleukin-1 level.

- **EFFECT ON INTERLEUKINS IL-2**

There were significant increases in the interleukin-2 level when compared with the control at $p \leq 0.05$ (fig 38a).There appears to be an initial significant increase in IL-2 level when compared with control at $p \leq 0.05$ at group 1(0.05mg/kg) and 2 (0.1mg/kg). This was followed by a drastic drop of the IL-2 at BPA treatment group 3 (0.2mg/kg). The observed decrease at this point was such that there was no significant difference between the IL-2 level of test group 3 (0.2mg/kg) and that of the control group at $p \leq 0.05$. Again, the IL-2 level significantly begin to increase at group 4 (0.3mg/kg) through to group 11 (1mg/kg), the observed increase was dose dependent, gradual and constantly rising with time. The BPA concentration that showed the least effect on IL-2 levels was 200µg/kg bw. The maximum effect was of IL-2 was observed at 1mg/kg bw (fig 38b).

There was a significant increase in interleukins-2 concentration at $p \leq 0.05$ when compared with the control for all the exposure doses except for for dose 0.2mg/kg which showed no significant effect on interleukin-2 level when compared with the control. Fig 38a revealed a characteristics dose dependent increase (0.05mg/kg to 0.1mg/kg, and 0.3mg/kg to 1mg/kg) across all the exposure weeks, except for 0.2mg/kg dose group that showed a decrease in the serum interleukin-2 level compared to other test groups. At weeks 12 and 13 the serum level for interleukin-2 for 0.05mg/kg test group were higher than those of 0.1mg/kg test group (fig.38a). There was a time dependent effect observed for the entire dose groups, except for dose 0.1mg/kg where week 11 showed the highest concentration of interluekins-2 and week 12 appeared between week 10 and 11, while week 13 drop below week 10 (fig.38b). The dose group 0.2mg/kg showed no sensitivity to time of exposure (fig.38b).

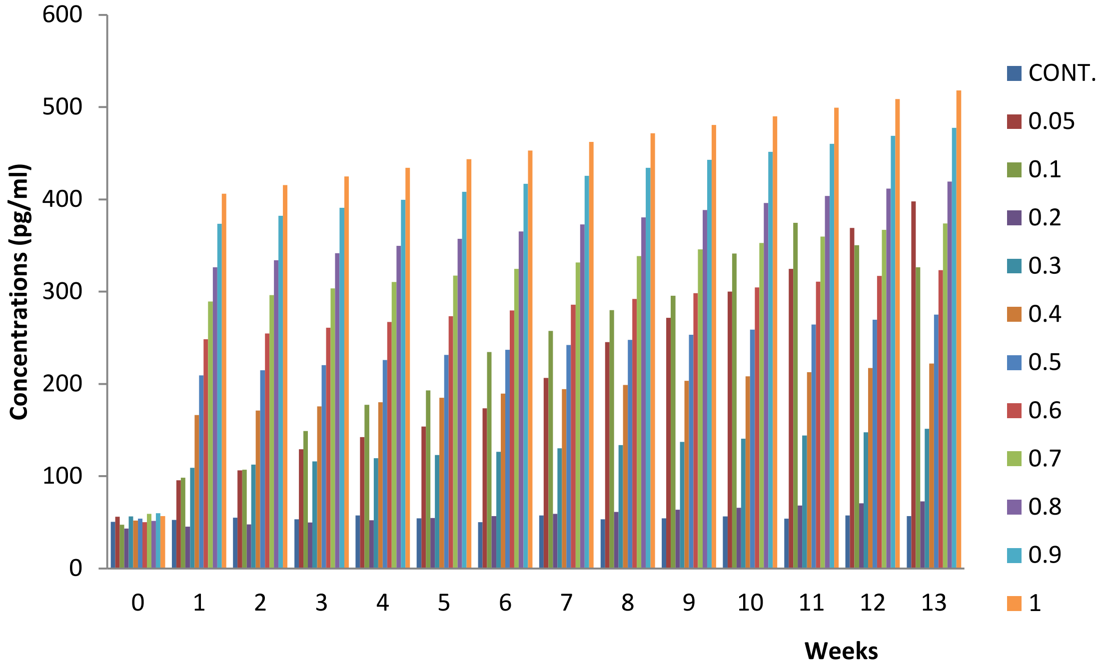

Fig. 38a; Bar chart of concentration against weeks (durations) for interleukin-2 level.

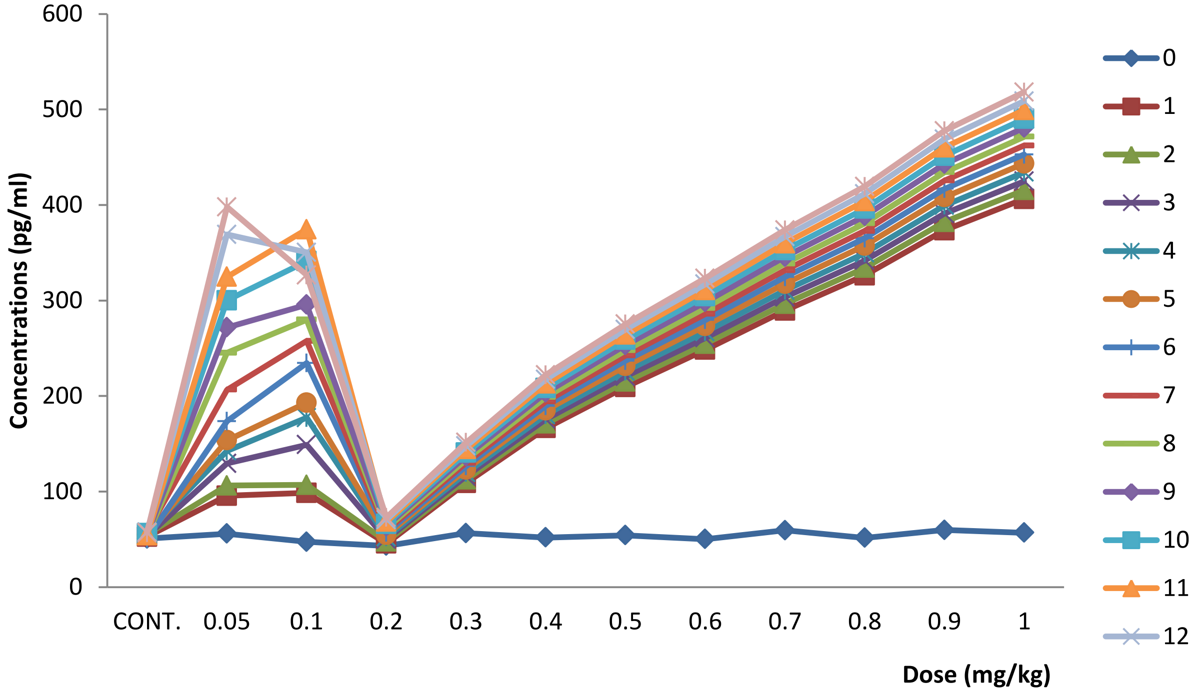

Fig. 38b; Graph of concentration against dose for interleukin-2 level.

- **EFFECT ON INTERLEUKINS IL-3**

There was a significant increase in the interleukin-3 level when compared with the control at $p \leq 0.05$. Group 1(0.05mg/kg) to 5(0.4mg/kg) at weeks 2, 5, 6, 7, 8, 9, 10, 12 and 13 showed a dose dependent effect (fig 39a). During the first and third weeks of the study, the test group showed similar response to BPA exposure; in that, in both instance, the group that received 0.05mg/kg showed high level of interleukin-3 relative to those of 0.1mg/kg group at weeks 1 and 3 (fig.39a). The test group that received 0.1mg/kg to 0.5mg/kg showed a dose dependent increase in serum levels on interleukin-3, except that in week 1, those of 0.5mg/kg decreased relative to those of 0.4mg/kg (fig.39b). Throughout the study, the test group that received 0.05mg/kg to 0.4mg/kg revealed a dose dependent increase in interleukins-3 level except at weeks 1 and 3, where it was observed that 0.05mg/kg test group showed higher levels of IL-3 relative to those of 0.1mg/kg; the test group that received 0.6mg/kg to 1mg/kg revealed a dose dependent increase in interleukins-3 level except at weeks 6, 9, 12 and 13 where the dose dependent effect was shown from from 0.5mg/kg test group (fig. 39a). In all instances the test group that received 0.5mg/kg showed a decreased level of interleukins-3 relative to those of 0.4mg/kg except at week 3 where its maximum effect was observed (fig. 39a). Fig. 39b showed all the test group revealed a time dependent effect, except for test group 0.05mg/kg to 0.4mg/kg which showed different response at weeks 1, 3, 4 and 11; 0.5mg/kg to 1mg/kg showed different response at weeks 3,4,6 and 12 (fig.39b). Generally, the test group 0.05mg/kg to 0.5mg/kg revealed that the level of IL-3 decreases with time, while those of 0.6mg/kg to 1mg/kg showed the reversed effect (fig. 39b). The maximum effect of BPA on IL-3 was revealed by 0.5mg/kg at week 3, and 0.1mg/kg at week 13 (fig. 39a and 39b).

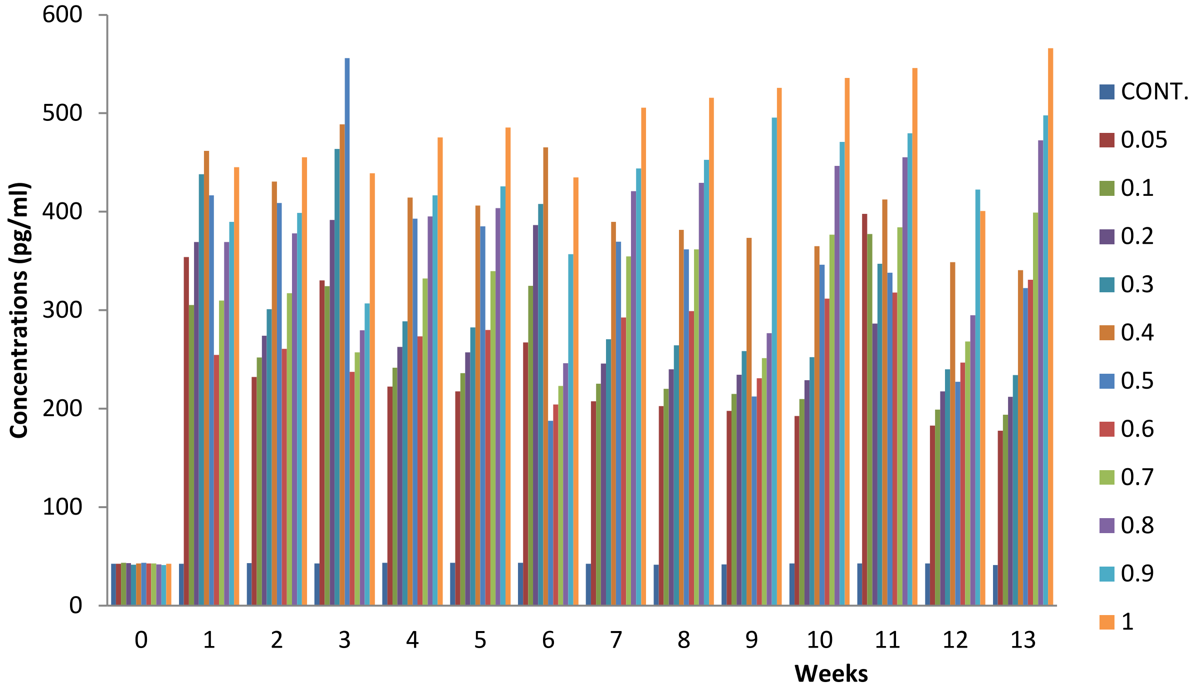

Fig 39a; Bar chart of concentration against weeks (durations) for interleukin-3 level.

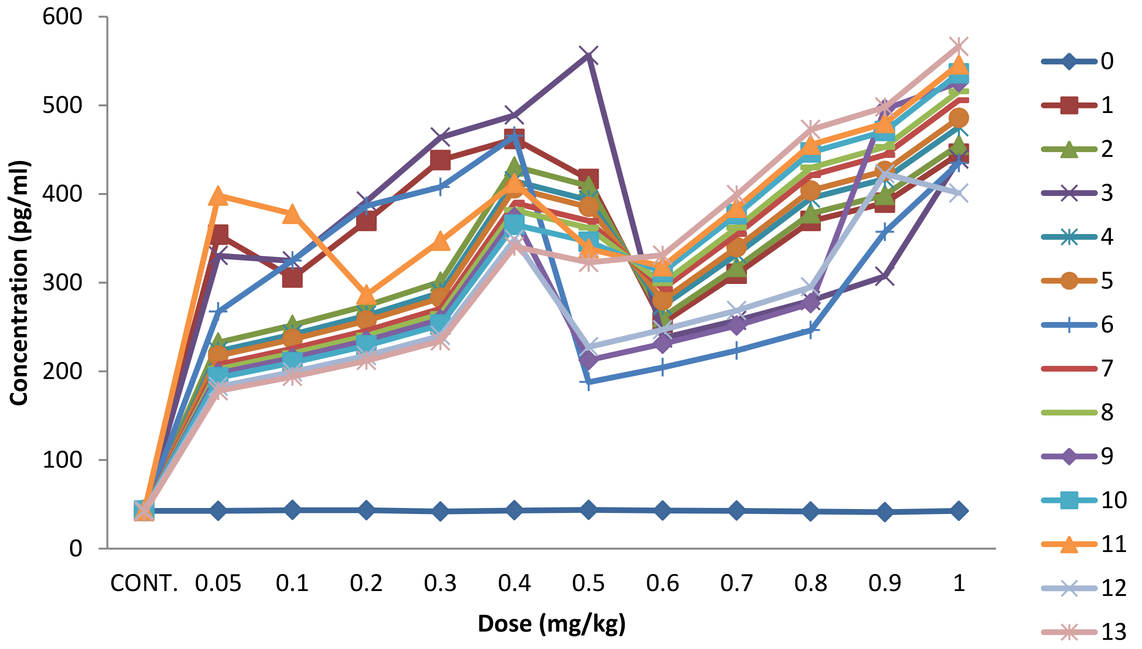

Fig 39b; Graph of concentration against dose for interleukin-3 level.

- **EFFECT ON INTERLEUKINS IL-4**

There was a significant increase in the interleukin-4 level when compared with the control at $p \leq 0.05$. In all the weeks, the test group that received 0.5mg/kg BPA showed the maximum effect of BPA on interleukin-4 (fig.40a). Although, no established pattern of response was observed, it was noted that during weeks 4 to 11 and 13, the test group that received 0.2mg/kg, 0.4mg/kg, 0.7mg/kg and 1mg/kg BPA generally showed a lower concenration in IL-4 when compared with their immediate counterparts, while during weeks 1 to 3 and 12, the test group that received 0.2mg/kg, 0.4mg/kg, 0.8mg/kg and 1mg/kg BPA showed a decrease in the IL-4 level when compared their immediate counterparts (fig. 40a). Fig. 40b showed that the entire dose of exposure to BPA showed mild sensitivity to time; there was a zigzag kind of response by the test groups. The test group 0.5mg/kg had the highest effect on interleukins-4 at week 2 (fig.40b).

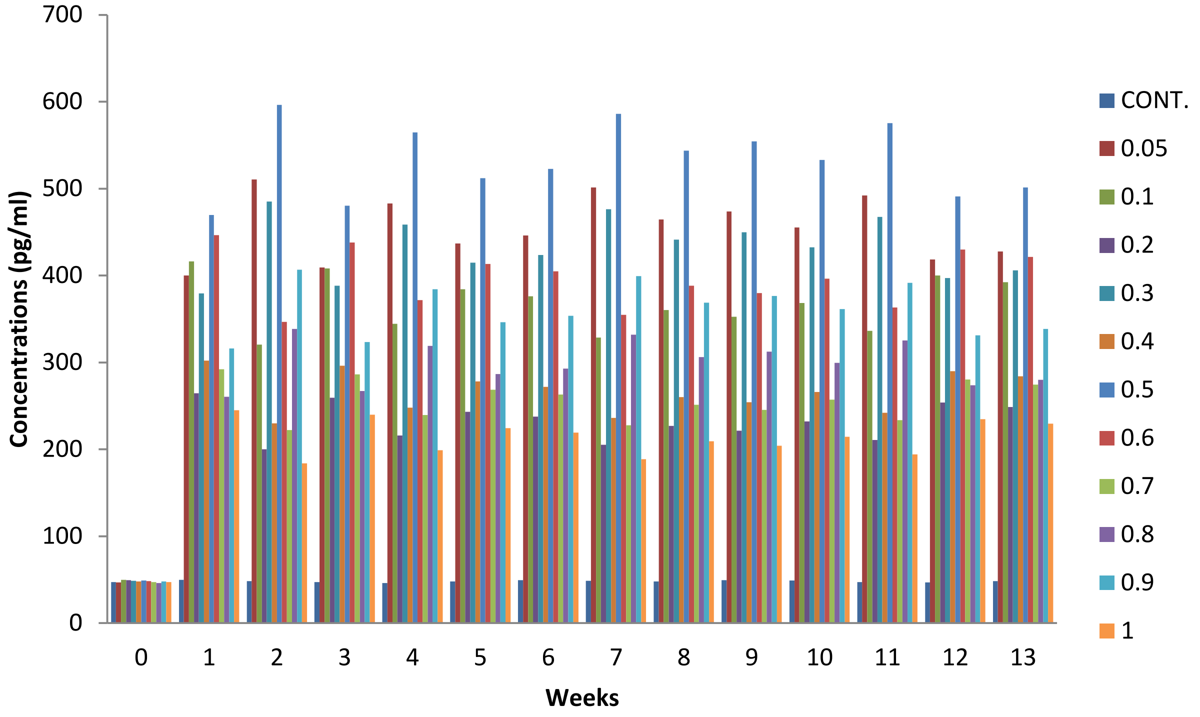

Fig. 40a; Bar chart of concentration against weeks (durations) for interleukin-4 level.

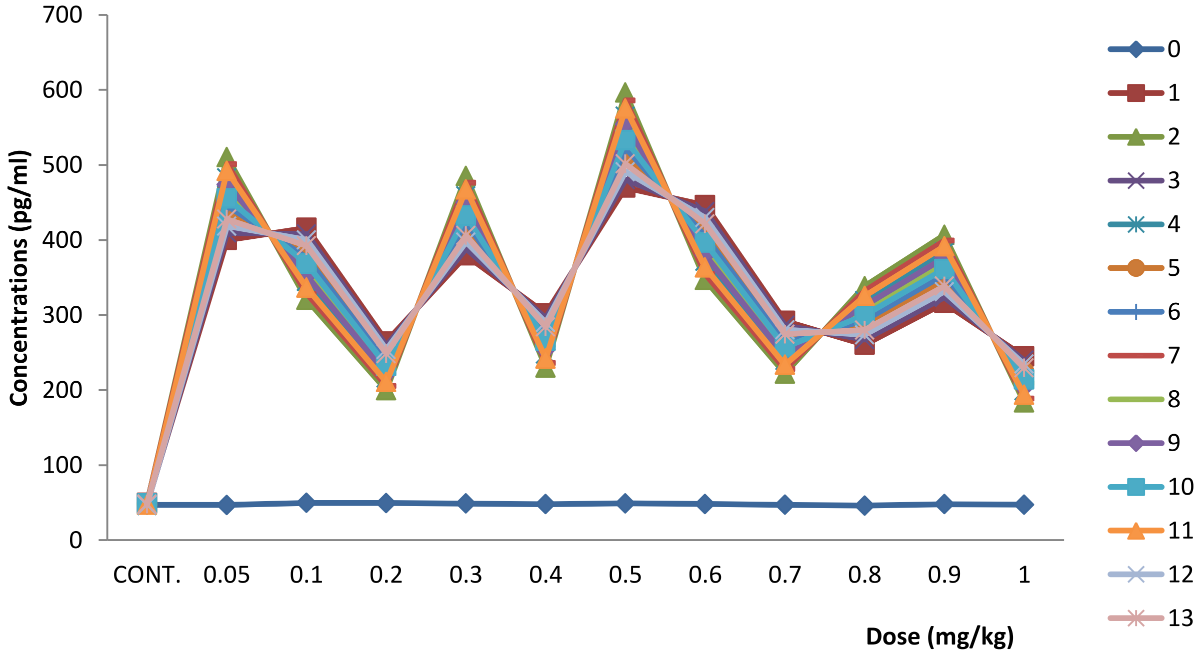

Fig. 40b; Graph of concentration against dose for interleukin-4 level.

- **EFFECT ON INTERLEUKINS IL-5**

There was a significant increase in the interleukin-5 level when compared with the control at $p \leq 0.05$. In all instances, the test group that received 1mg/kg of BPA, showed the highest effect of BPA on interleukins-5 except during week 2 of the experiment where higher level of IL-5 was exhibited by the group that was administered 0.8mg/kg and 0.9mg/kg BPA (fig. 41a). During weeks 1, 2, 4, 6 to 12; the entire test group showed a similar response pattern, in that, there was a dose dependent increase in intterleukins-5 by the groups that were dministered 0.05mg/kg to 0.4mg/kg and 0.7mg/kg to 1mg/kg except at week 2 where those of 1mg/kg showed a decreasing effect (fig.41a). Throughtout the course of the study, the test group 0.5mg/kg and 0.6mg/kg showed a dosewise decrease in the concentration of interleukins-5, except at week 5 where those of 0.5mg/kg showed a further decrease relative to those of 0.6mg/kg (fig.41a). The weekly characteristics as shown in fig. 41b, showed that exposure to BPA were time sensitive to time of exposure, except at weeks 3, 5 for test dose of 0.05mg/kg to 0.4mg/kg, and weeks 5, 13 for 0.5mg/kg; weeks 5 and 10 for 0.6mg/kg; weeks 2, 5 and 10 for 0.7mg/kg to 0.9mg/kg; and weeks 5 and 10 for 1mg/kg were different response were observed (fig. 41b).

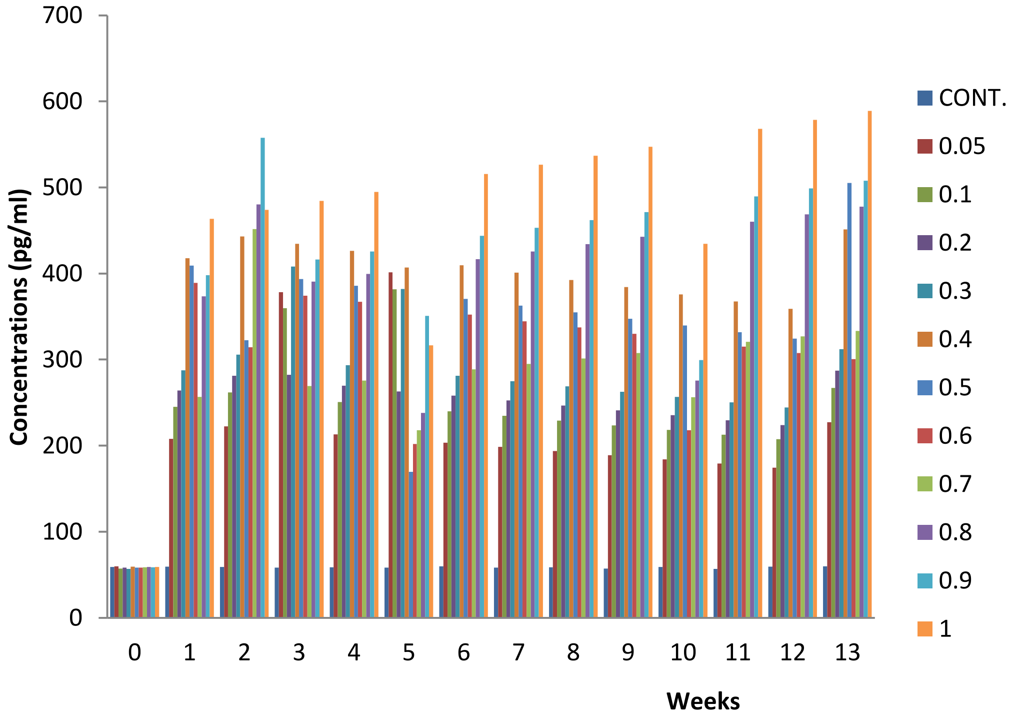

Fig. 41a; Bar chart of concentration against weeks (durations) for interleukin-5 level.

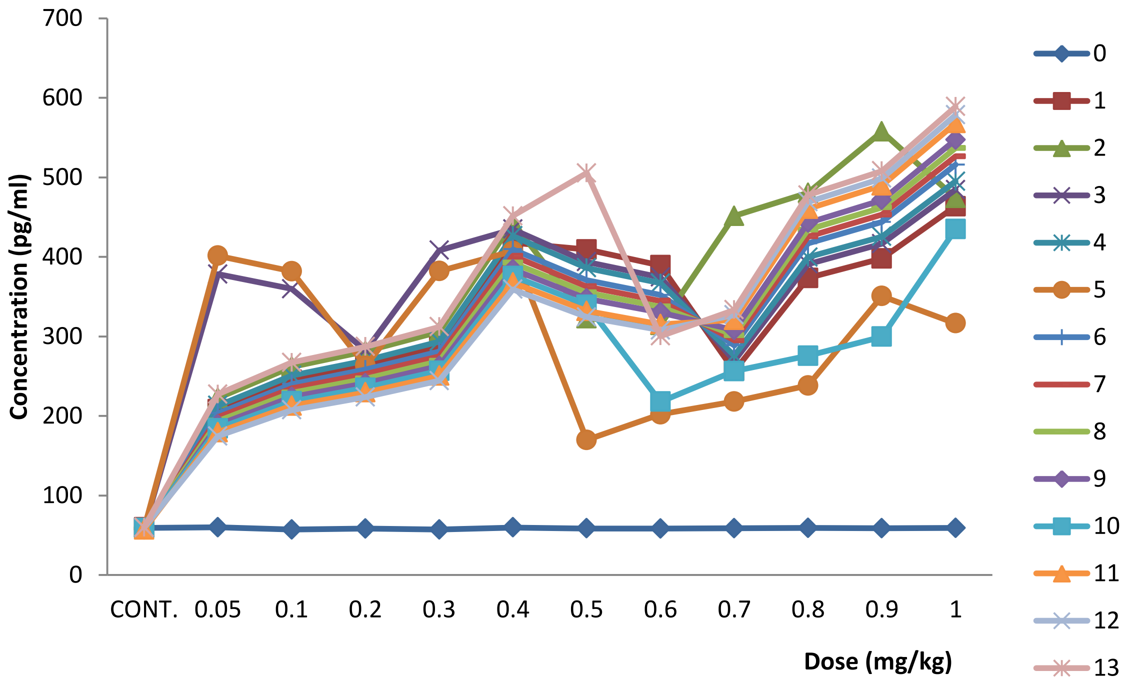

Fig. 41b; Graph of concentration against dose for interleukin-5 level.

- **EFFECT ON INTERLEUKINS IL-6**

There was significant increase in the interleukin-6 level when compared with the control at $p \leq 0.05$. A dose dependent increase in interleukin-6 was observed throughout the time of exposure to BPA for the test groups that received 0.2mg/kg to 0.6mg/kg and 0.7mg/kg to 0.9mg/kg (fig. 42a). At weeks 1 and 2, the test group of 1mg/kg showed a relatively higher concentration of interleukin-6 compared to the 0.9mg/kg test group while at weeks 3 to 13, the 1mg/kg test group showed a decreased level of IL-6 relative those of 0.9mg/kg (fig. 42a). At weeks 1 to 9, the group administered 0.1mg/kg BPA showed higher interleukin-6 concentration relative to those of 0.05mg/kg, while for weeks 10 to 13 the reverse was the case (fig. 42a). The exposure to BPA at graded doses showed sensitivity to time of exposure while the test group of 0.05mg/kg, 0.5mg/kg, 0.6mg/kg, 0.8mg/kg and 0.9mg/kg showed that the concentration of interleukins-6 increases with time, while the test group 0.1mg/kg to 0.4mg/kg, 0.7mg/kg and 1mg/kg showed that the concentration of IL-6 decreased over time (fig. 42b). The dose 0.6mg/kg showed the highest effect at week 13 (fig.42b).

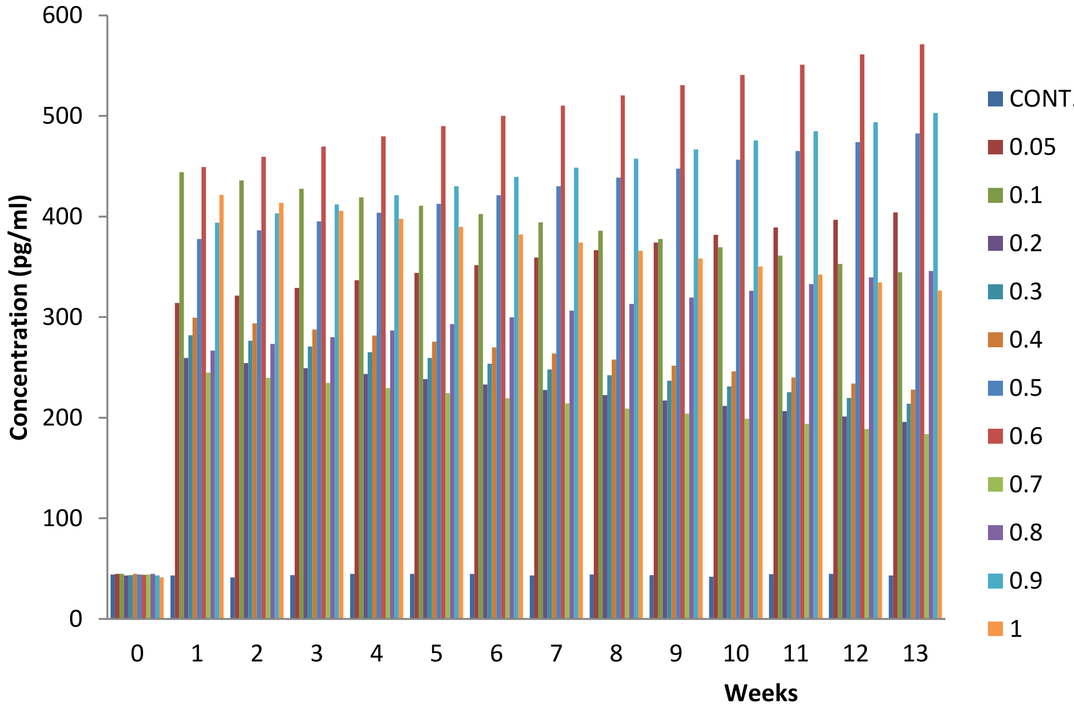

Fig. 42a; Bar chart of concentration against weeks (durations) for interleukin-6 level.

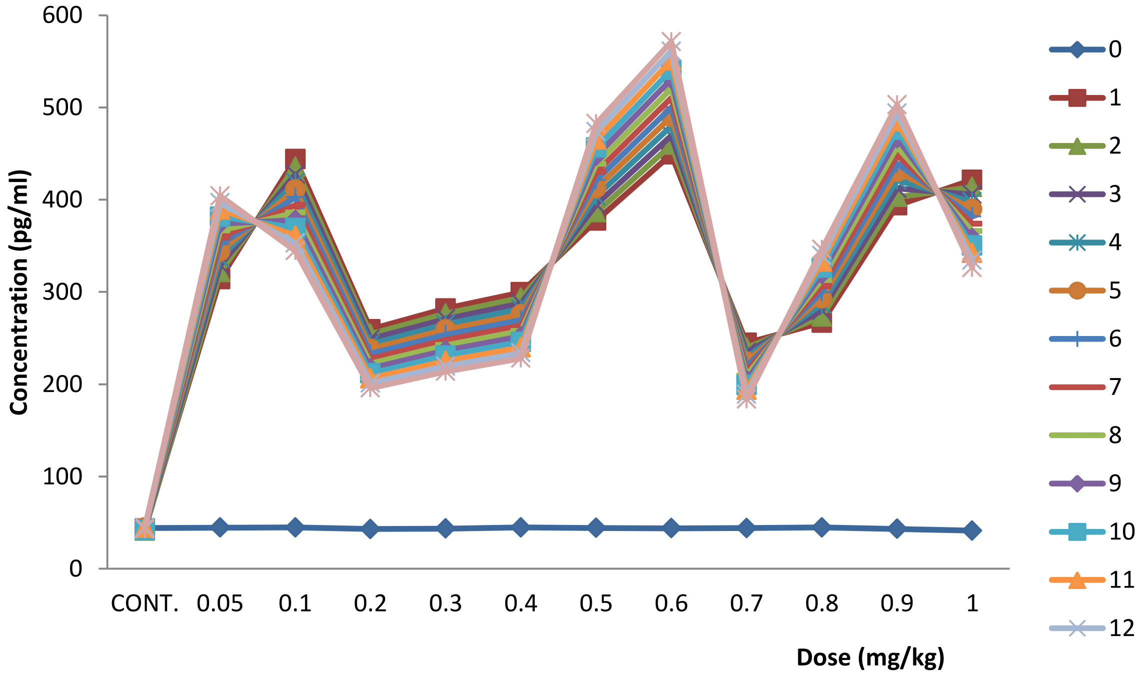

Fig. 42b; Graph of concentration against dose for interleukin-6 level.

- **EFFECT ON INTERLEUKINS IL-7**

There was significant increase in the interleukin-7 level when compared with the control at $p \leq 0.05$. Throughout the exposure period, the test groups that were exposed to 0.05mg/kg to 0.4mg/kg showed dose dependent increases in the serum levels of interleukin-7 except for week 11 where those of 0.4mg/kg decreased relative to those 0.3mg/kg (fig. 43a). Also, the test group that were given 0.6mg/kg to 1mg/kg of BPA showed dose dependent increases in IL-7 except in weeks 2, 6 and 7 (fig. 43a). In week 11, the exposure dose of 0.3mg/kg had the maximum effect on interleukin-7 (fig. 43a and 43b). The weekly profile showed sensitivity to time, although it was not time dependent (fig. 43b).

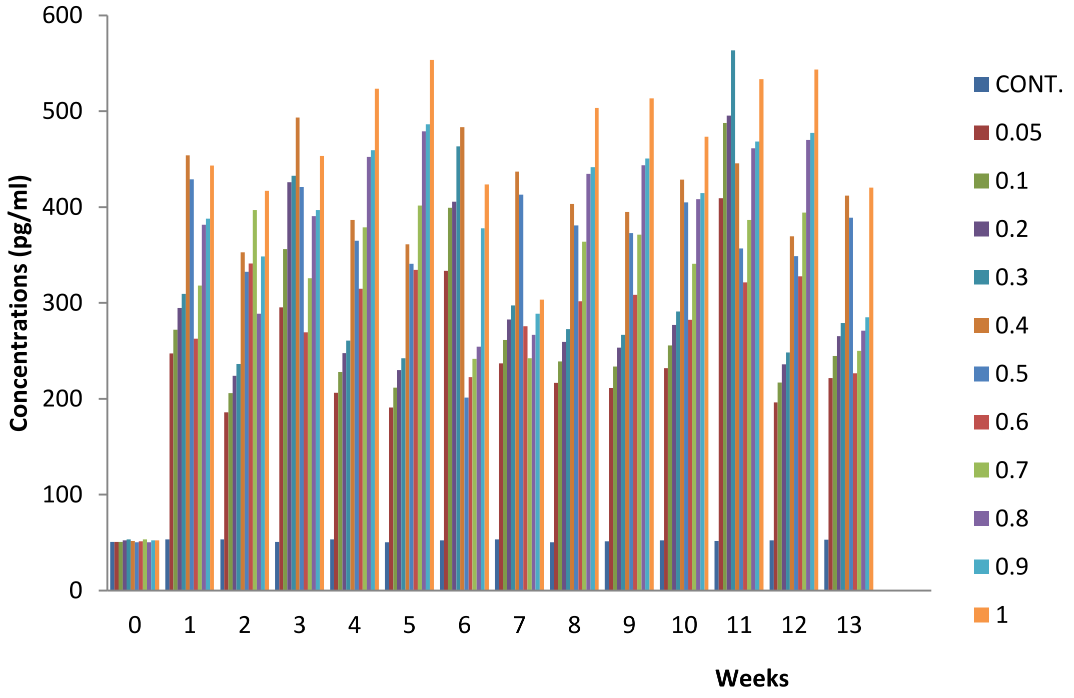

Fig. 43a; Bar chart of concentration against weeks (durations) for interleukin-7 level.

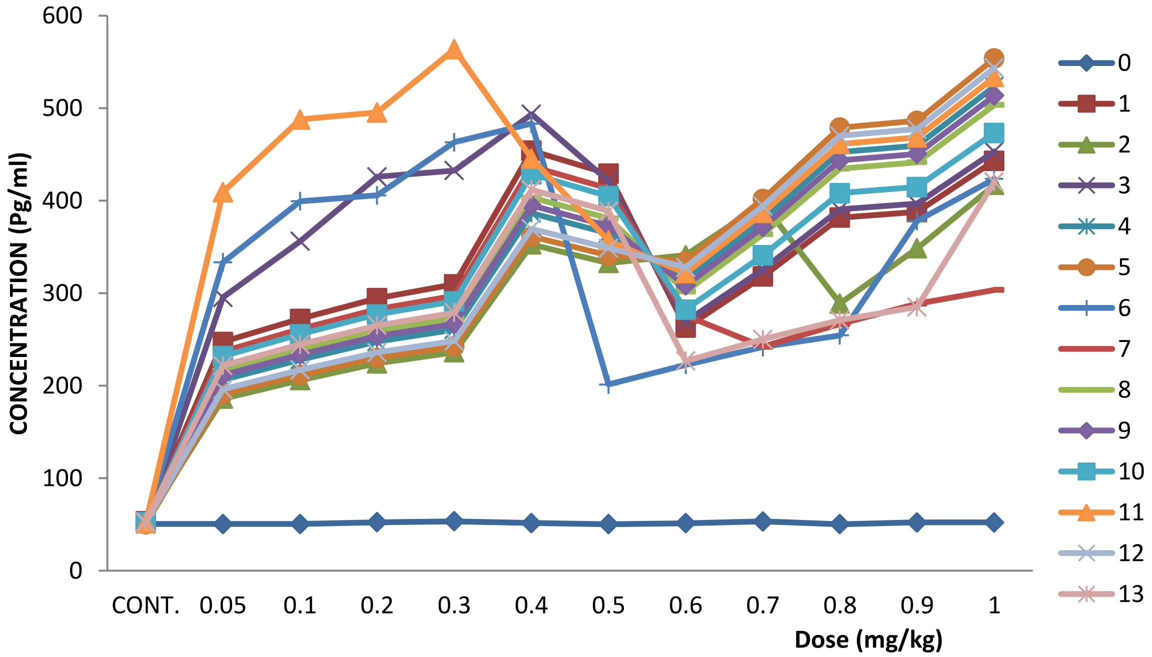

Fig. 43b; Graph of concentration against dose for interleukin-7 level.

- **EFFECT ON INTERLEUKINS IL-8**

There was significant increase in the interleukin-8 level when compared with the control at $p \leq 0.05$. Although no particular pattern of response was revealed by this result (fig 44a and 44b), it was observed that the test group exposed to 0.2mg/kg of BPA showed the maximun effect of BPA on interleukins-8 (fig. 44a), the test group of 0.5mg/kg showed the lowest effect of BPA exposure on IL-8 relative to other test groups, except in week 3 (fig. 44a) while fig. 44b reveals a time sensitive response in IL-8 following BPA exposure (fig. 44b). Four peaks were observed in fig. 44b; the first was at 0.05mg/kg BPA with week 4 as its maximum, secondly at 0.2mg/kg and 0.6mg/kg with week 13 as its maximum and 0.8mg/kg with week 2 as its maximum (fig. 44b).

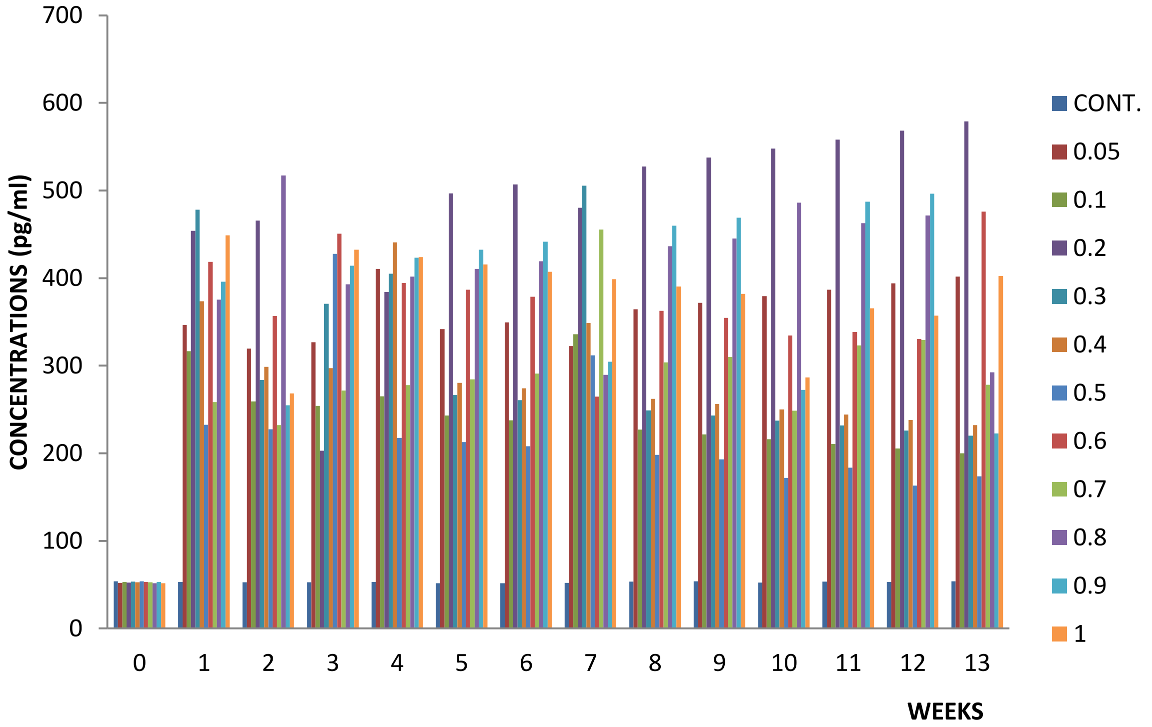

Fig. 44a; Bar chart of concentration against weeks (durations) for interleukin-8 level.

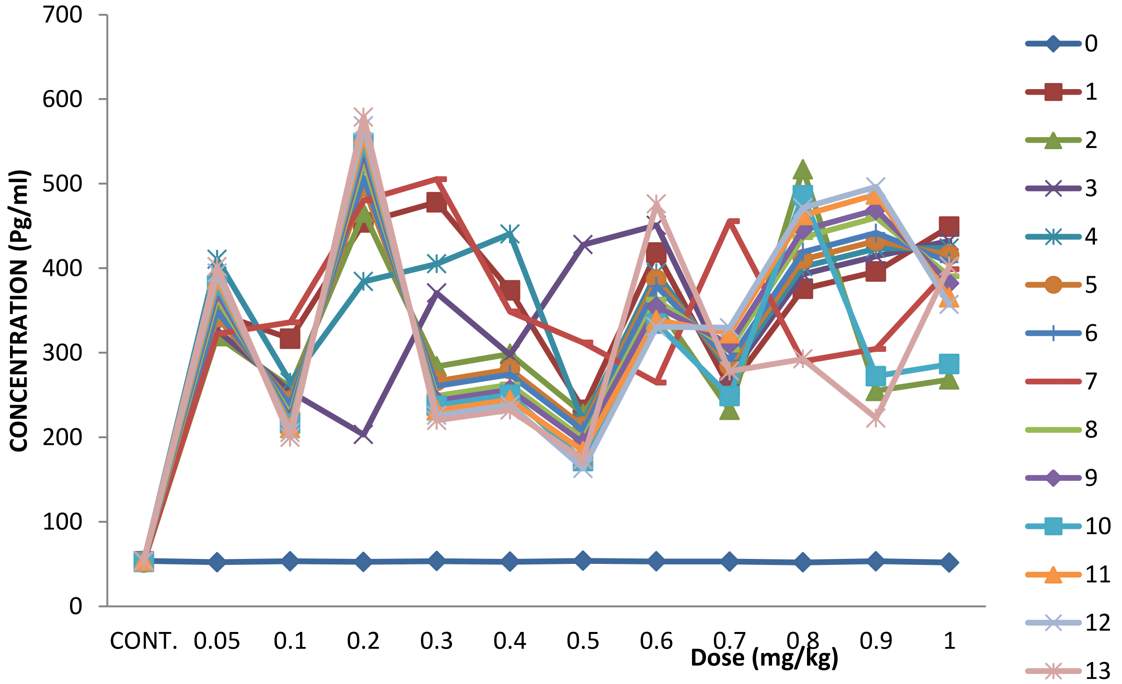

Fig. 44b; Graph of concentration against dose for interleukin-8 level.

- **EFFECT ON INTERLEUKINS IL-9**

There was significant increase in the interleukin-9 level when compared with the control at p≤0.05 (fig 45b). In all instances, the group that were exposed to 1mg/kg BPA showed the highest level of interleukin-9 with hihgest value of (568.62±0.005) in week 13 (fig.45a), it was observed that, while the test groups of 0.7mg/kg to 1mg/kg BPA were showing increasing IL-9 level over time, with its lowest effect in week 1 and highest effect in week 13; the test groups 0.05mg/kg to 0.6mg/kg were showing a decreasing effect with the lowest effect observed in week 13 and highest effect in week 1 (fig 45a and 45b). The entire groups responded to BPA exposure in similar manner (fig.45a). The test group 0.05mg/kg to 0.4mg/kg and 0.7mg/kg to 1mg/kg showed dose dependent increase in IL-9 level, while 0.5mg/kg and 0.6mg/kg showed dose dependent decrease (fig. 45a and 45b). The effect of the exposure to BPA on interleukin-9 concentration were time dependent and showed sensitivity to time (fig. 45b).

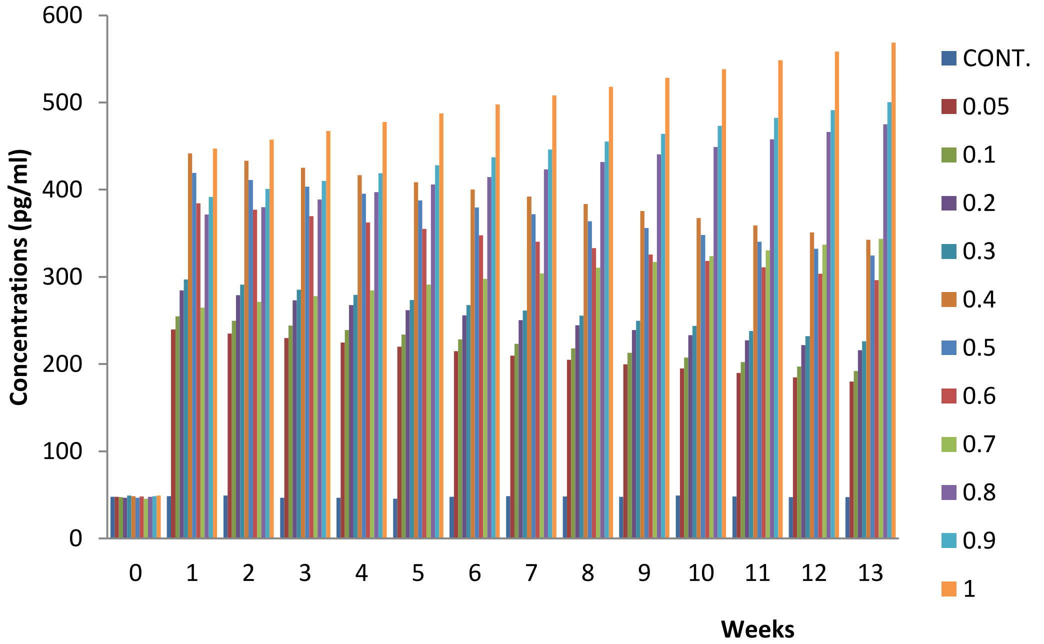

Fig. 45a; Bar chart of concentration against weeks (durations) for interleukin-9 level.

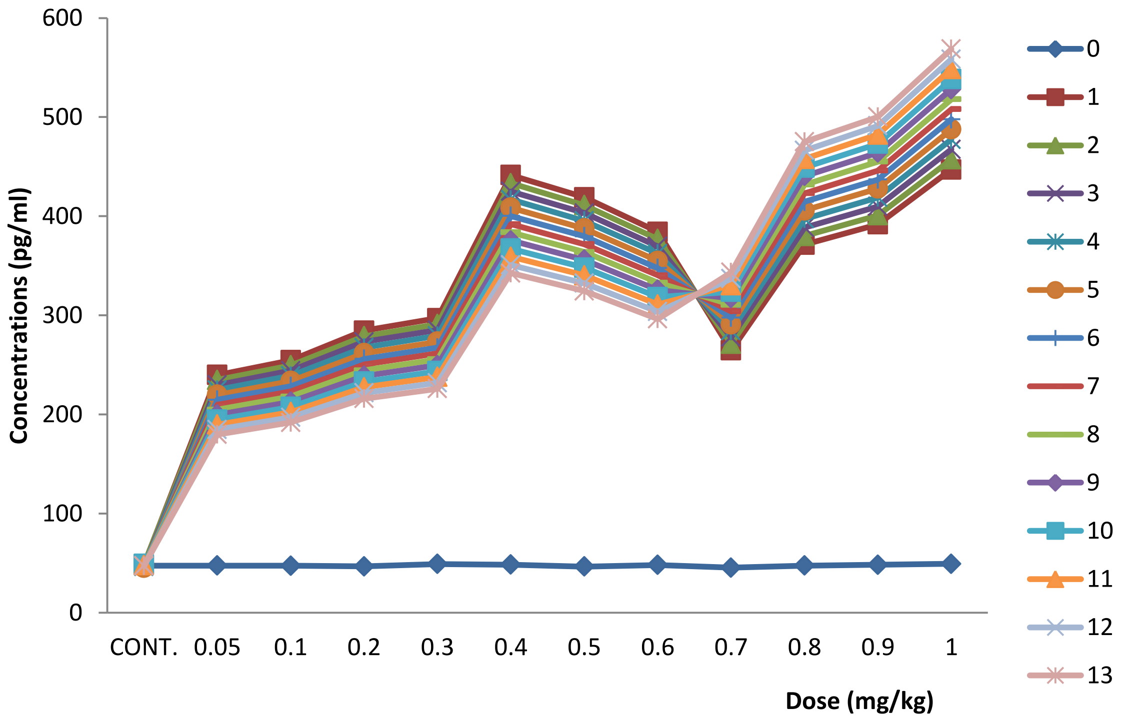

Fig. 45b; Graph of concentration against dose for interleukin-9 level.

- **EFFECT ON INTERLEUKINS IL-10**

There was significant increase in the interleukin-10 level when compared with the control at $p \leq 0.05$ (fig 46a). Throughout the exposure period, the test group that were exposed to 0.05mg/kg to 0.3mg/kg showed dose dependent and consistent decrease in the serum interleukins-10 levels (fig.46a). The test groups exposed to 0.6mg/kg to 1mg/kg of BPA revealed dose dependent and consistent increase in the level of interleukin-10 (fig. 46a). In weeks 1 to 8, the test group of 0.4mg/kg showed a lower level of IL-10 compared to those of group 0.5mg/kg, while at weeks 9 to 13, the group exposed to 0.4mg/kg of BPA showed a higher level of IL-10 relative to thossse of 0.5mg/kg (fig. 46a). Fig. 46b showed that IL-10 level after exposure to graded doses of BPA was sensitive to time of exposure. The concentration of interleukin-10 increases with increase in time (fig. 46b) except at the dose group of 0.5mg/kg where the reverse effect was observed (fig. 46b).

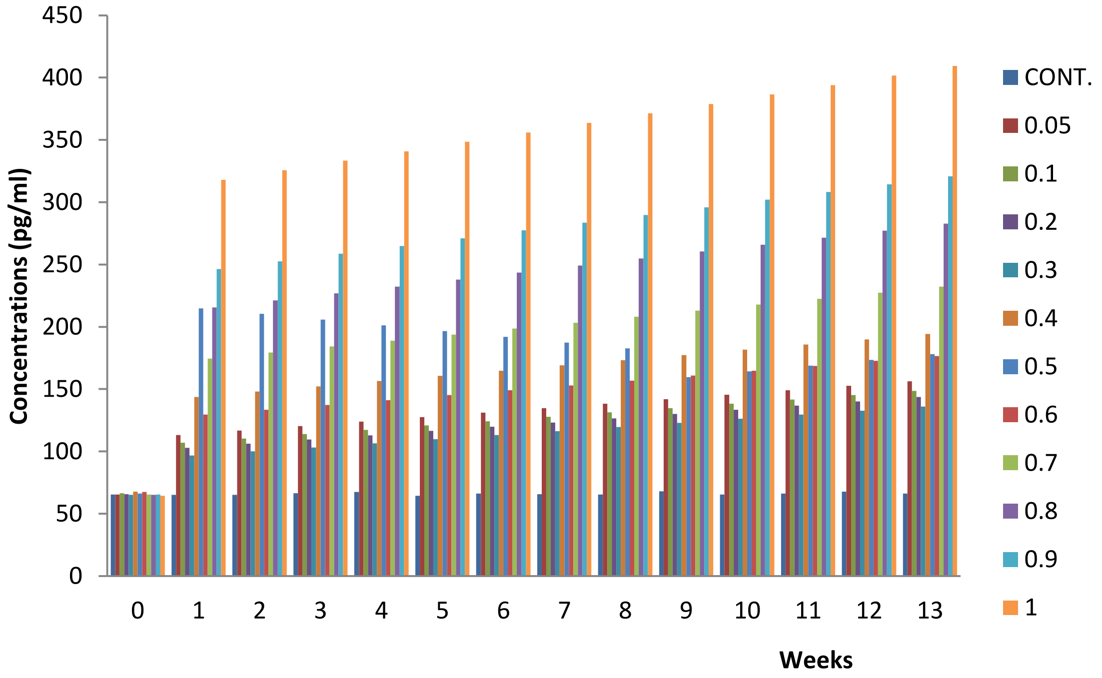

Fig. 46a; Bar chart of concentration against weeks (durations) for interleukin-10 level.

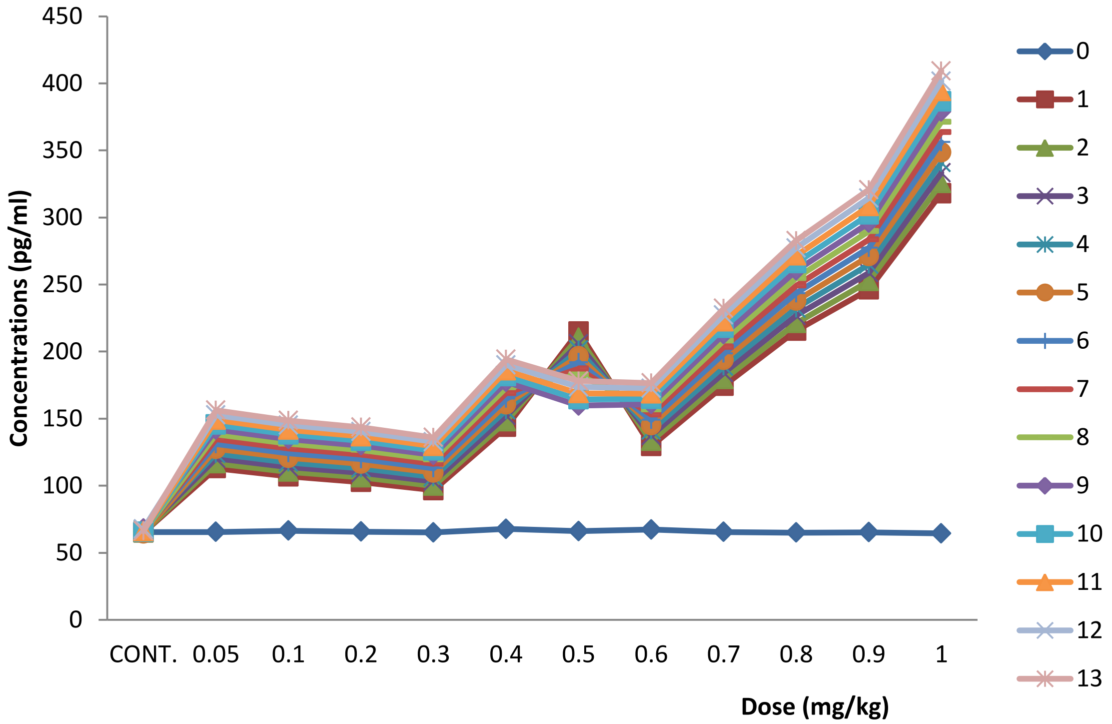

Fig. 46b; Graph of concentration against dose for interleukin-10 level.

- **EFFECT ON INTERLEUKINS IL-11**

There was significant increase in the interleukin-11 level when compared with the control at $p \leq 0.05$ (fig 47a). Throughout the exposure to BPA, the entire exposure groups showed the pattern of response (fig. 47a); In all instances, the test groups exposed to 0.05mg/kg of BPA showed higher interleukin-11 level relative to those of 0.1mg/kg (fig. 47a). The test groups exposed to 0.1mg/kg to 0.5mg/kg and 0.6mg/kg to 1mg/kg, both showed a dose dependent increase in level of interleukin-11, except in weeks 1 to 5, where the test group exposed to 0.1mg/kg showed a higher level of interleukin-11 relative to those 0.2mg/kg (fig.47a).

The weekly profile revealed that at all weeks, there was a decrease in interleukin-11 concentration by the test groups of 0.05mg/kg to 0.1mg/kg over time (fig. 47b). The test group that received 0.2mg/kg of BPA showed no sensitivity to time of exposure (fig. 47b). A dose dependent effect was observed from the test group 0.3mg/kg to 1mg/kg and the concentration of IL-11 at those point increased with time of exposure (fig. 47b).

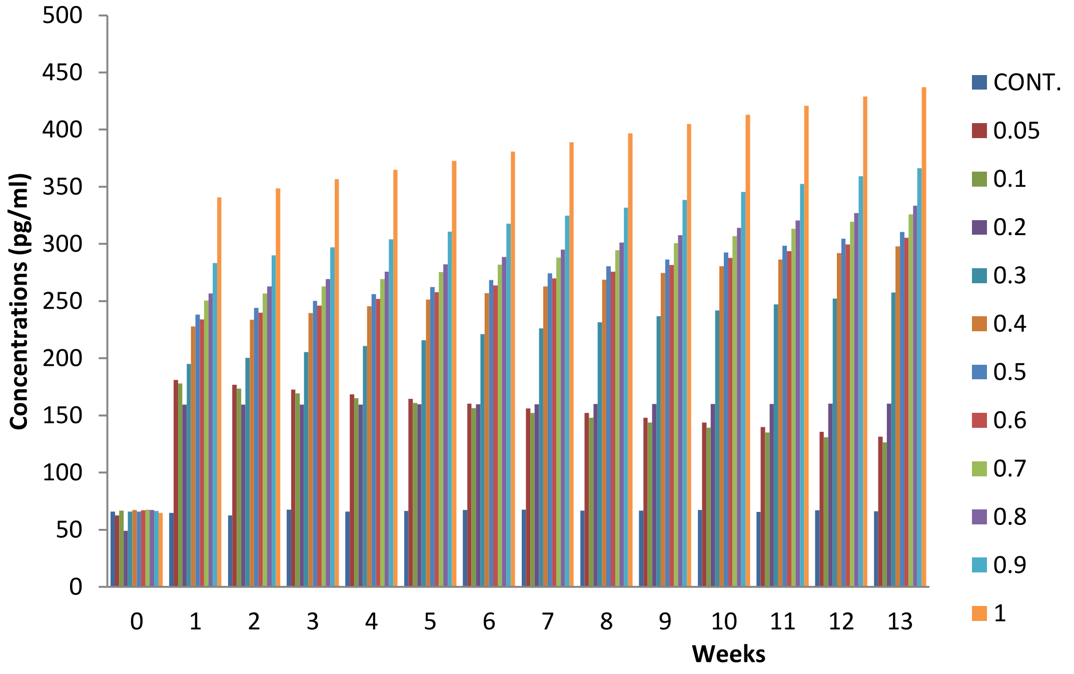

Fig. 47a; Bar chart of concentration against weeks (durations) for interleukin-11 level.

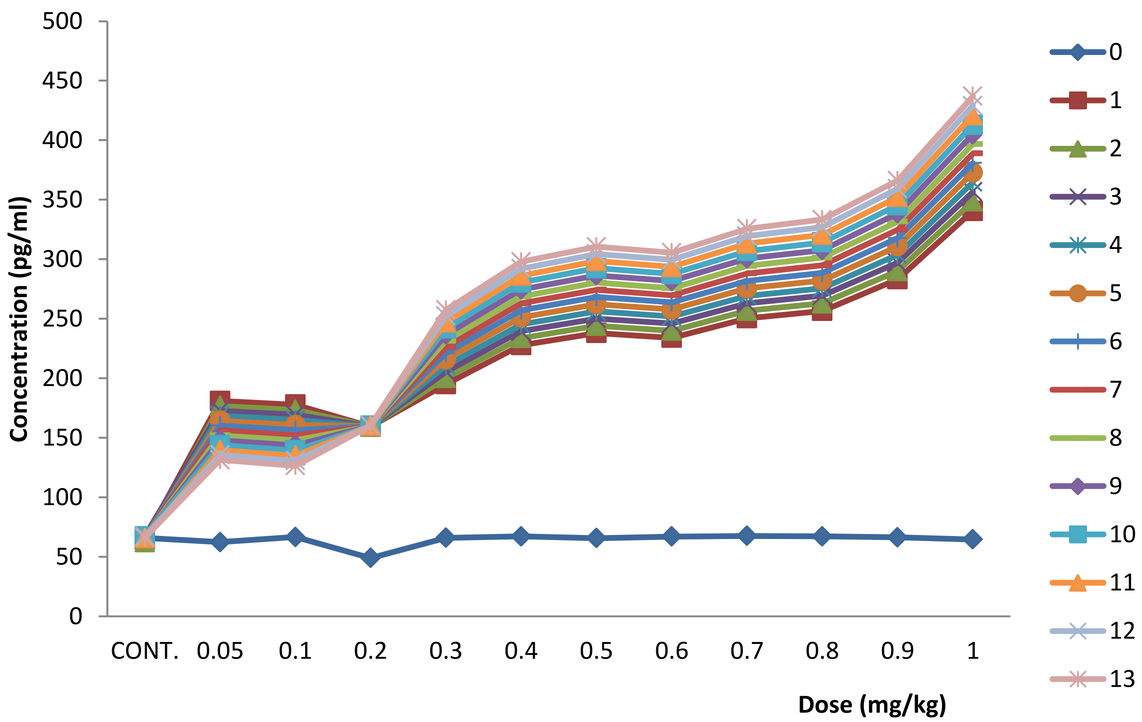

Fig. 47b; Graph of concentration against dose for interleukin-11 level.

- **EFFECT ON INTERLEUKINS IL-12**

There was significant ($p \leq 0.05$) increase in the interleukin-12 level when compared with the control (fig.48a). In all cases and time of exposure, the entire test group showed gradual decreases over time, with their highest effect observed in week 1 and lowest effect in week 13 (fig. 48a). The group that were administered 1mg/kg of BPA gave the maximum effect on IL-12 level throughout the experiment duration (fig. 48a). It was observed that, as the dose groups that were exposed to 0.05mg/kg to 0.6mg/kg showed consistent dose dependent decrease in IL-12 level (fig. 48a), those that received 0.7mg/kg to 1mg/kg of BPA showed consistent dose dependent increase in serum level of IL-12 (fig.48a). There was an initial steep rise at group 1 (0.05mg/kg), this was followed by a slight gradual fall in IL-12 level as the treatment was sustained up to group 7 (0.6mg/kg) (fig. 48b); then there was an increase which was steady and gradual with time of exposure sustained and the least effect of BPA treatment on IL-12 level was observed at Group 7 (0.6mg/kg) (fig 48b). The concentration of interleukin-12 decreased with the duration of exposure (fig. 48a and 48b).

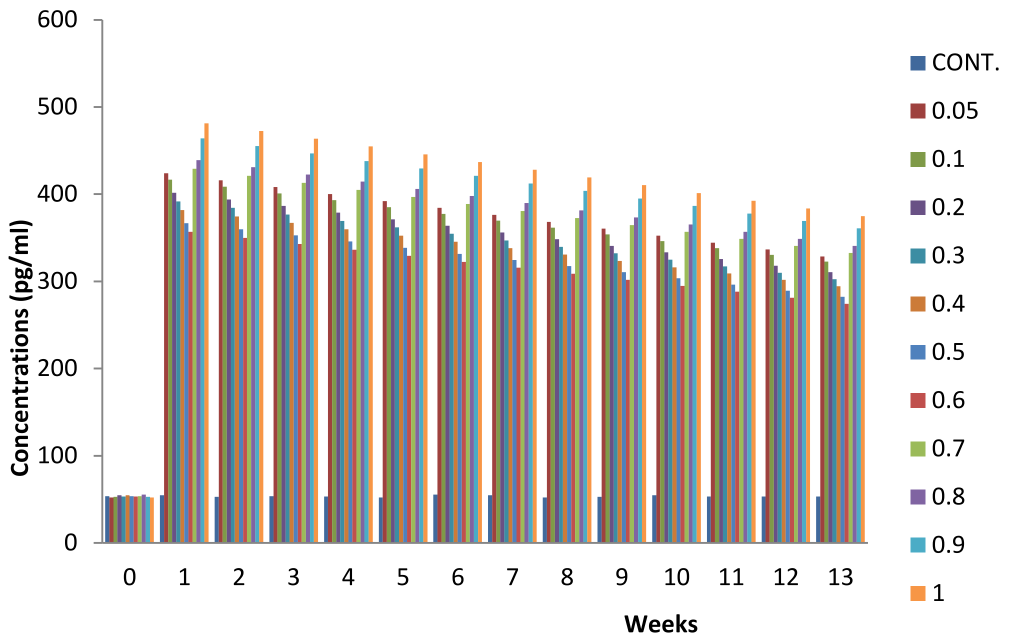

Fig. 48a; Bar chart of concentration against weeks (durations) for interleukin-12 level.

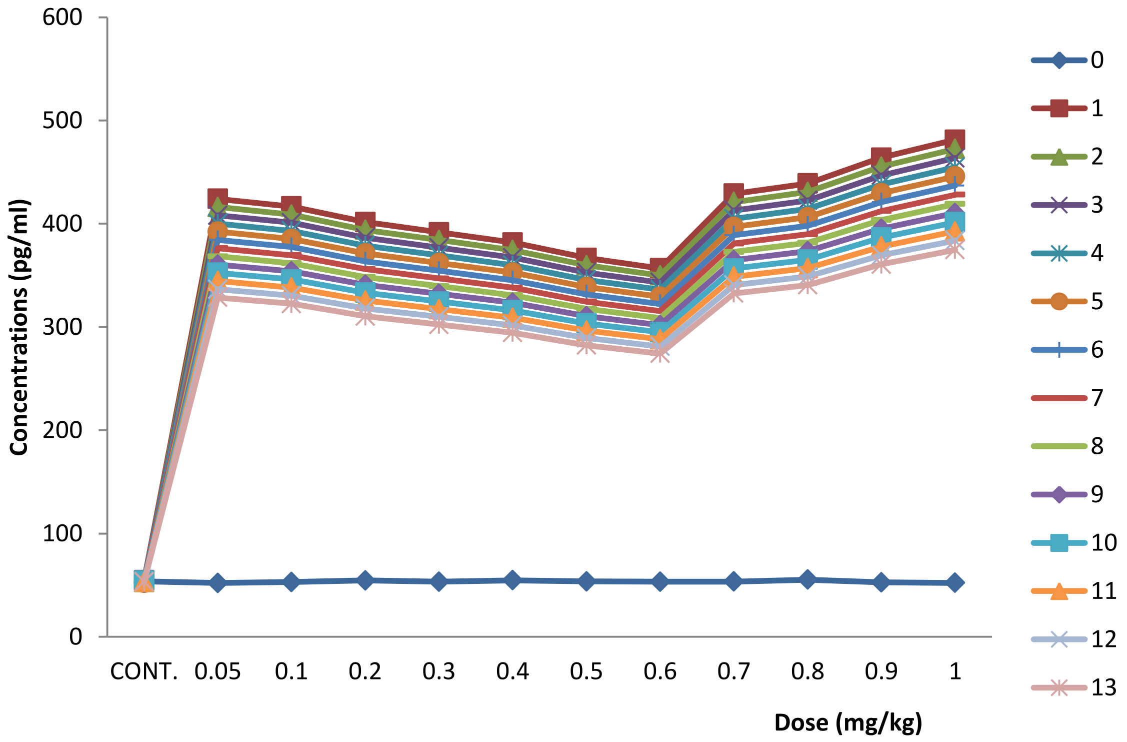

Fig. 48b; Graph of concentration against dose for interleukin-12 level.

- **EFFECT ON INTERLEUKINS IL-13**

There was significant increase in the interleukin-13 level when compared with the control at $p \leq 0.05$ (see fig.49a). The study revealed that the test groups exposed to0.05mg/kg of BPA had lower level of interleukin-13 relative to those of 0.1mg/kg (fig. 49a). There was dose dependent decrease revealed by the test group exposed to 0.1mg/kg to 0.5mg/kg of BPA at weeks 1 to 3 (fig. 49a). The test groups that were exposed to 0.6mg/kg to 1mg/kg of BPA revealed dose dependent increases in serum interleukin-13 level throughout the exposure time (fig.49a). The concentration of interleukin-13 decreases with exposure time (fig.49b). The exposure to graded doses of BPA showed time sensitivity (fig. 49b); the exposed doses of 0.1mg/kg and 0.5mg/kg showed mild sensitivity to time, while those of 0.6mg/kg to 1mg/kg showed a steady, consistent and gradual increase (fig. 49b).

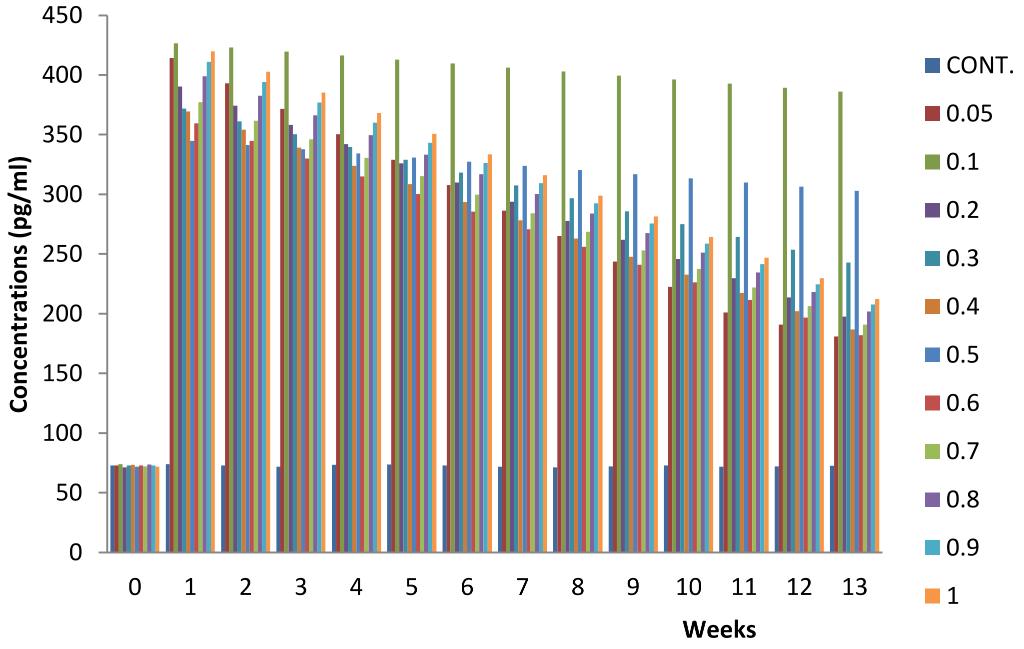

Fig.49a; Bar chart of concentration against weeks (durations) for interleukin-13 level.

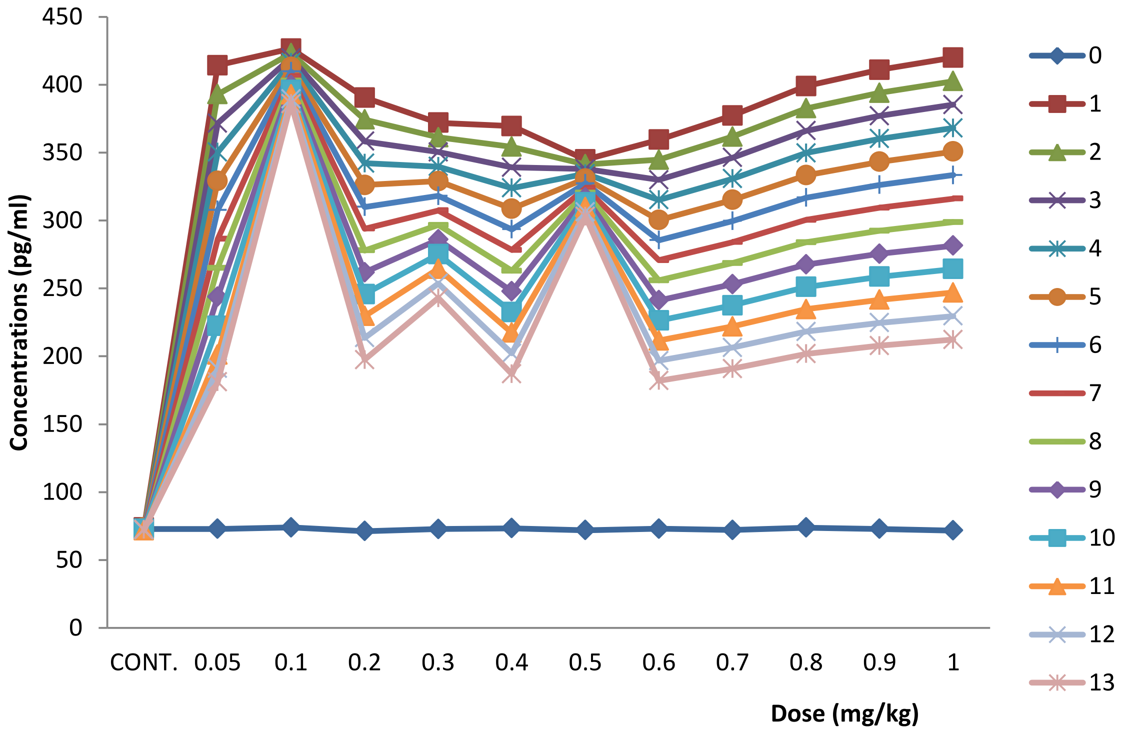

Fig.49b; Graph of concentration against dose for interleukin-13 level.

- **EFFECT ON INTERLEUKINS IL-14**

There was significant increase in the interleukin-14 level when compared with the control at $p \leq 0.05$. The first week recorded the highest effect of BPA on IL-14 level,which consistently decreased through from week 13 where the least effect was observed (fig.50a). The BPA treatment group 11 exhibit the more pronounced effect on IL-14 level across all the group, while group 1(0.05mg/kg) showed the minimal effect (fig.50b). In all instances, the entire exposure doses showed dose dependent increase iin serum IL-14 level with the dose group of 1mg/kg of BPA triggering the highest (maximum) effect after BPA exposure (fig.50a). It was observed that as the duration of exposure increases, there was a consistent decrease in IL-14 level throughout the study, with week 1 exhibiting the highest concentration of of IL-14 and week 13 the lowest IL-14 level (fig. 50a and 50b). The change in interleukin-14 showed sensitivity to time of exposure (fig 50b), which was gradual and consistent.

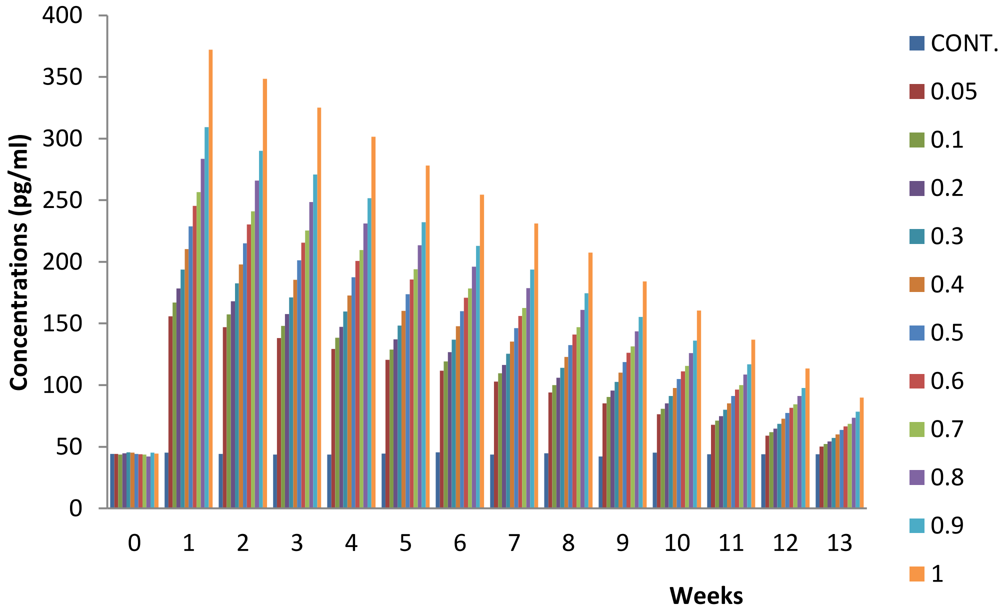

Fig. 50a; Bar chart of concentration against weeks (durations) for interleukin-14 level.

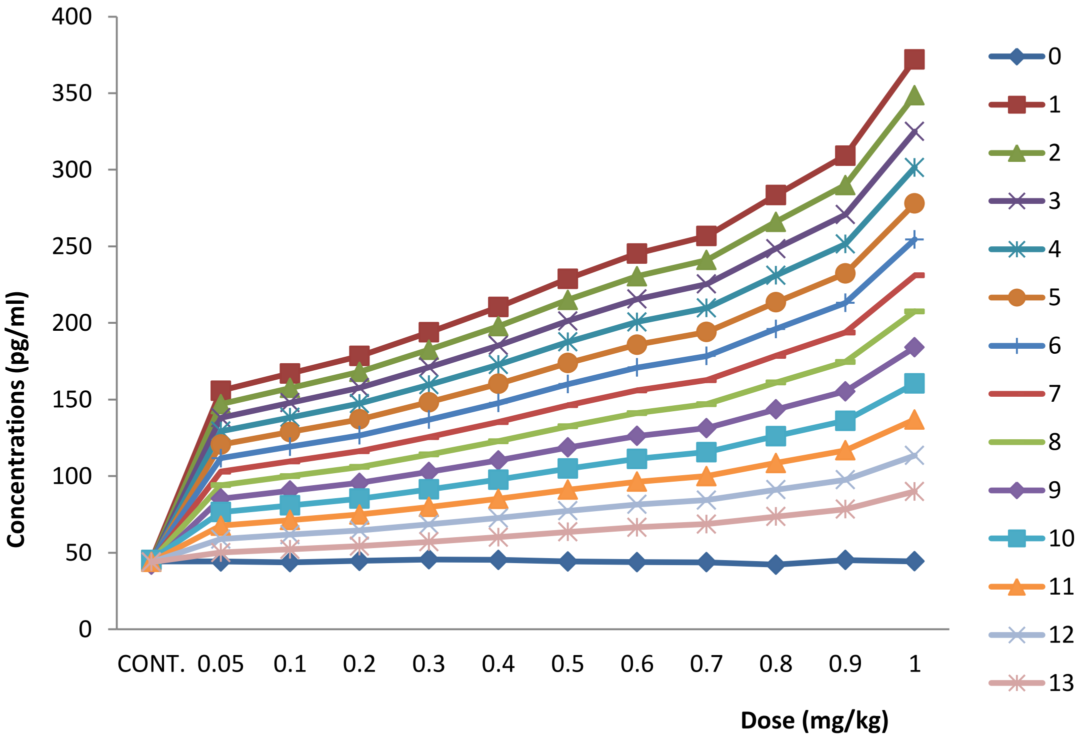

Fig. 50b; Graph of concentration against dose for interleukin-14 level.

- **EFFECT ON INTERLEUKINS IL-15**

There was significant increase in the interleukin-15 level when compared with the control at p≤0.05 (fig 51a). The interleukin-15 result revealed that BPA at 0.5mg/kg to 1mg/kg trigered dose dependent increases in the serum concentration of interleukin-15 throughout the study weeks (fig. 51a) except that the interleukin-15 level showed by 0.8mg/kg BPA test group decreased relative to those of 0.9mg/kg test group (fig. 51a). The group exposed to 0.8mg/kg decreased over time, with its highest effect at week 1 and its least effect at week 13 (fig. 51a and 51b). The dose group that were administered 0.05mg/kg to 0.2mg/kg showed a dose dependent increase throughout the experiment duration except at the third (3^{rd}) week of the study where the test group 0.1mg/kg decreased relative to those of 0.2mg/kg (fig. 51a).

The group exposed to 0.05mg/kg to 0.7mg/kg showed a direct proportional increase with the the time of the study (fig.51b), while those exposed to 0.8mg/kg to 1mg/kg showed an inverse relationship with the time of exposure (fig.51b).

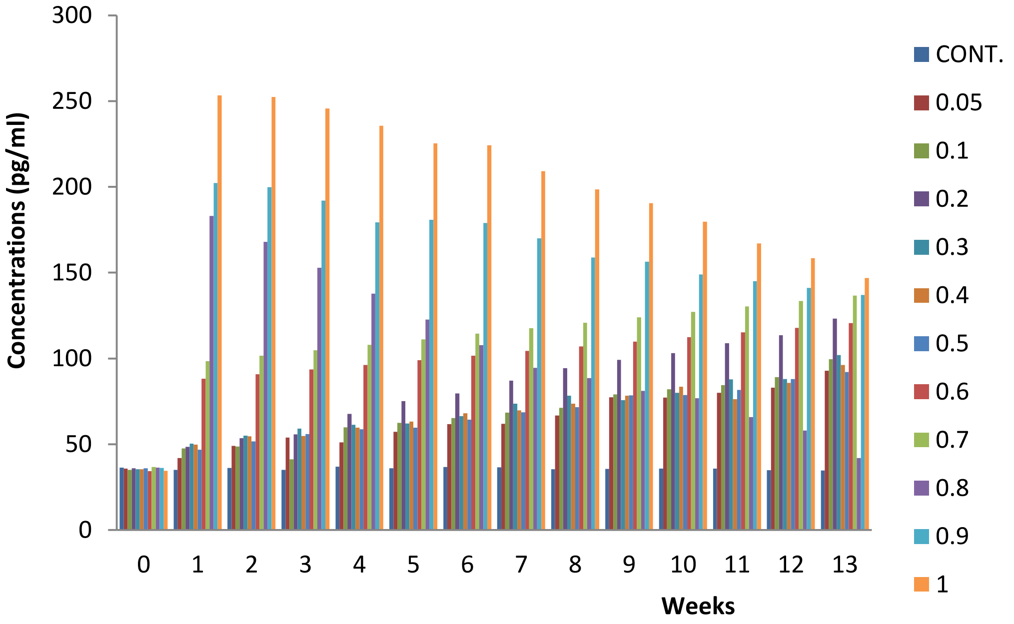

Fig 51a; Bar chart of concentration against weeks (durations) for interleukin-15 level.

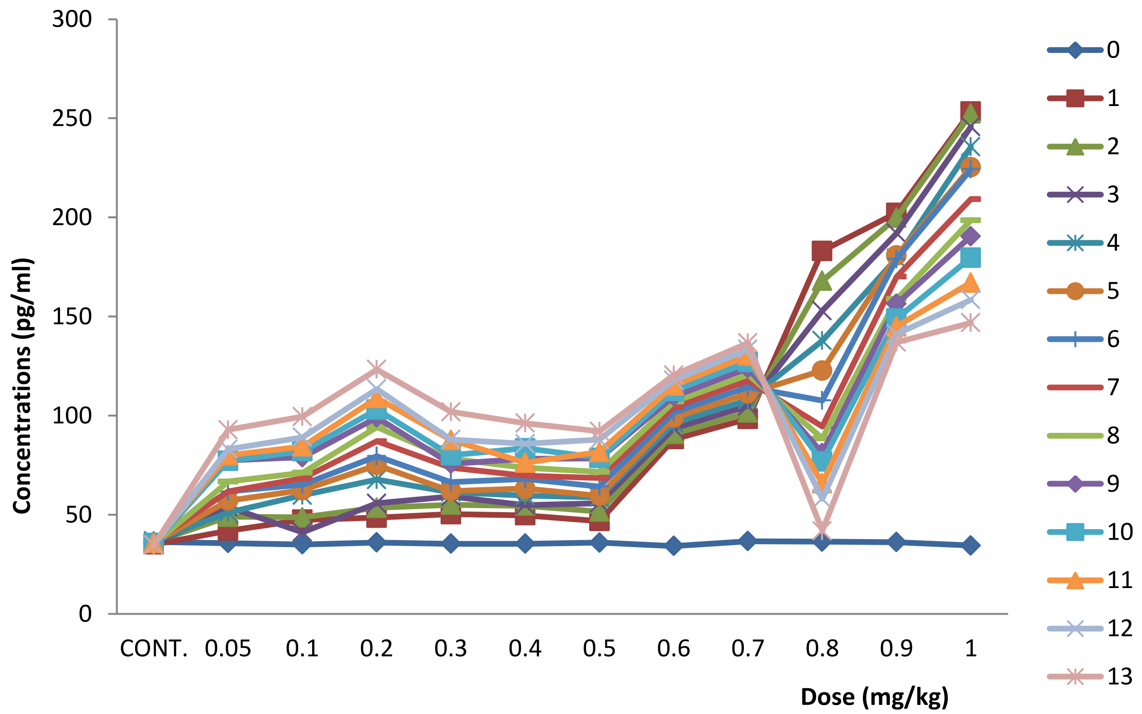

Fig 51b; Graph of concentration against dose for interleukin-15 level.

- **EFFECT ON INTERLEUKINS IL-16**

There was significant increase in the interleukin-16 level when compared with the control at $p \leq 0.05$ (fig 52a). The interleukin-16 result revealed that BPA at 0.4mg/kg to 1mg/kg trigered dose dependent increases in the serum concentration of interleukin-16 throughout the study weeks (fig. 52a) except that the interleukin-16 level showned by 0.8mg/kg BPA test group decreased relative to those of 0.9mg/kg test group at weeks 11 to 13 (fig. 52a). The group exposed to 0.8mg/kg to 1mg/kg decreased over time, with their highest effect at week 1 and least effect at week 13 (fig. 52a and 52b). The groups that were administered 0.05mg/kg to 0.2mg/kg showed a dose dependent increase throughout the experiment duration except at the second (2^{nd}) week of the study where the test group 0.1mg/kg decreased relative to those of 0.2mg/kg (fig. 52a).

The group exposed to 0.05mg/kg to 0.7mg/kg showed a direct proportional increase with the the time of the study (fig.52b), while those exposed to 0.8mg/kg to 1mg/kg showed an inverse relationship with the time of exposure (fig.52b).

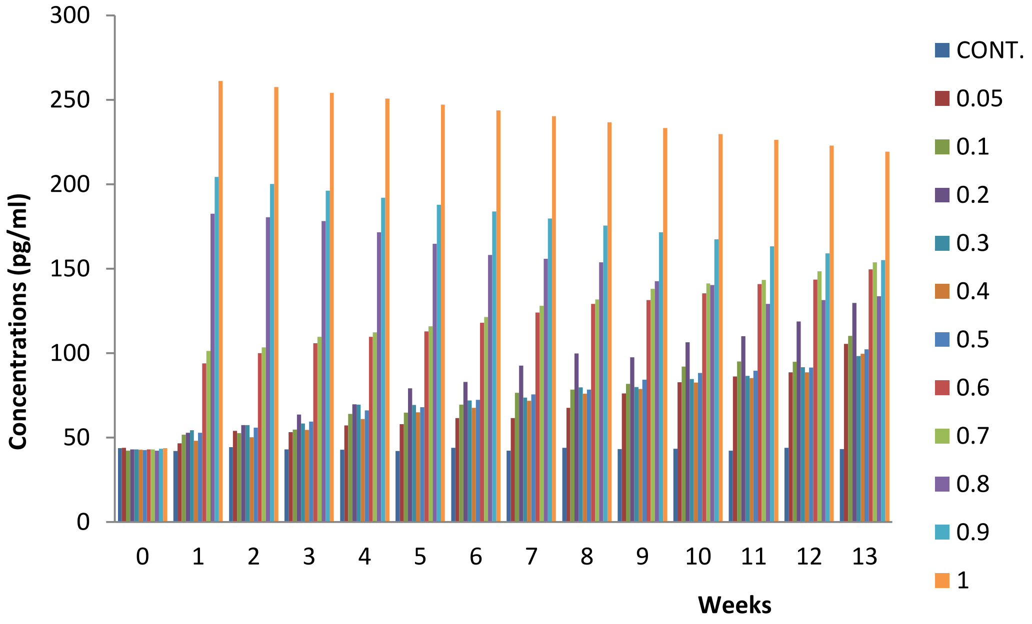

Fig. 52a; Bar chart of concentration against weeks (durations) for interleukin-16 level.

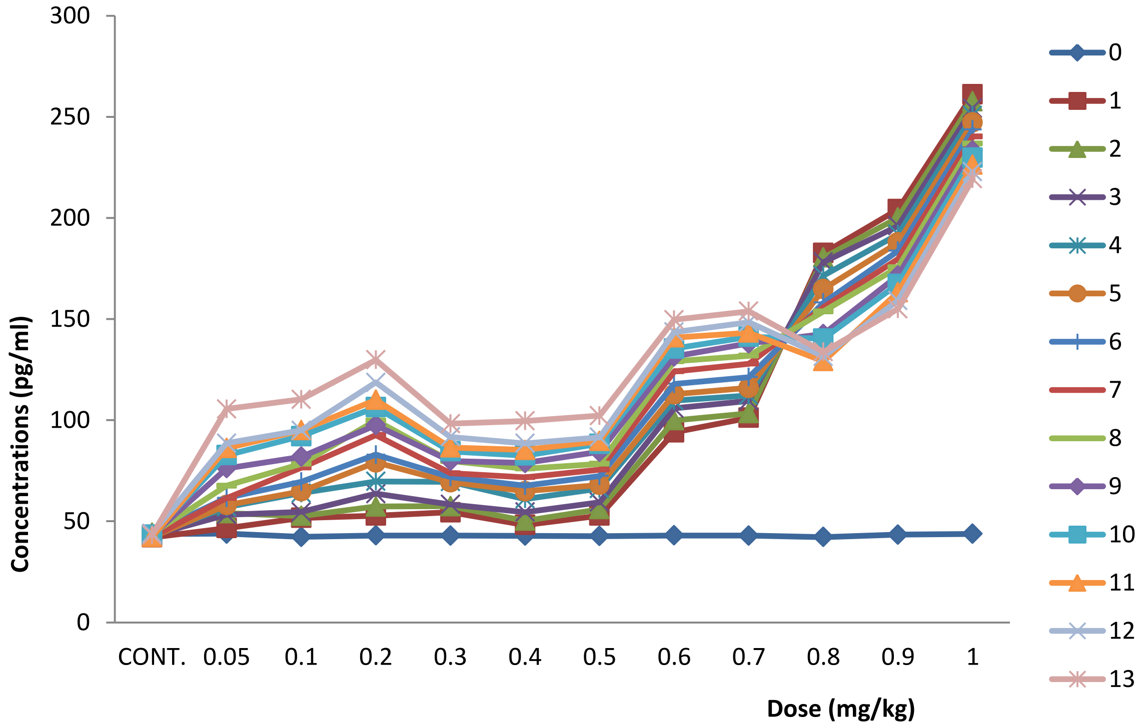

Fig. 52b; Graph of concentration against dose for interleukin-16 level.

- **EFFECT ON INTERLEUKINS IL-17**

There was significant increase in the interleukin-17 level when compared with the control at p≤0.05 (fig. 53a). The established pattern of response observed in fig. 53a revealed that the exposure doses of 0.05mg/kg to 0.3mg/kg and those of 0.5mg/kg to 1mg/kg showed dose dependent increases in the conceatration of interleukin-17, except in week 1 where the test group of 0.5mg/kg showed a slight increase relative to those of 0.6mg/kg (fig.53a). The group exposed to0.4mg/kg of BPA showed a declining effect in interleukin-17 when compared with those of 0.3mg/kg, except at weeks 12 and 13 where they appearedhigher relative to those of 0.3mg/kg (fig.53a). The exposure group that revealed the maximum effect of BPA on interleukin-17 are 1mg/kg, then 0.9mg/kg and 0.8mg/kg (fig. 53a). The concentration of interleukin-17 decreases with time (fig. 53b) and the effect of BPA on IL-17 was sensitive to time except at the test groups 0.2mg/kg and 0.6mg/kg that showed no sensitivity to time (fig. 53b).

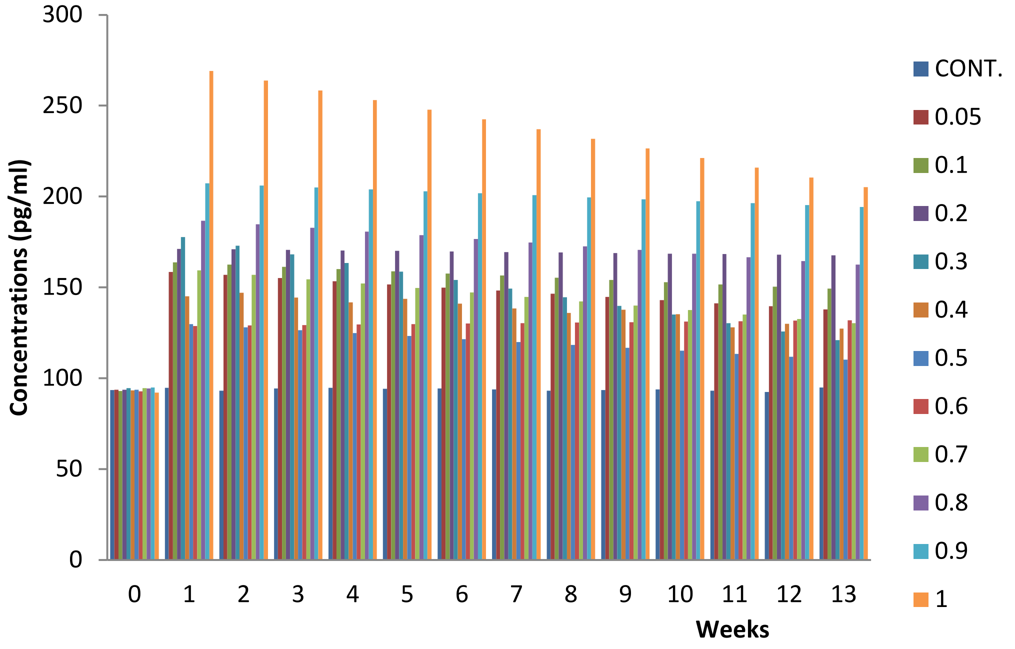

Fig. 53a; Bar chart of concentration against weeks (durations) for interleukin-17 level.

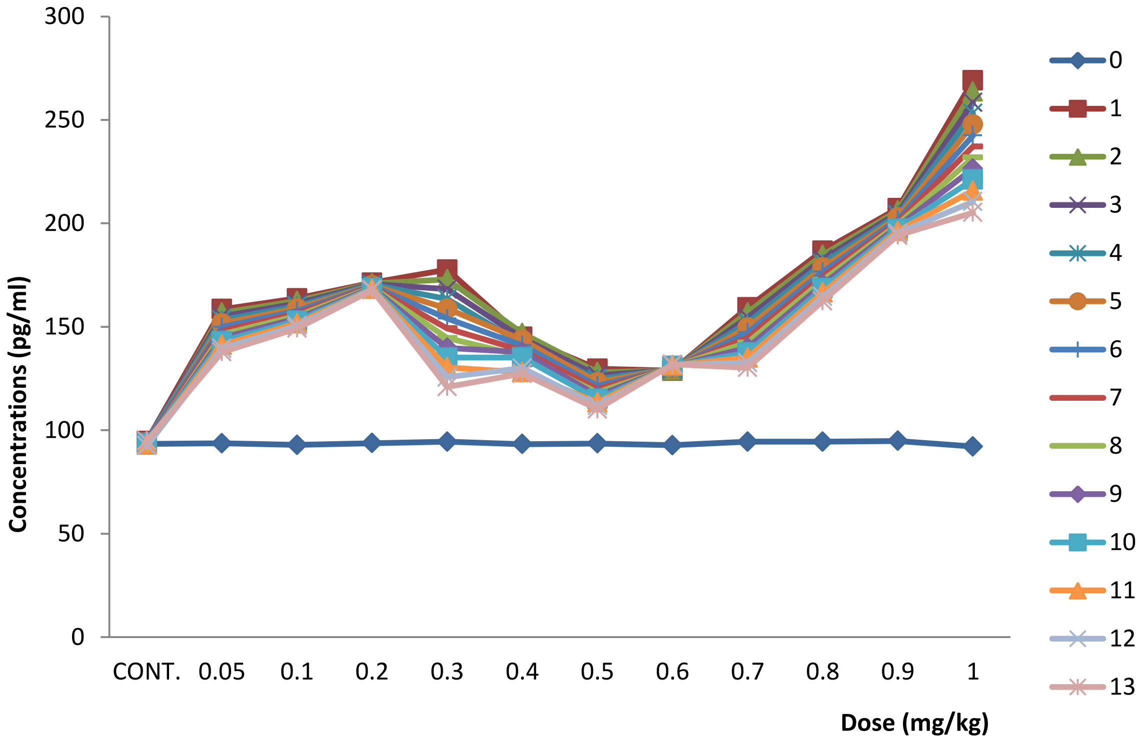

Fig. 53b; Graph of concentration against dose for interleukin-17 level.

- **EFFECT ON INTERLEUKINS IL-18**

There was significant ($p \leq 0.05$) increase in the interleukin-18 level in the BPA treated group when compared with the control (fig 54a).There was a steady gradual increase in BPA-groups 1(0.05mg/kg) to 3(0.2mg/kg); this was followed by a sharp increase in the IL-18 level at group 4(0.3mg/kg); a steady gradual decrease with time was seen from group 5(0.4mg/kg) through to group 8(0.7mg/kg); the IL-18 level then begin to increase again at group 9(0.8mg/kg) to 11(1mg/kg)(fig 54b). BPA tends to trigger the highest level of IL-18 in week 1, and it decreases with time, as week 13 recorded the least levels of IL-18 across all the group(fig.54a). The study revealed that the test group exposed to 0.05mg/kg to 0.3mg/kg of BPA showed consistent dose dependent increase in serum interleukin-18 level throughout the exposure time (fig. 54a). Initially, these increase were higher than that of week 0 and control, but as from weeks 8 to 13, it was observed that the concentration of interleukin-18 gradually fell below those of week 0 and control (fig.54a and 54b). The test groups exposed to 0.4mg/kg to 0.7mg/kg showed a consistent decrease in the interleukin-18 level throughout the study (fig. 54a). The groups that were administered 0.8mg/kg to 1mg/kg showed dose dependent increases in the concentration of IL-18 (fig. 54a). As the time of exposure increases, the concentration of interleukin-18 decreased for the entire test groups (fig. 54a and 54b). The test groups exposed to 0.4mg/kg to 0.7mg/kg showed consistent decreases in the interleukin-18 throughout the study. The groups that were administered 0.8mg/kg to 1mg/kg showed a dose dependent increase (fig. 54a).

As the time of exposure increases, the concentration of interleukin-18 decreases for the entire test group (fig. 54b). The maximum effect of BPA on IL-18 was observed at week 1 and the minimal effect at week 13 (fig.54b). The week 13 of the test group 0.4mg/kg to 0.9mg/kg were less than or equal to that of week 0 (fig. 54b); the week 12 of test group 0.7mg/kg was also equal to that of week 0 (fig.54b)

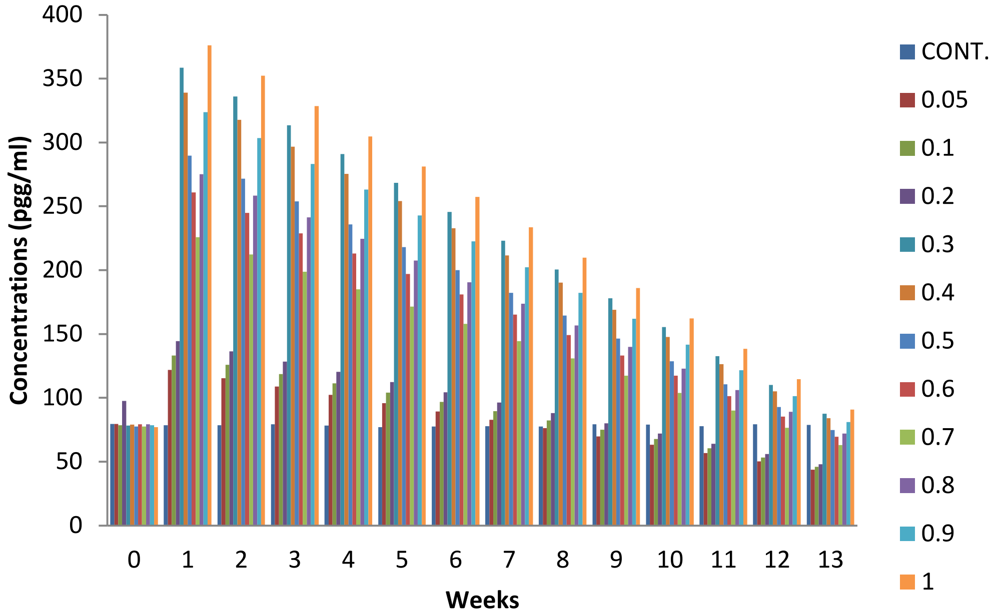

Fig. 54a; Bar chart of concentration against weeks (durations) for interleukin-18 level.

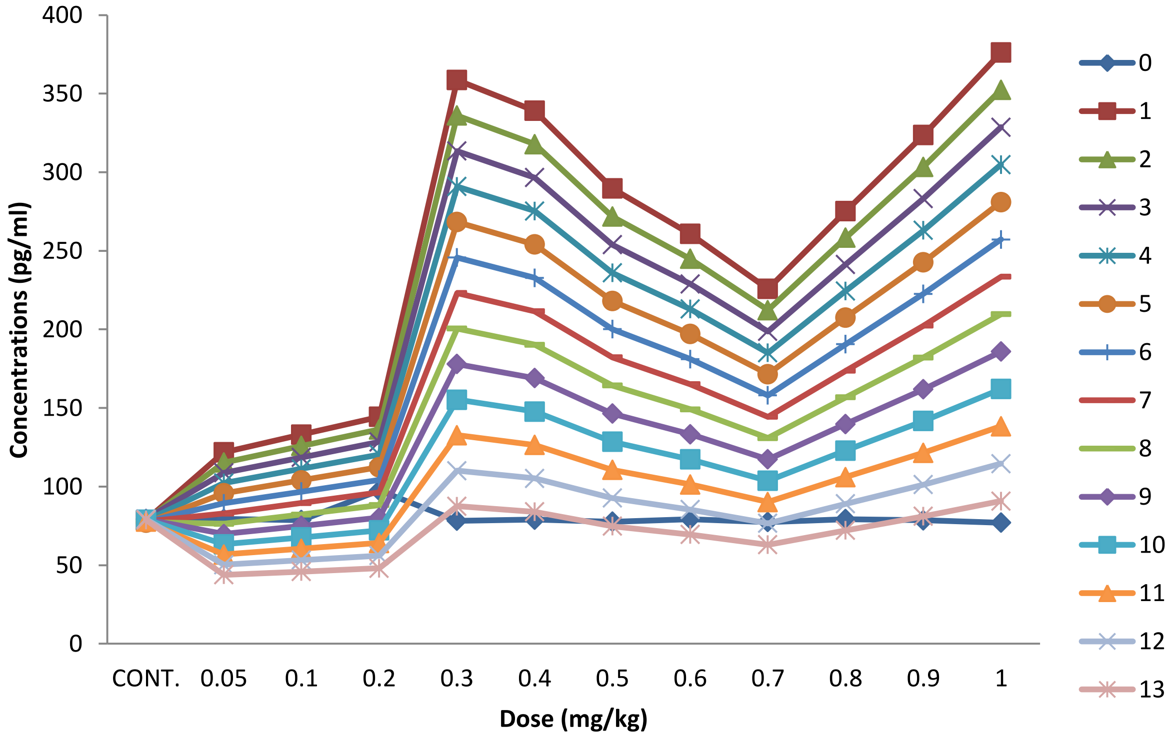

Fig. 54b; Graph of concentration against dose for interleukin-18 level.

- **EFFECT ON INTERLEUKINS IL-21**

There was significant increase in the interleukin-21 level when compared with the control at $p \leq 0.05$ (fig 55a); the increases in IL-21 level was gradual and steady with time (fig. 55a); BPA treated group 6 (0.5mg/kg) gave the maximum effect on the IL-21 level that was peak (fig. 55a and 55b). Afterward, the level of IL-21 tends downhill at a constant and steady manner with time (fig 55b).

The study revealed that there was dose dependent increases in the level of interleukin-21 by the test groups exposed to 0.05mg/kg to 0.5mg/kg of BPA (fig. 55a), with 0.5mg/kg as the peak throughout the duration of the experiment (fig.55a). As from weeks 7 to 13, it was observed that 0.1mg/kg BPA dose group increased interleukin-21 relative to those of 0.2mg/kg (fig. 55a). At weeks 11 to 13, the test group 0.1mg/kg to 0.3mg/kg showed a decrease in the concentration of interleukin-21 (fig. 55a). The groups that were administered 0.6mg/kg to 1mg/kg showed dose dependent decreases in the interleukins-21 level throughout the study time except at weeks 10 to 13, where those of 0.9 and 1mg/kg showed relatively slight increases (fig.55a).

As the duration of exposure increases, the concentration of interleukin-21 increases (fig 55a and 55b). Figure 55b showed that exposure to BPA causes time sensitive interleukin-21 level, all the weeks of exposures peaks at 0.5mg/kg test group (fig. 55b).

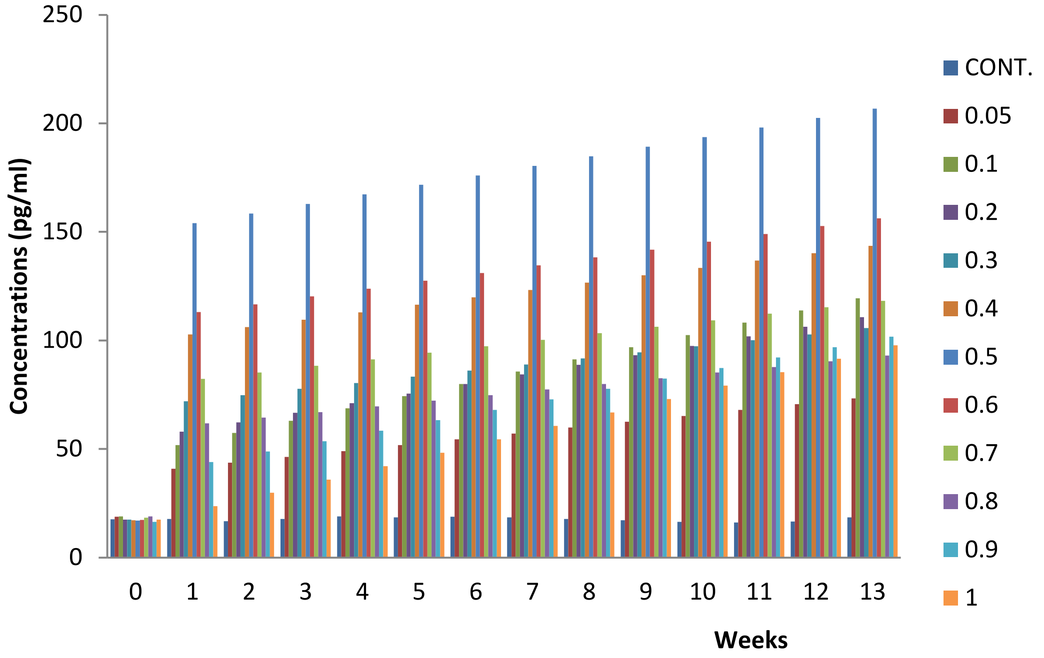

Fig 55a; Bar chart of concentration against weeks (durations) for interleukin-21 level.

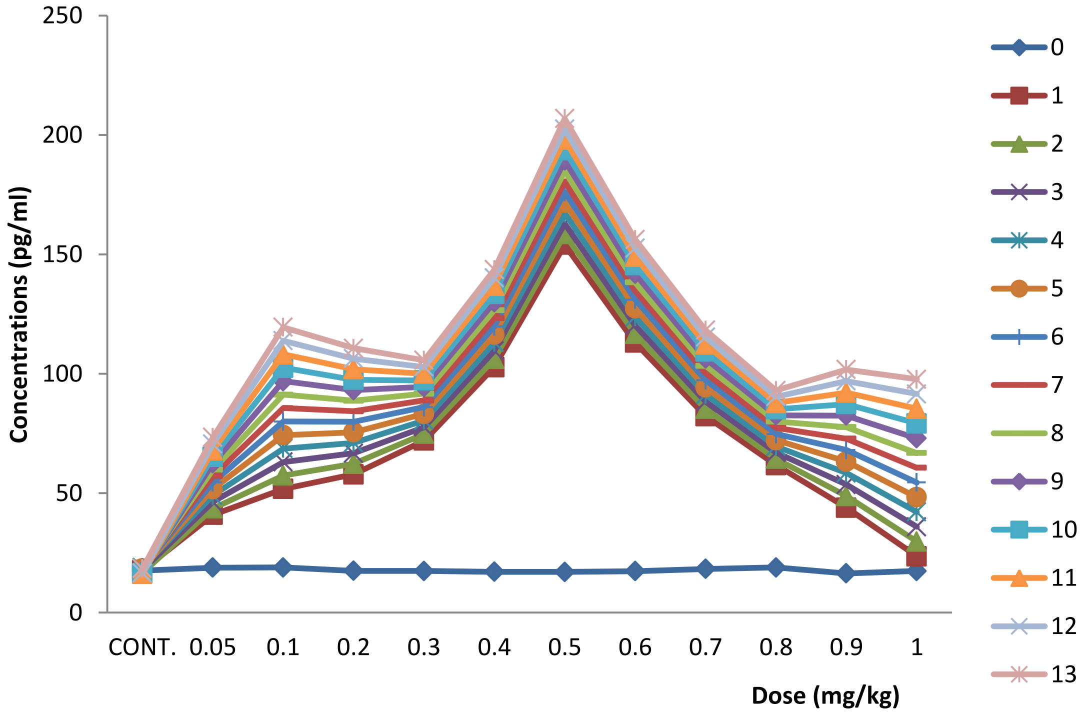

Fig 55b; Graph of concentration against dose for interleukin-21 level.

- **EFFECT ON INTERLEUKINS IL-22**

There was significant increase in the interleukin-22 level when compared with the control at $p \leq 0.05$ (fig.56a). The result of the study showed that the groups exposed to 0.05mg/kg of BPA to 0.3mg/kg of BPA revealed a dose dependent increase but in weeks 1 to 3, the test group of 0.05mg/kg showed higher level of interleukin-22 relative to those of 0.1mg/kg (fig. 56a). The maximum effect of BPA exposure on interleukin-22 was revealed the test group exposed to 0.7mg/kg of BPA (fig. 56a and 56b). As the duration of exposure increases, the concentration of interleukin-22 increases (fig. 56b). There were three (3) peaks established by this result by test group 0.3mg/kg, 0.5mg/kg and 0.7mg/kg (fig. 56b) and the response of interleukin-22 to BPA was time sensitive.

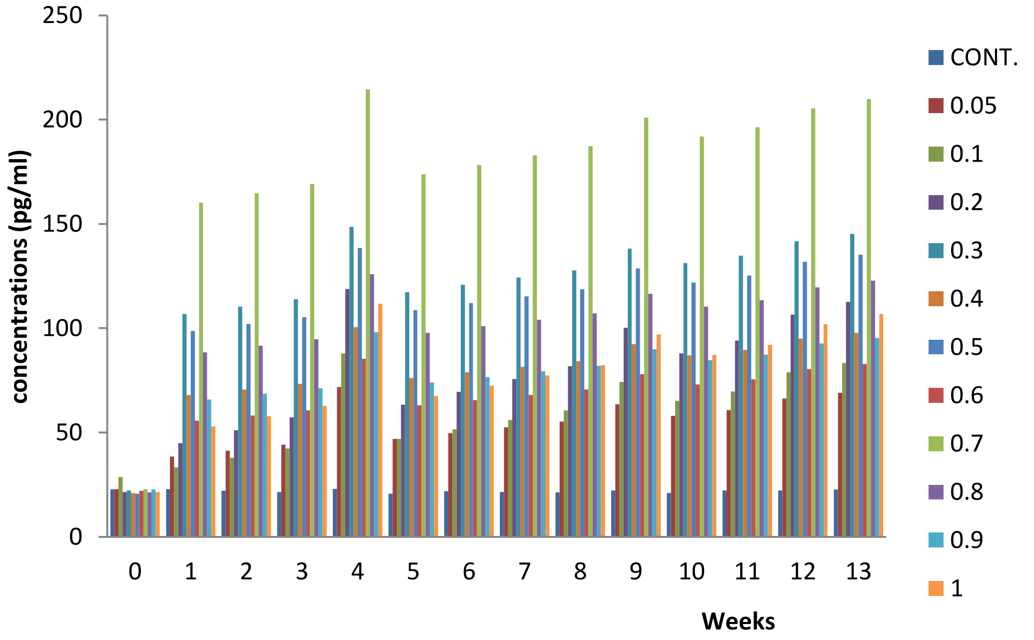

Fig. 56a; Bar chart of concentration against weeks (durations) for interleukin-22 level.

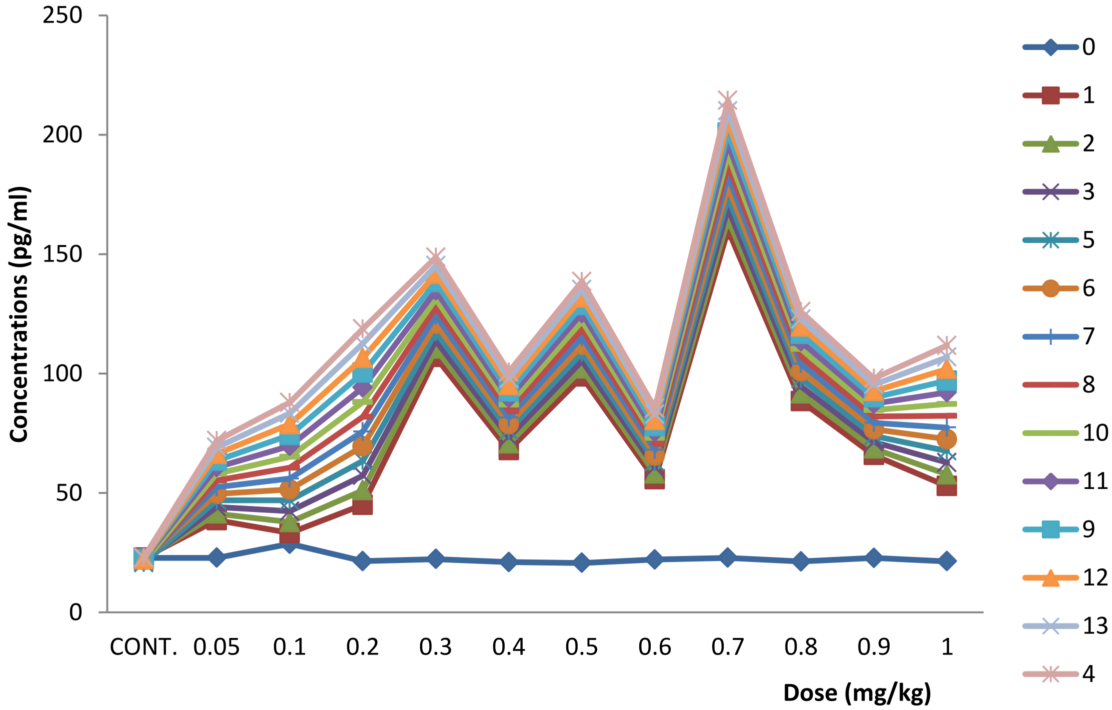

Fig. 56b; Graph of concentration against dose for interleukin-22 level.

- **EFFECT ON INTERLEUKINS IL-23**

There was significant increase in the interleukin-23 level when compared with the control at $p \leq 0.05$(fig 57a). The result of interleukin-23 revealed that during the weeks 1,3-5, 7-9 and 13, the groups that were exposed to 0.05mg/kg to 0.5mg/kg of BPA showed dose dependent increases in serum level of interleukin-23 but weeks 5,7-9 and 13, the test group administered 0.1mg/kg showed increased level of interleukin-23 relative to those exposed to 0.2mg/kg (fig.57a), while the group exposed to 0.6mg/kg to 1mg/kg of BPA showed dose dependent decrease in the serum concentration of interleukin-23 except at week 13 where the test group that received 1mg/kg of BPA showed higher interleukin-23 level relative to that of 0.9mg/kg (fig.57a). At the second week of the study, dose dependent decrease were revealed by the groups that received 0.05mg/kg to 0.5mg/kg and 0.6mg/kg to 1mg/kg (fig.57a). The test group that were exposed to 0.05mg/kg, 0.1mg/kg, 0.5mg/kg and 1mg/kg peaked at weeks 2, 11, 13, and 6 respectively (fig.57b)

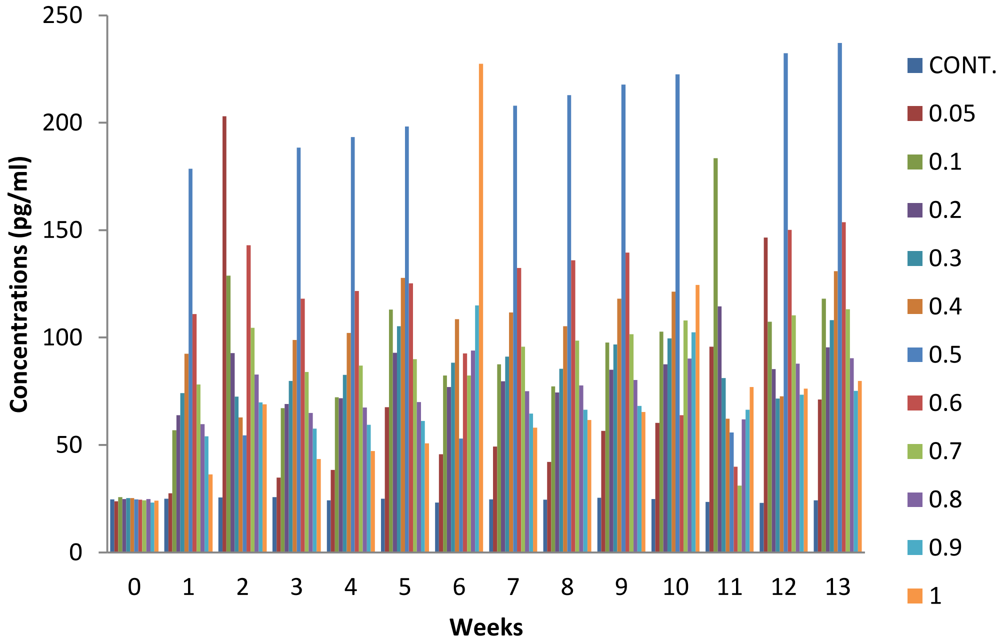

Fig. 57a; Bar chart of concentration against weeks (durations) for interleukin-23 level.

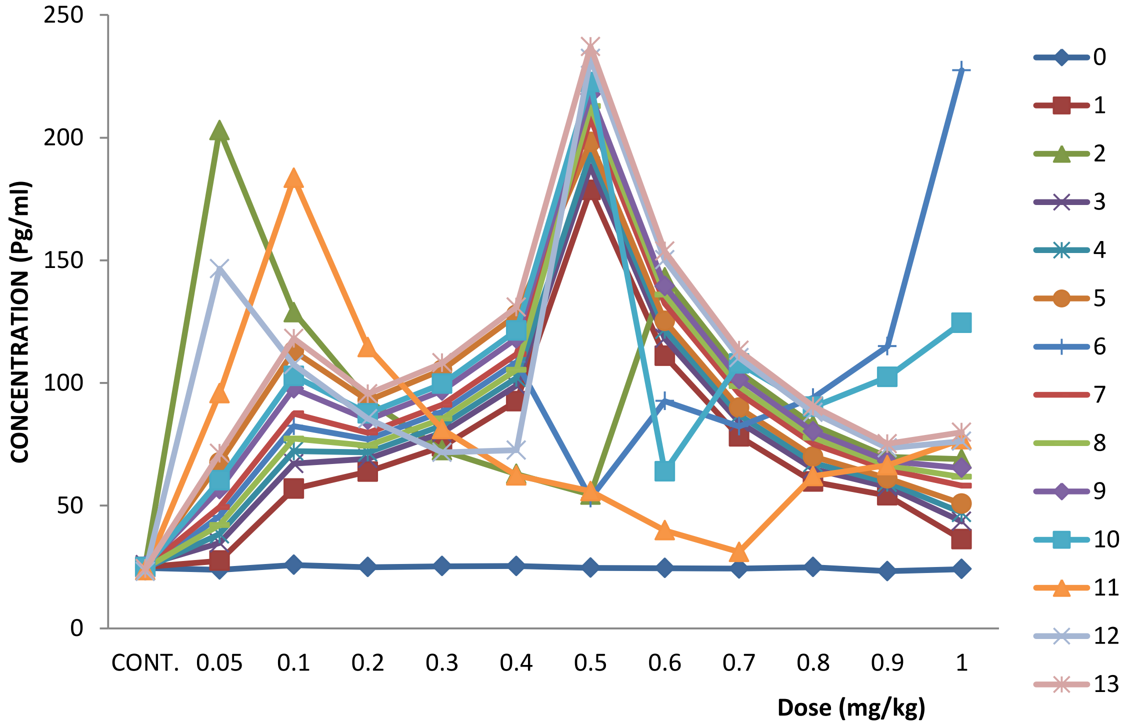

Fig. 57b; Graph of concentration against dose for interleukin-23 level.

- **EFFECT ON INTERLEUKINS 25**

There was an initial gradual and steady significant increase in the interleukin-25 level with time when compared with the control at $p \leq 0.05$ (fig 58a). As the treatment was sustained over a period of duration, it increases to it maximum peak at BPA treatment group 6 (0.5mg/kg); and from there, it starts to decrease constantly but gradually through to group 11 (1mg/kg). As the duration of the study increases, the weekly effect increases (fig 58b).There was a significant increase in the interleukin-25 level when compared with the control.

The study revealed that there was dose dependent increase in the level of interleukin-25 by the test groups exposed to 0.05mg/kg to 0.5mg/kg of BPA (fig. 58a), with 0.5mg/kg as the peak throughout the duration of the experiment (fig.58a). As from weeks 6 to 13, it was observed that 0.05mg/kg BPA dose group increased interleukin-25 relative to those of 0.1mg/kg (fig. 58a). The group that were administered 0.6mg/kg to 1mg/kg showed dose dependent decrease in the interleukins-25 level throughout the study time except in weeks 10 to 13, where those of 0.9mg/kg showed relatively slight increases (fig.58a). As the duration of exposure increases, the concentration of interleukin-25 increases (fig 58a and 58b). Figure 58b showed that exposure to BPA causes time sensitive interleukin-25 level, all the weeks of exposures peaks at 0.5mg/kg test group (fig. 58b).

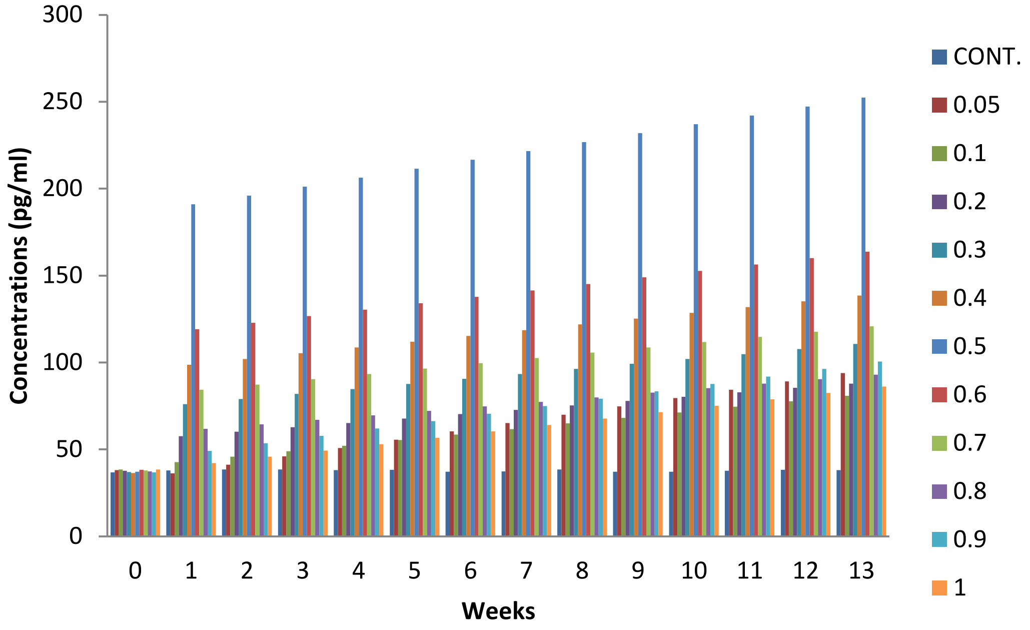

Fig 58a; Bar chart of concentration against weeks (durations) for interleukin-25 level.

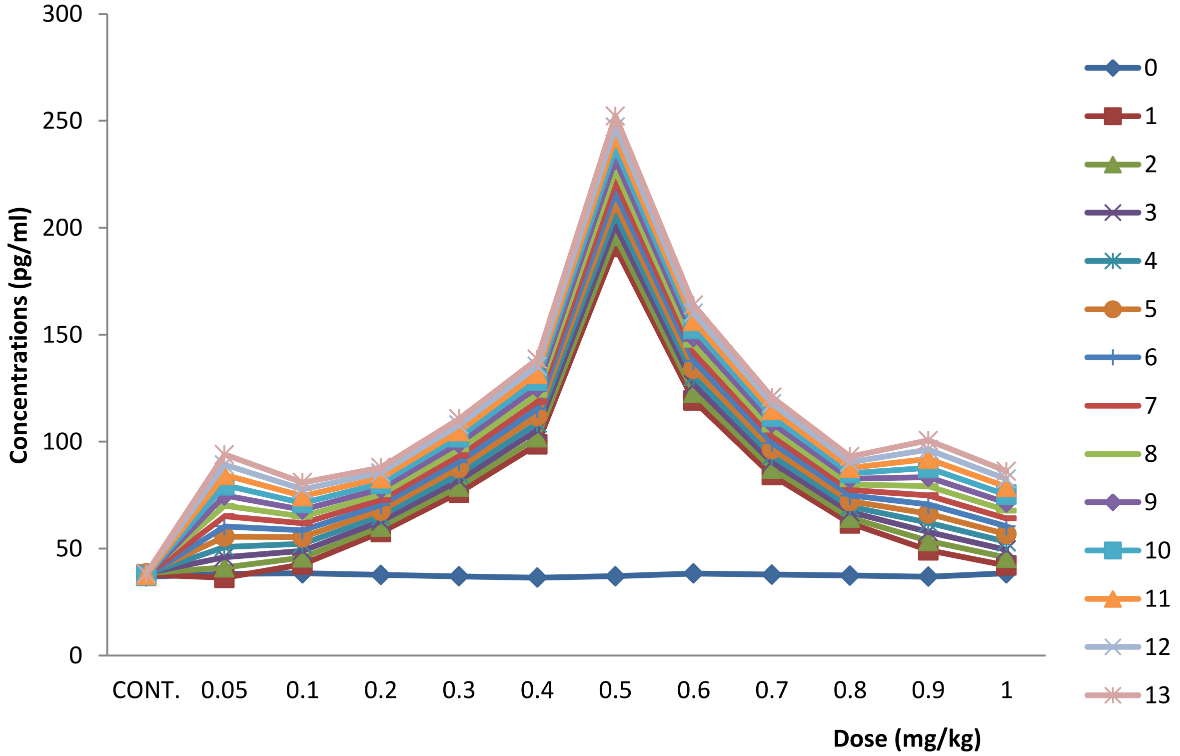

Fig 58b; Graph of concentration against dose for interleukin-25 level.

- **EFFECT ON INTERLEUKINS IL-26**

There was significant increase in the interleukin-26 level when compared with the control at $p \leq 0.05$ (fig 59a). The result of interleukin-26 showed two (2) pattern of response to BPA exposure (fig.59a). The first pattern of response was observed at the first (1st) to seventh (7th) weeks of the study (fig. 59a). The second (2nd) pattern of response at eight (8th) to thirteenth (13th) weeks of exposure (fig. 59a).

In weeks 1 to 7, the entire groups showed similar pattern of response; a dose dependent increase was revealed by the groups that were exposed to 0.05mg/kg to 0.5mg/kg of BPA (fig. 59a), but at weeks 4 to 7, the group that received 0.2mg/kg of BPA showed a decrease in interleukin-26 relative to those of 0.3mg/kg group (fig. 59a). A dose dependent decrease in interleukin-26 level was observed in the groups that were administered 0.8mg/kg to 1mg/kg (fig.59a). Throughout the duration of the study, the groups that were exposed to 0.6mg/kg and 0.7mg/kg showed a dose dependent decrease in interleukin-26 level (fig. 59a).

In weeks 8 to 13, the entire dose group showed similar pattern of response; the groups exposed to 0.05mg/kg to 0.1mg/kg and 0.2mg/kg to 0.4mg/kg, revealed dose dependent increases in interleukin-26 level (fig. 59a). During the period of the study, the test group that received 0.5mg/kg showed a decreased interleukin-26 level relative to those of 0.4mg/kg and 0.6mg/kg (fig. 59a). The test groups of 0.8mg/kg to 1mg/kg of BPA showed dose dependent increases in interleukin-26 except in weeks 8 and 9 where those of 1mg/kg group showed a slight decrease in the level of interleukin-26 relative to those of 0.9mg/kg (fig 59a). The maximum effect of BPA on interleukin-26 was revealed by the test groups that received 0.8mg/kg of BPA and 0.5mg/kg of BPA (fig.59a and 59b), where the prominent peaks were observed (fig. 59b). The 1mg/kg of BPA test group showed a time sensitive and time dependent effect (fig. 59b).

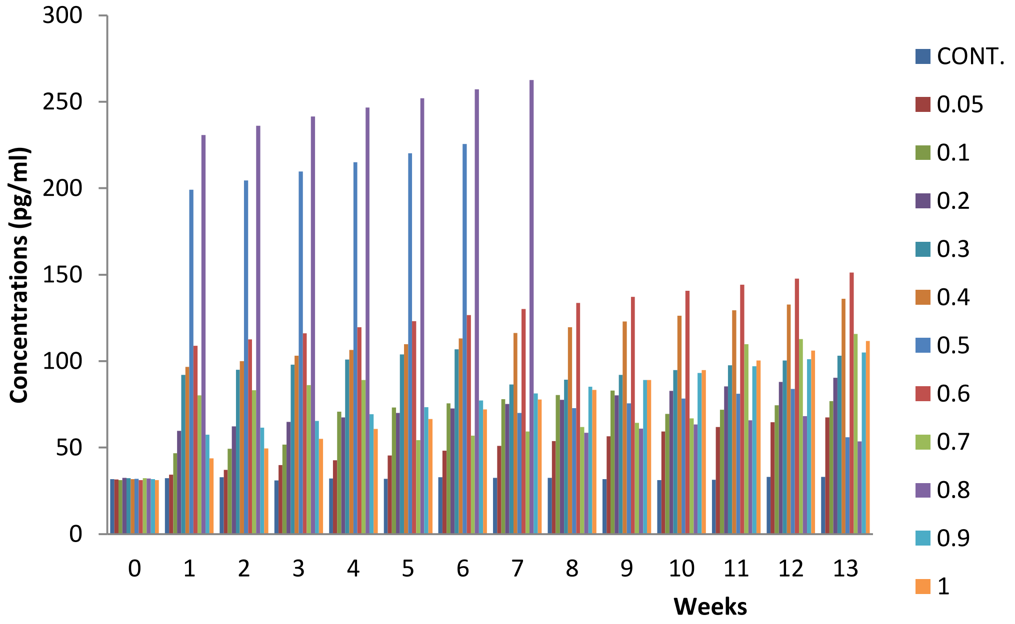

Fig 59a; Bar chart of concentration against weeks (durations) for interleukin-26 level.

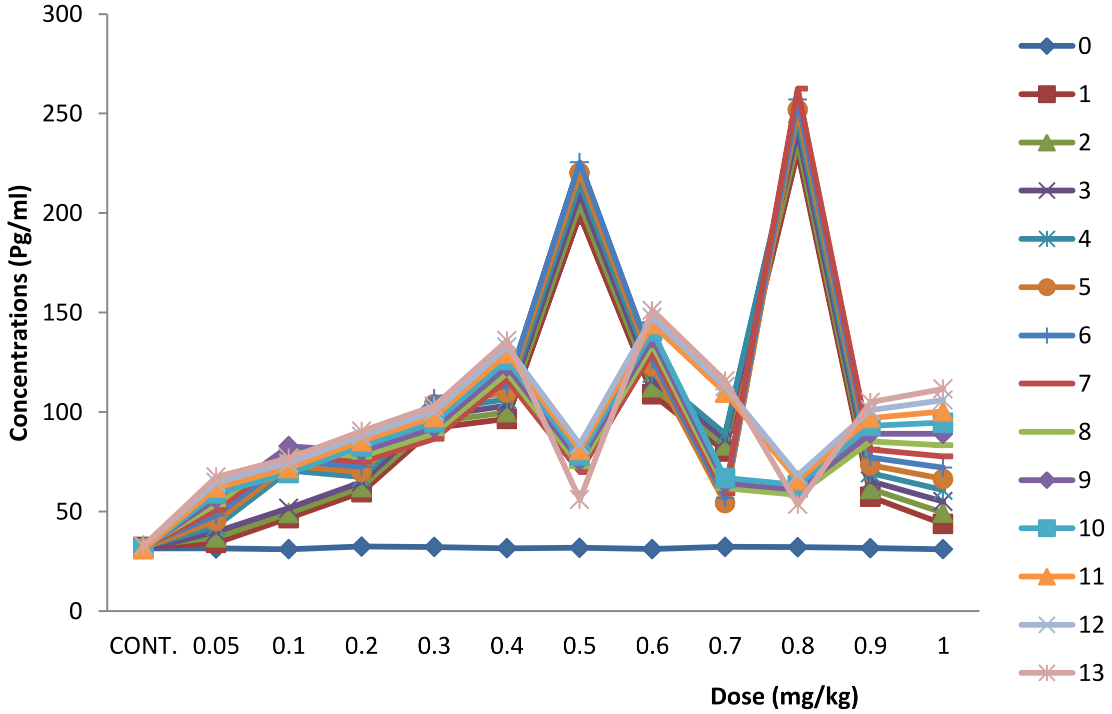

Fig 59b; Graph of concentration against dose for interleukin-26 level.

- **EFFECT ON INTERLEUKINS IL-27**

There were significant increases in the interleukin-27 level when compared with the control at $p \leq 0.05$ (fig 60a). The interleukin-27 result showed no particular response pattern over time (fig. 60a and 60b), it was observed week 7 where the dose group exposed to 0.4mg/kg of BPA showed high level of interleukin-27 (fig. 60a). The group that received 1mg/kg of BPA revealed the maximum effect of BPA on interleukin-27 except at weeks 7 and 11 where 0.4mg/kg and 0.7mg/kg revealed the maximum effect of BPA on interleukin-27 (fig. 60a). The test group exposed to 0.05mg/kg, 0.4mg/kg, 0.6mg/kg, 0.7mg/kg and 1mg/kg of BPA revealed their maximum effect of BPA on interleukin-27 at week 7, week 7, week 13, week 11, and week 13 respectively (fig. 60b).

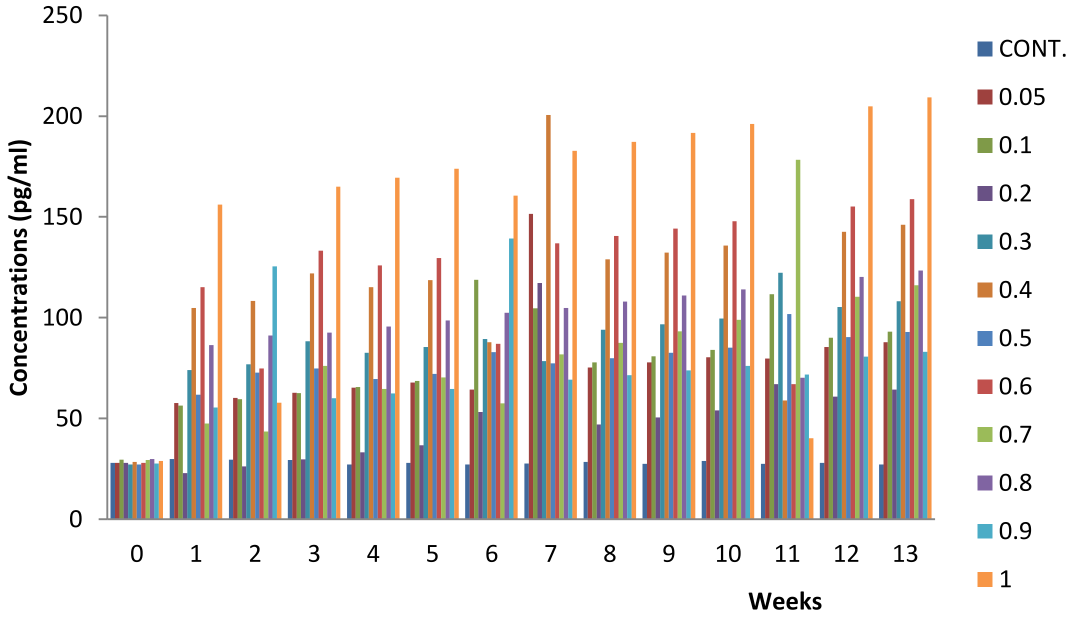

Fig. 60a; Bar chart of concentration against weeks (durations) for interleukin-27 level.

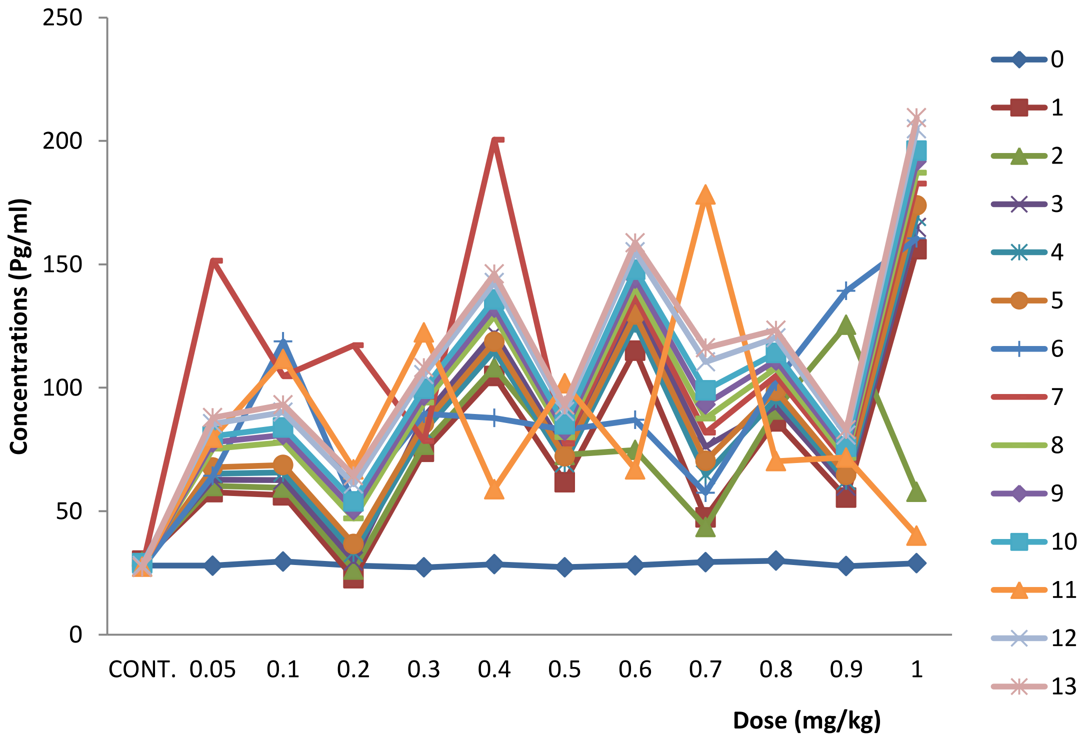

Fig. 60b;Graph of concentration against dose for interleukin-27 level.

- **EFFECT ON INTERLEUKINS 30**

There was significant decrease in the interleukin-30 level when compared with the control at $p \leq 0.05$ (fig 61a), except for group 11(1mg/kg) which showed significant increase in interleukin 30 (fig 61b). The result of interleukin-30 showed an established pattern of behaviour in response to BPA exposure. Throughout the exposure duration, the groups that were exposed to 0.05mg/kg to 0.2mg/kg of BPA showed dose dependent decrease in interleukin-30, then the groups that were exposed to 0.3mg/kg to 0.5mg/kg and 0.7mg/kg to 1mg/kg of BPA showed increased levels of interleukin-30 (fig. 61a). The dose of 0.6mg/kg showed increased level of interleukin-30 relative to those of 0.7mg/kg group throughout the duration of the study (fig. 61a). The maximum effect of BPA on interleukin-30 was revealed by the 1mg/kg dose group (fig.61a and 61b).

In fig.61b, it was revealed that the entire weeks of exposure for the test groups 0.05mg/kg to 0.3mg/kg were below that of the control and week 0, except in weeks 11 to 13 that were slighly above that of control and week 0 (fig. 61b). For the test groups 0.4mg/kg, 0.6mg/kg to 0.8mg/kg, the first six weeks of exposure, the interleukin-30 level were suppressed below that of control and week 0 (fig. 61b). As the time of exposure increases, the concentration of interleukin-30 increases (fig. 61b).

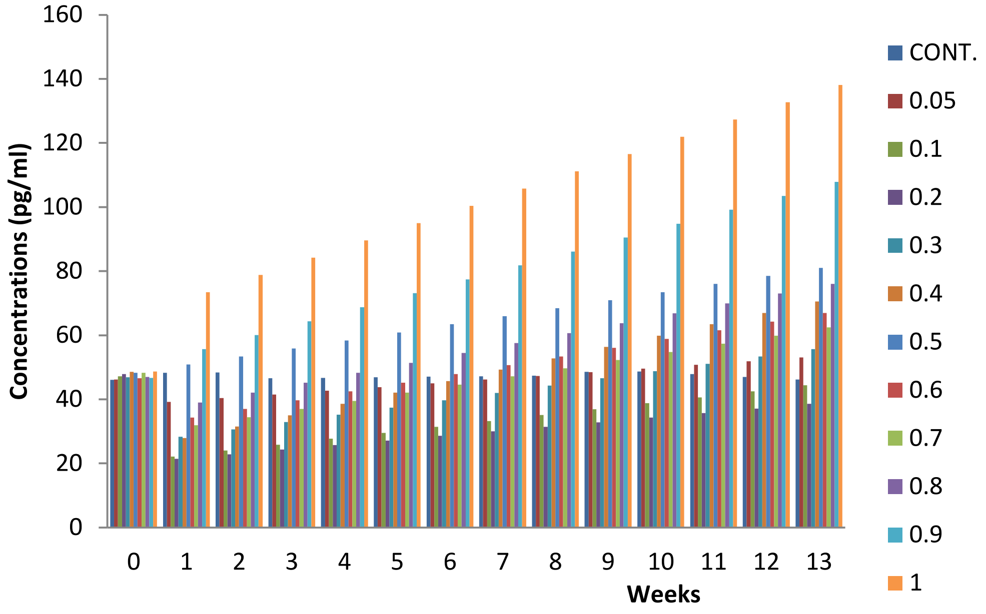

Fig. 61a; Bar chart of concentration against weeks (durations) for interleukin-30 level.

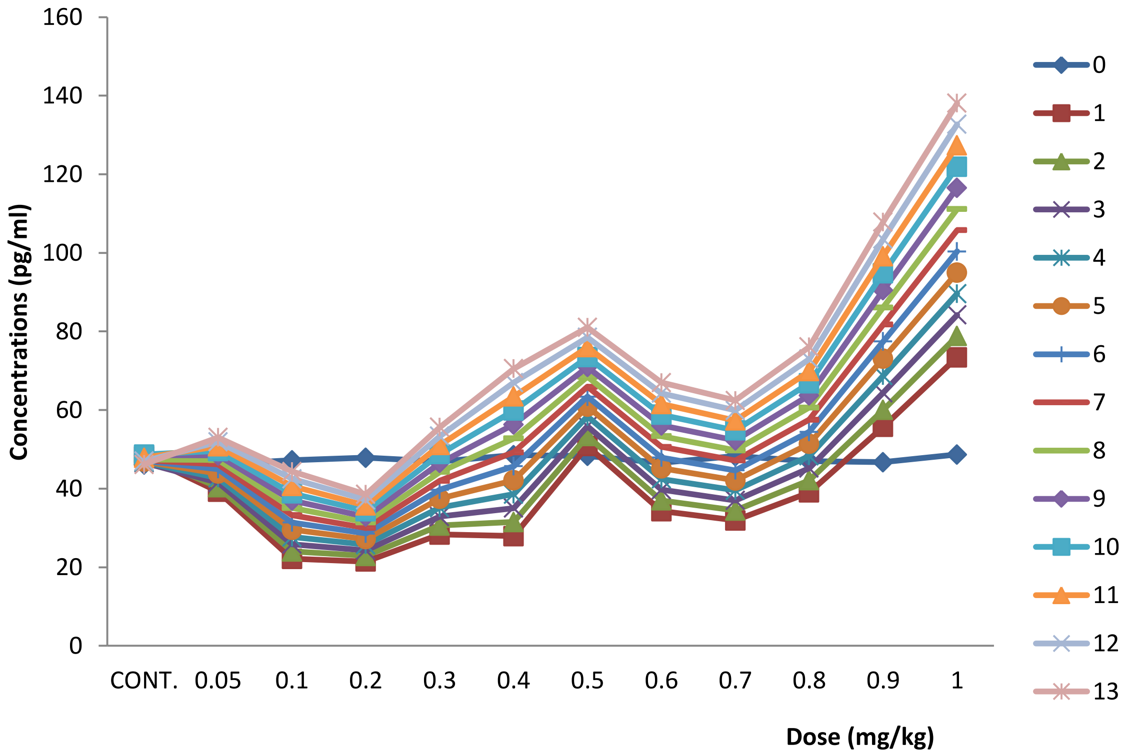

Fig. 61b;Graph of concentration against dose for interleukin-30 level.

- **EFFECT ON INTERLEUKINS IL-31**

There was significant suppression of interleukin-31 level when compared with the control at $p \leq 0.05$ in group 1(0.05mg/kg) – 6(0.5mg/kg) (fig 62a). There was significant ($p \leq 0.05$) sharp increase of interleukins 31 at groups 7 (0.6mg/kg). Groups 8(0.7mg/kg) – 10(0.9mg/kg) showed significant ($p \leq 0.05$) increases in weeks 5 to 13, but group 11(1mg/kg) showed a significant ($p \leq 0.05$) increase in the interleukins(fig 62b). The result of interleukin-31 showed an established pattern of behaviour in response to BPA exposure. Throughout the exposure duration, the groups that were exposed to 0.05mg/kg to 0.2mg/kg showed dose dependent decrease in interleukin-31 except in week 10 where test group of 0.05mg/kg showed a decrease in interleukin-31 relative to that of 0.1mg/kg(fig. 62a), then the groups that were exposed to 0.3mg/kg to 0.6mg/kg and 0.7mg/kg to 1mg/kg of BPA showed dose dependent increase in serum levels of interleukin-31 (fig. 62a). The maximum effect of BPA on interleukin-30 was revealed by the 0.6mg/kg and 1mg/kg dose group (fig.62a and 62b).

In fig.62b, it was revealed that the entire weeks of exposure for the test groups 0.05mg/kg to 0.4mg/kg were below that of the control and week 0, except that weeks 11 to 13 were slighly above that of control and week 0 for the test groups 0.05mg/kg, but weeks 8 to 13 were slightly above that of control for the test groups 0.3mg/kg and 0.4mg/kg (fig. 62b). As the time of exposure increases, the concentration of interleukin-31 increases (fig. 62b).

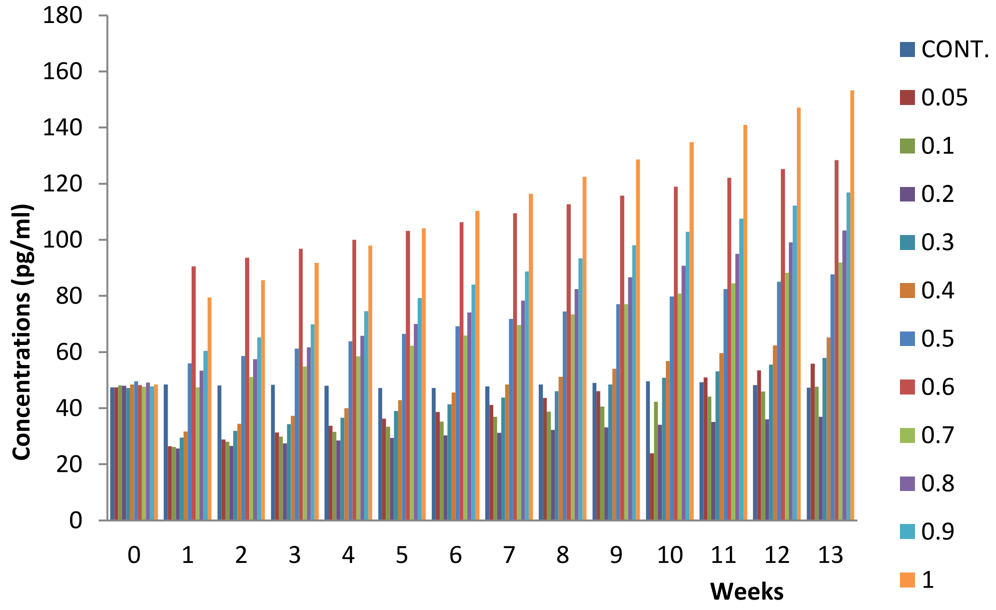

Fig. 62a; Bar chart of concentration against weeks (durations) for interleukin-31 level.

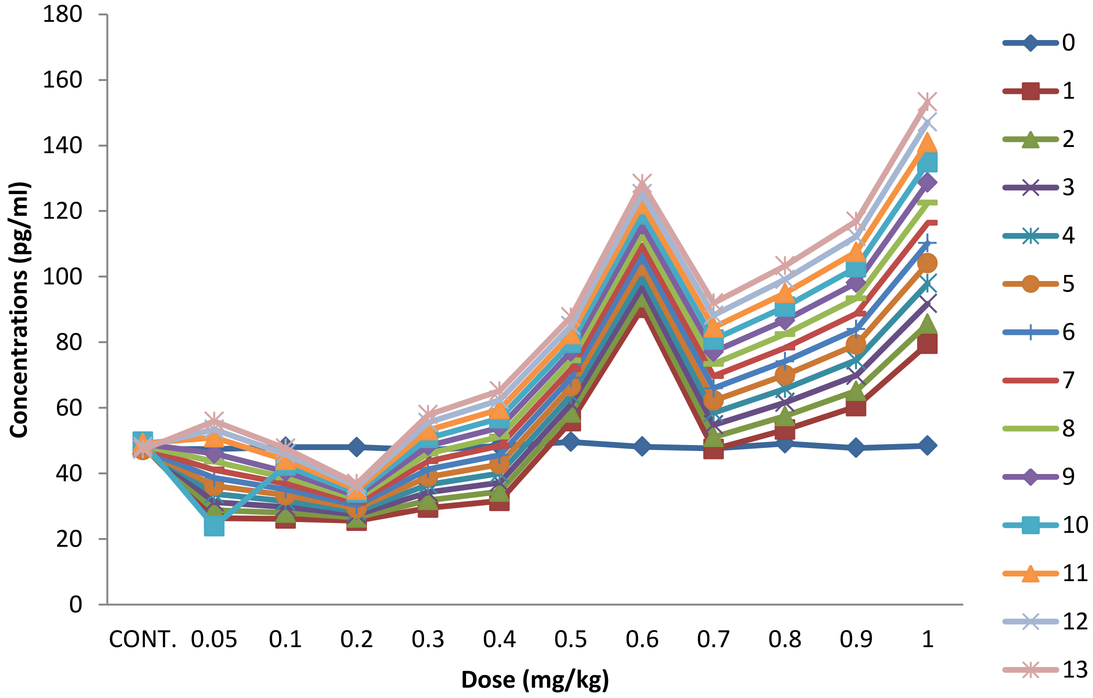

Fig. 62b;Graph of concentration against dose for interleukin-31 level.

- **EFFECT ON INTERLEUKINS IL-32**

There was significant increase in the interleukin-32 level when compared with the control at $p \leq 0.05$ (fig 63a). BPA seems to suppress interleukin 32 at groups 1(0.05mg/kg) to 5(0.4mg/kg); also there was a significant decrease observed at group 3 (0.2mg/kg) (fig 63b). The result of interleukin-32 showed an established pattern of behaviour in response to BPA exposure. Throughout the exposure duration, the groups that were exposed to 0.05mg/kg to 0.2mg/kg showed dose dependent decrease in interleukin-32 except in week 5 where test group of 0.05mg/kg showed a decrease in interleukin-32 relative to that of 0.1mg/kg(fig. 63a), then the groups that were exposed to 0.3mg/kg to 0.7mg/kg and 0.8mg/kg to 1mg/kg of BPA showed dose dependent increase in serum levels of interleukin-32 (fig. 63a). The maximum effect of BPA on interleukin-30 was revealed by the 0.7mg/kg and 1mg/kg dose group (fig.63a and 63b).

In fig.63b, it was revealed that some weeks of exposure for the test groups 0.05mg/kg to 0.4mg/kg were below that of the control and week 0, except that weeks 8 to 13 were slighly above that of control and week 0 for the test groups 0.05mg/kg and 0.3mg/kg, but weeks 5 to 13 were slightly above that of control for the test group 0.4mg/kg (fig. 63b). As the time of exposure increases, the concentration of interleukin-32 increases (fig. 63b).

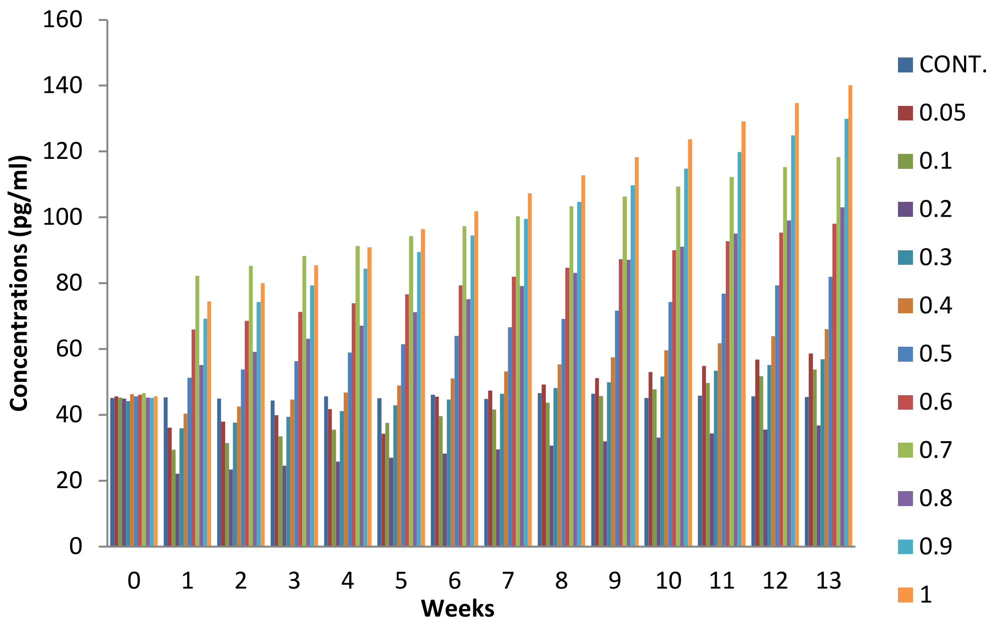

Fig. 63a; Bar chart of concentration against weeks (durations) for interleukin-32 level.

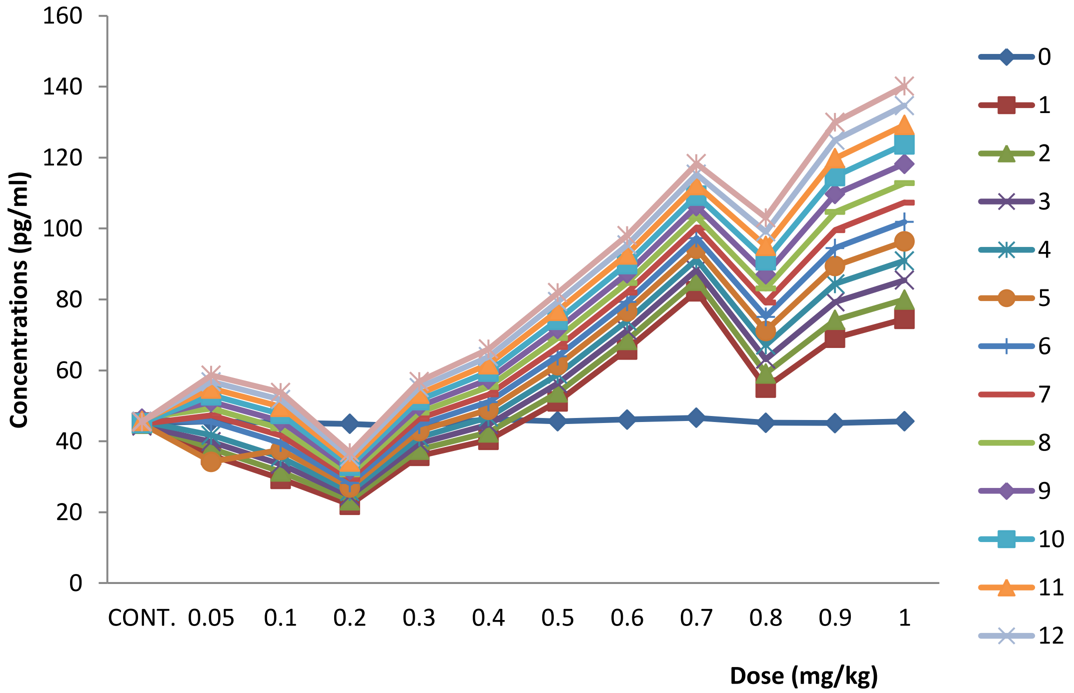

Fig. 63b;Graph of concentration against dose for interleukin-32 level.

- **EFFECT ON INTERLEUKINS IL-33**

There were significant dose dependent increases in the interleukin-33 level across the weeks at $p \leq 0.05$ (fig 64b).

Throughout the duration of exposure to BPA, the entire exposure groups showed the same pattern of response (fig. 64a); In all instances, the test group exposed to 0.05mg/kg of BPA showed higher interleukin-33 level relative to those of 0.1mg/kg (fig. 64a). The test groups exposed to 0.1mg/kg to 1mg/kg showed dose dependent increases in level of interleukin-33, except in weeks 6 to 13, where the test group exposed to 0.1mg/kg showed a higher level of interleukin-33 relative to those 0.2mg/kg (fig. 64a).

The weekly profile revealed that in all weeks of exposure, there was a decrease in interleukin-33concentrations by the test groups of 0.05mg/kg to 0.1mg/kg over time (fig. 64b). The test group that received 0.2mg/kg of BPA showed no sensitivity to time of exposure (fig. 64b). A dose dependent effect was observed from the test groups of 0.3mg/kg to 1 mg/kg and the concentrations of IL-33 at those point increased with time of exposure (fig. 64b).

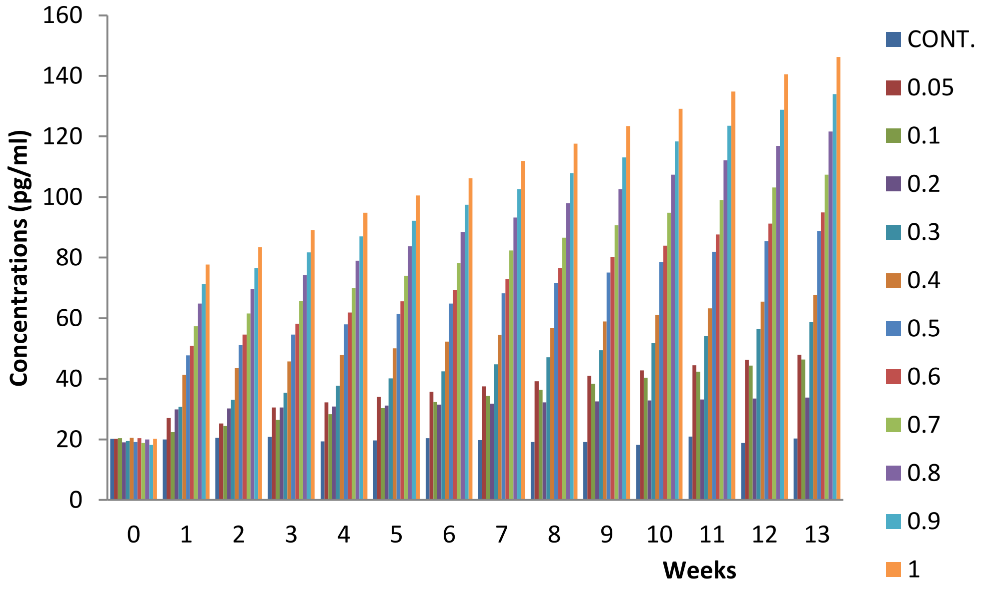

Fig. 64a; Bar chart of concentration against weeks (durations) for interleukin-33 level.

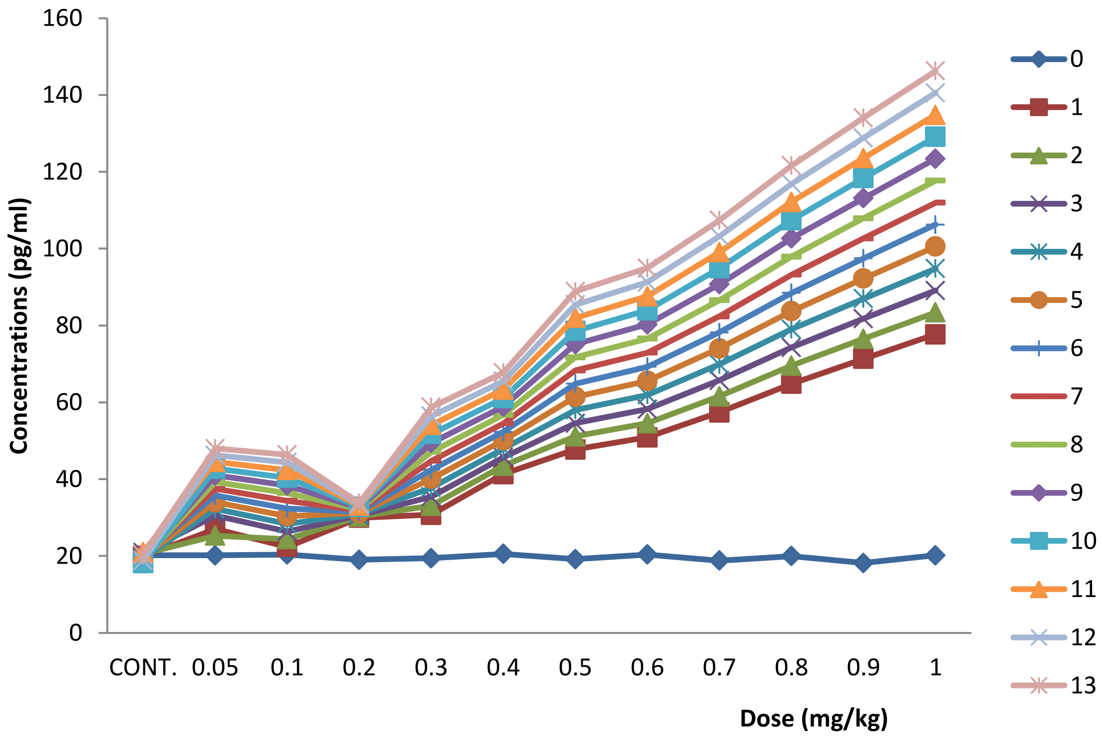

Fig. 64b;Graph of concentration against dose for interleukin-33 level.

1.6 EFFECT OF BISPHENOL A ON INFLAMMATORY MARKERS

a) HYDROGEN PEROXIDASE

There was significant decrease in the hydrogen peroxidase level when compared with the control at $p \leq 0.05$ (fig 65a). The highest concentrations of hydrogen peroxidase was observed at group 11 (1mg/kg) in week 1, although it still falls below the week 0 values (fig 65b). The result of hydrogen peroxidase showed that over the duration of the study, the test group exposed to 0.1mg/kg showed lower serum level of hydrogen peroxidase relative to those exposed to 0.05mg/kg except in weeks 7 to 9, where the reversed effect was observed (fig. 65a). The test group exposed to 0.7mg/kg of BPA showed lower serum hydrogen peroxidase concentration relative to those exposed to 0.6mg/kg except in weeks 1 to 3, where the reversed effect was observed. (fig.65a) Although, the weekly profile showed no characteristic pattern of behaviour, fig. 65b revealed that groups that were given 0.3mg/kg, 0.4mg/kg, 0.9mg/kg and 1mg/kg of BPA showed peaks at weeks 13, 6, 10 and 1 respectively (fig. 65b).

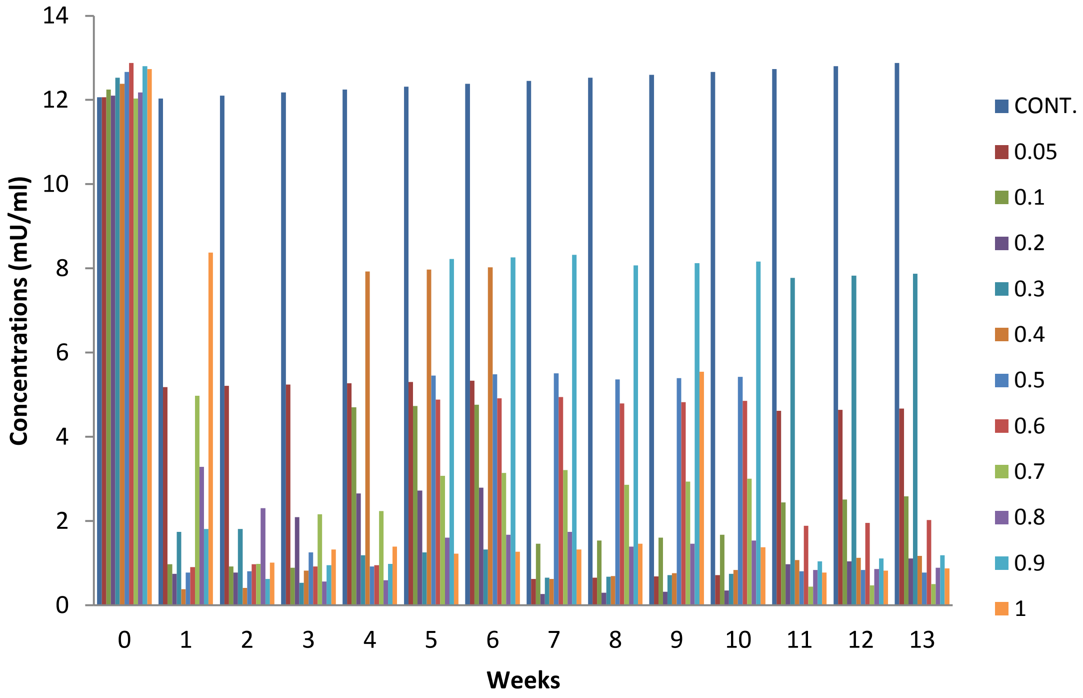

Fig. 65a; Bar chart of concentration against weeks (durations) for hydrogen peroxidase level.

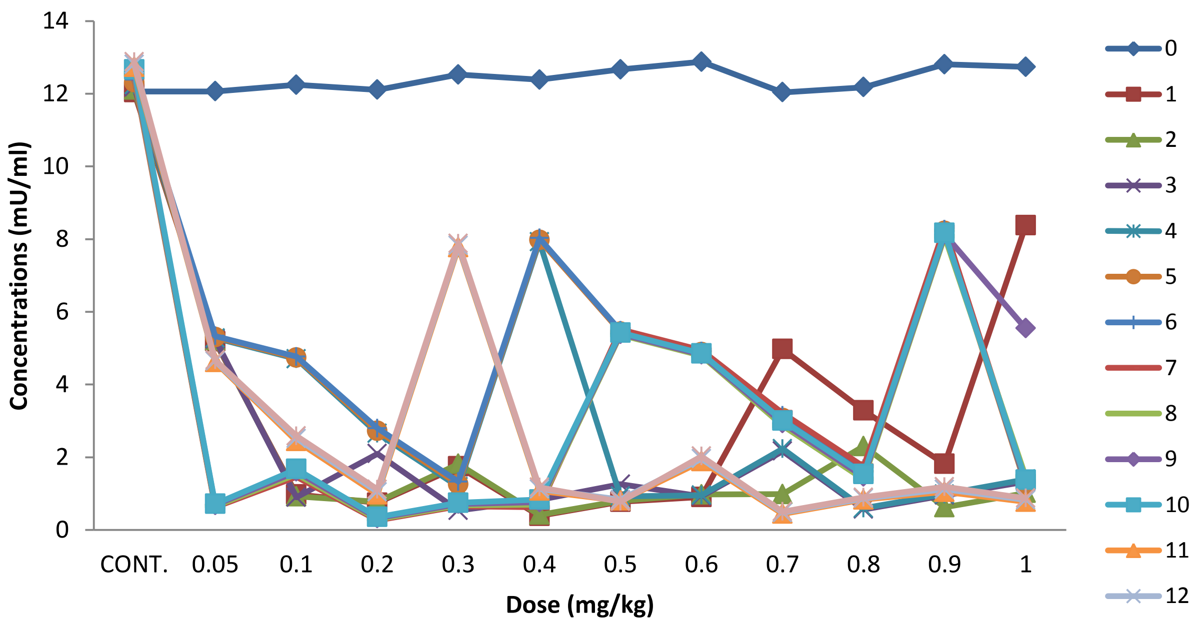

Fig. 65b;Graph of concentration against dose for hydrogen peroxidase level.

- **HYDROGEN PEROXIDE**

There were significant dose dependent increases in the hydrogen peroxide level when compared with the control and week 0 at $p \leq 0.05$ (fig 66a). The weekly effect was only observed at group 11 (1mg/kg)(fig 66b). There was dose dependent increase in the serum concentration of hydrogen peroxide which was consistent over time (fig 66a). The group that received 1mg/kg of BPA showed the highest level of hydrogen peroxide throughout the study (fig. 66a). The change in hydrogen peroxide level after exposure to graded doses of BPA showed no time sensitivity, except for the group 11 of 1mg/kg of BPA (fig. 66b).

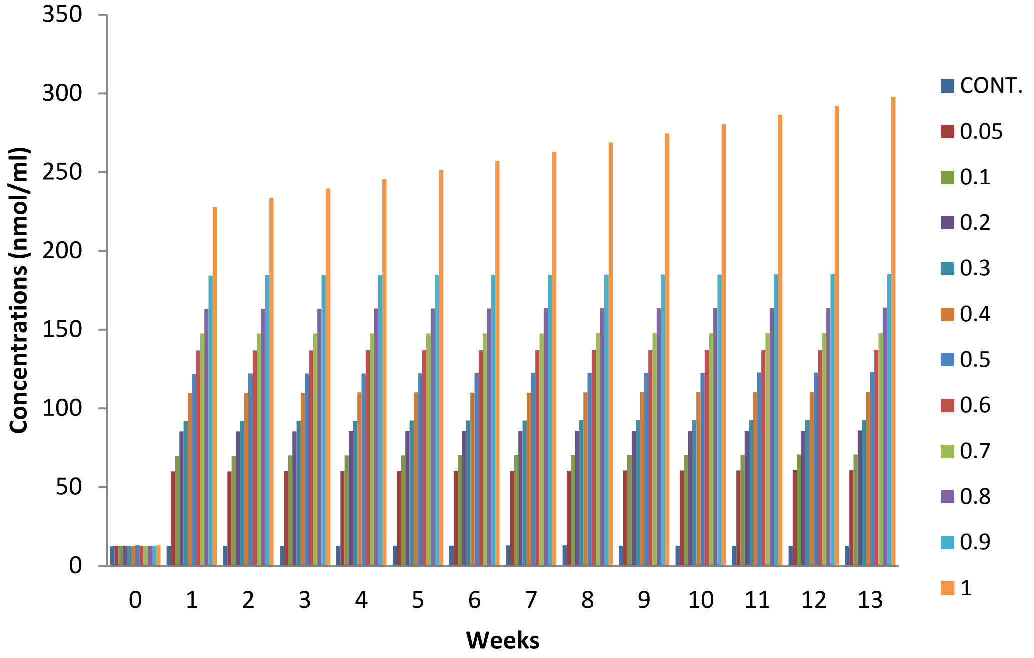

Fig 66a; Bar chart of concentration against weeks (durations) for hydrogen peroxide level.

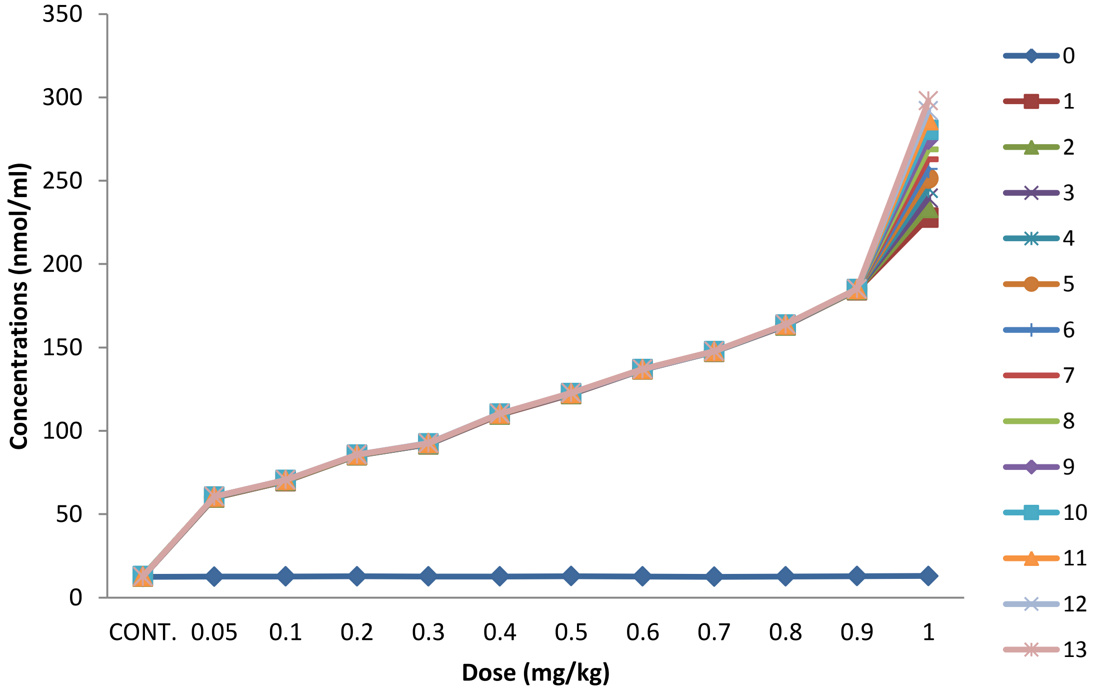

Fig 66b;Graph of concentration against dose for hydrogen peroxide level.

- **MYELOPEROXIDASE**

There were significant dose dependent decreases in the myeloperoxidase level in all the test groups, when compared with the control and week 0, at $p \leq 0.05$ (fig 67a); except for group 1 (0.05mg/kg) and 2 (0.1mg/kg) which showed no significance (fig 67b). The result of myeloperoxidase revealed that throughout the exposure duration, dose dependent decreases were shown by the group that were administered 0.05mg/kg to 0.3mg/kg of BPA, except in week 7 where the effect extended to the group that received 0.4mg/kg of BPA, and weeks 10 and 13, where the group exposed to 0.3mg/kg of BPA showed slightly higher myeloperoxidase level relative to the group that received 0.2mg/kg BPA (fig.67a). In weeks 3 and 5, the dose dependent effect were not observed (fig.67a).

The groups that were administered 0.05mg/kg, 0.2mg/kg, 0.4mg/kg, 0.6mg/kg and 0.7mg/kg revealed that BPA has no effect or had minimal effect on myeloperoxidase at weeks 4, 6, 8, 9, 11 to 13, for 0.05mg/kg; week 3 for 0.2mg/kg, week 5 and 12 for 0.4mg/kg, weeks 5 and 11 for 0.6mg/kg and 0.7mg/kg (fig. 67b). Although, the weekly profile revealed that effect of BPA on myeloperoxidase was time sensitive, but not time dependent (fig.67b).

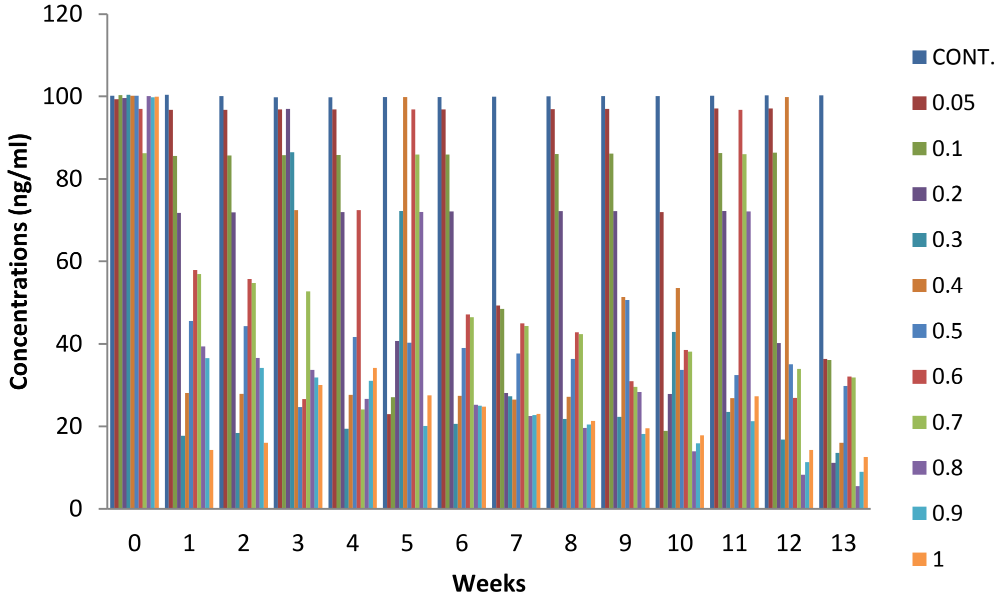

Fig 67a; Bar chart of concentration against weeks (durations) for myeloperoxidase level.

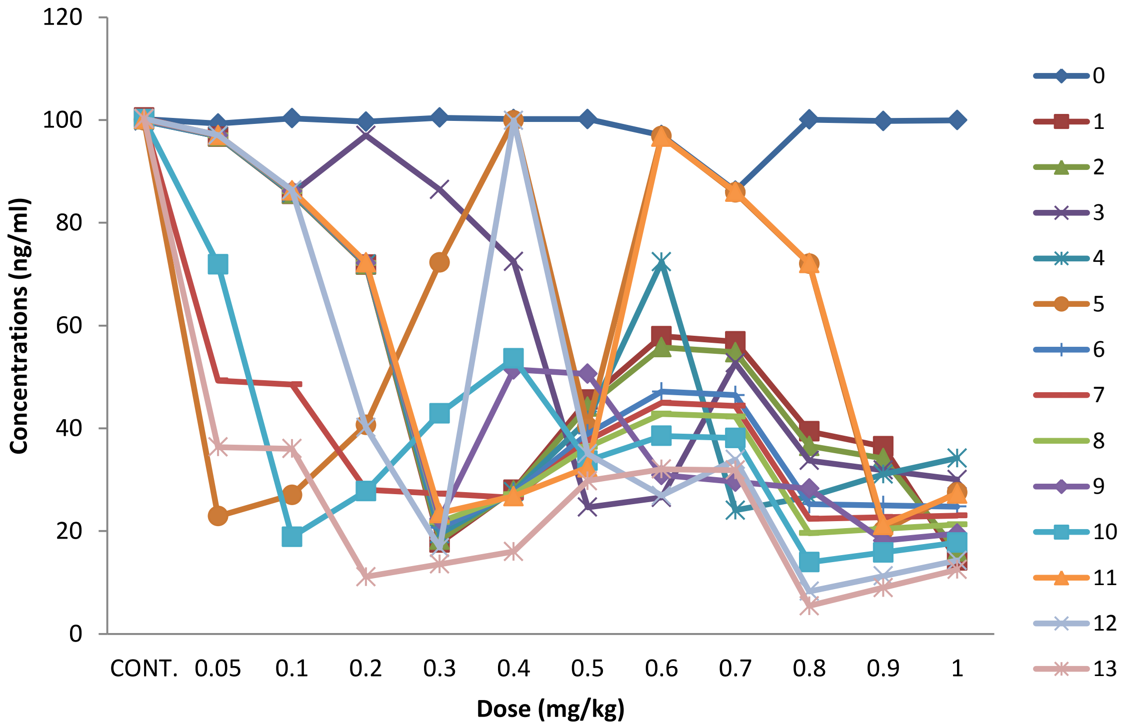

Fig 67b;Graph of concentration against dose for myeloperoxidase level.

4.7 EFFECT OF BISPHENOL A ON OXIDATIVE STRESS BIOMARKERS

4.7.1 RESULT OF ENDOGENOUS ANTIOXIDANTS

- **SUPERIOXIDE DISMUTASE (SOD)**

There were significant decrease in the SOD concentration in all the test groups when compared with the control at p≤0.05(fig 68a). Throughout the duration of the study, the group that were administered 0.05mg/kg of BPA showed lower serum levels of superoxide dismutase relative to that of the group that received 0.1mg/kg of BPA (fig. 68a). Also it was observed that throughout the study, the group that were exposed to 0.3mg/kg of BPA showed a decrease in SOD serum concentration reative to those of 0.4mg/kg (fig. 68a). The weekly characteristic profile (fig. 68b) displayed that, there was time sensitivity in the response of SOD after exposure to BPA, but the sensitivity was not time dependent (fig. 68b). There was no particular pattern of response established in all instances and he concentration fluntuate at various point of measurement (fig 68b).

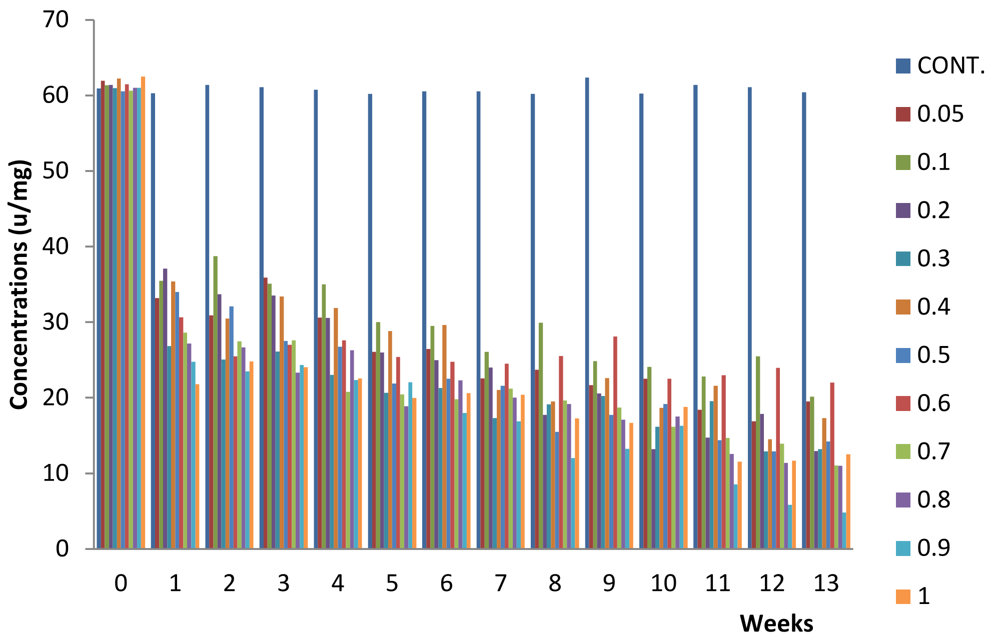

Fig. 68a; Bar chart of concentration against weeks (durations) for Superoxide Dismutase SOD level.

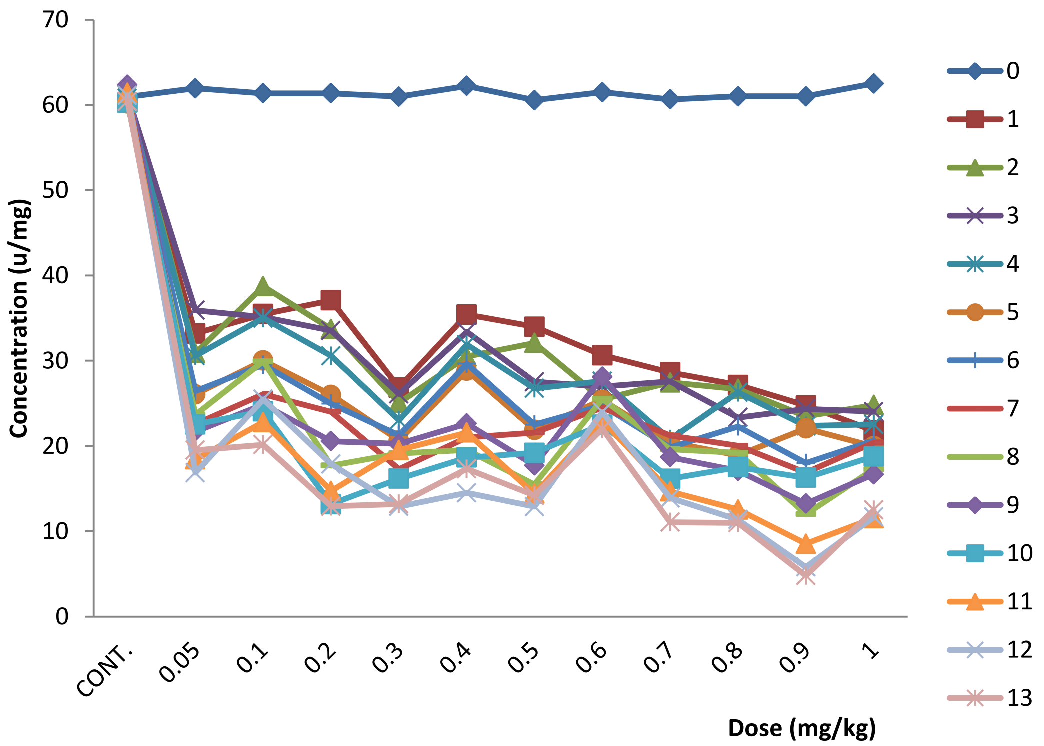

Fig. 68b;Graph of concentration against dose for Superoxide dimutase (SOD) level.

- **CATALASE**

There were significant decreases in the catalase concentration in all the BPA exposed groups, when compared with the control $p \leq 0.05$ (fig 69a). The result for catalase concentrations revealed that the group exposed to 0.05mg/kg of BPA showed lower catalase level relative to those exposed to 0.1mg/kg of BPA (fig.69a). A dose dependent decrease in catalase level was reveale by the groups that were exposed to 0.8mg/kg to 1mg/kg of BPA, throughtout the period of the study (fig.69a). A dose dependent decrease was shown by the groups that were administered 0.8mg/kg to 1mg/kg of BPA throughout the duration of the study (fig. 69a). The various weeks of exposure varied acrossed the groups (fig 69b). All the exposure groups showed time sensitivity except those of 0.7mg/kg and 0.9mg/kg (fig.69b). The groups that received 0.05mg/kg and 0.8mg/kg showed a decrease in catalase level over time, while that of 1mg/kg of BPA showed increases in catalase level with time (fig. 69b).

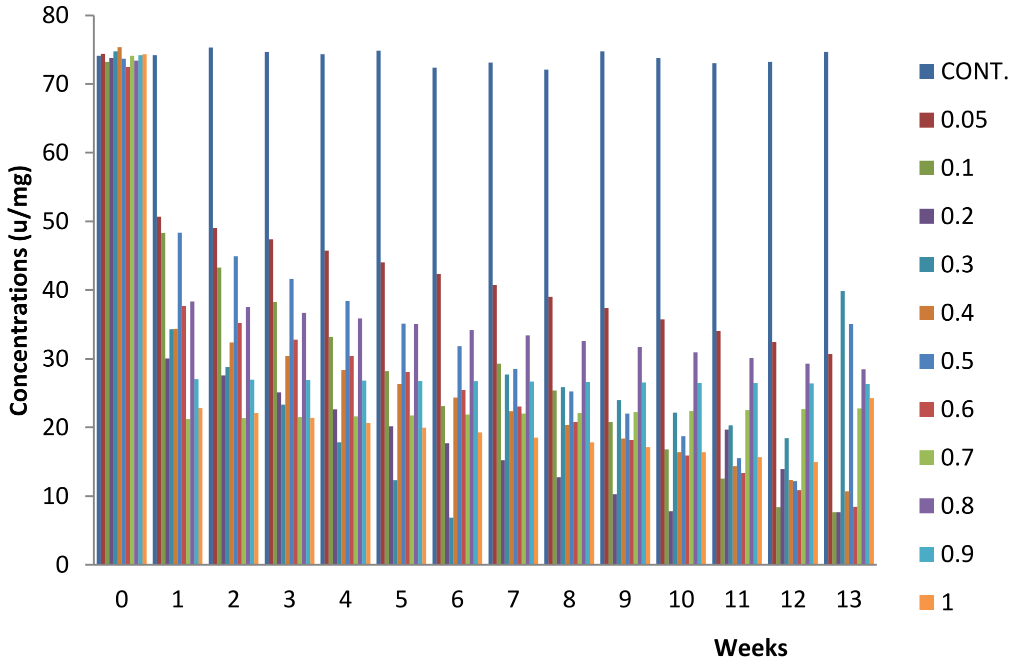

Fig. 69a; Bar chart of concentration against weeks (durations) for catalase level.

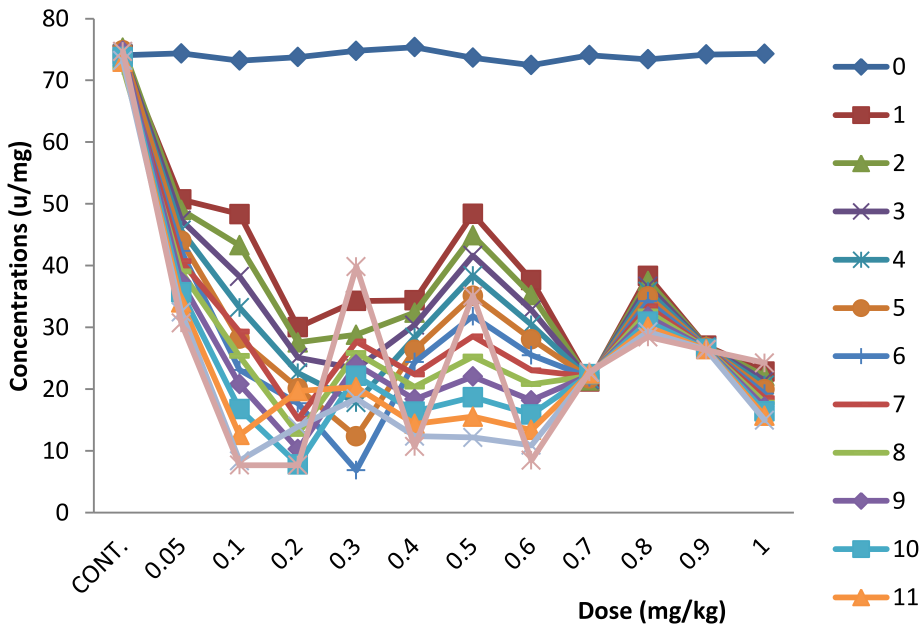

Fig. 69b; Graph of concentration against dose for catalase level.

- **TOTAL ANTIOXIDANT CAPACITY**

There was significant decrease in the total antioxidant capacity level in all the test groups when compared with the control $p \leq 0.05$ (fig 70a). At group 11 (1mg/kg), the decreases was more pronounced acrossed the time of exposure (fig 70a). As the time of exposure increases the level of total antioxidant capacity decreases (fig. 70b).

The result of total antioxidant capacity revealed that, in the first(1^{st}) and second (2^{nd}) weeks of exposure, the entire test groups showed a dose dependent decrease in total antioxidant capacity level (fig.70a). At the third(3^{rd}) and fourth (4^{th}) weeks, the same effect was observed but those of 0.3mg/kg group showed higher total antioxidant capacity level relative to that of 0.2mg/kg group (fig. 70a). As from the fifth(5^{th}) to thirteenth (13^{th}) week, the test groups exposed to 0.3mg/kg to 0.6mg/kg and 0.7mg/kg to 1 mg/kg of BPA showed dose dependent decreases in the total antioxidant capacity level (fig. 70a). The groups exposed to 0.3mg/kg, 0.5mg/kg, 0.7mg/kg to 0.9mg/kg showed no sensitivity to time of exposure (fig. 70b).

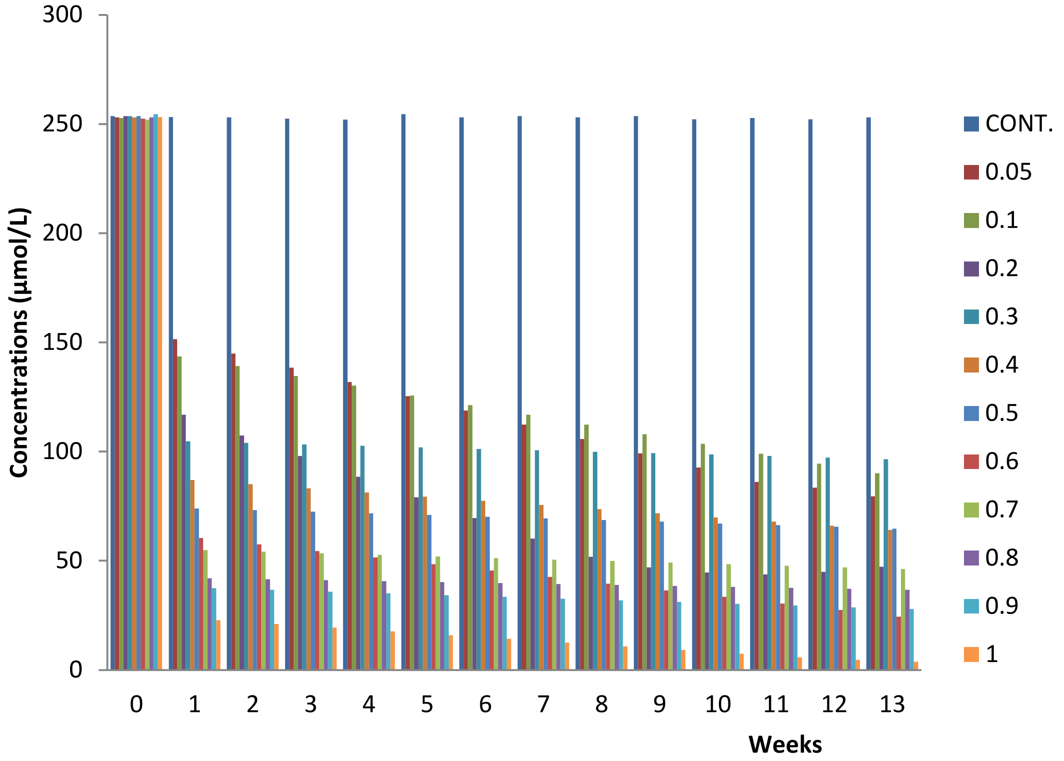

Fig. 70a; Bar chart of concentration against weeks (durations) for total antioxidant capacity level.

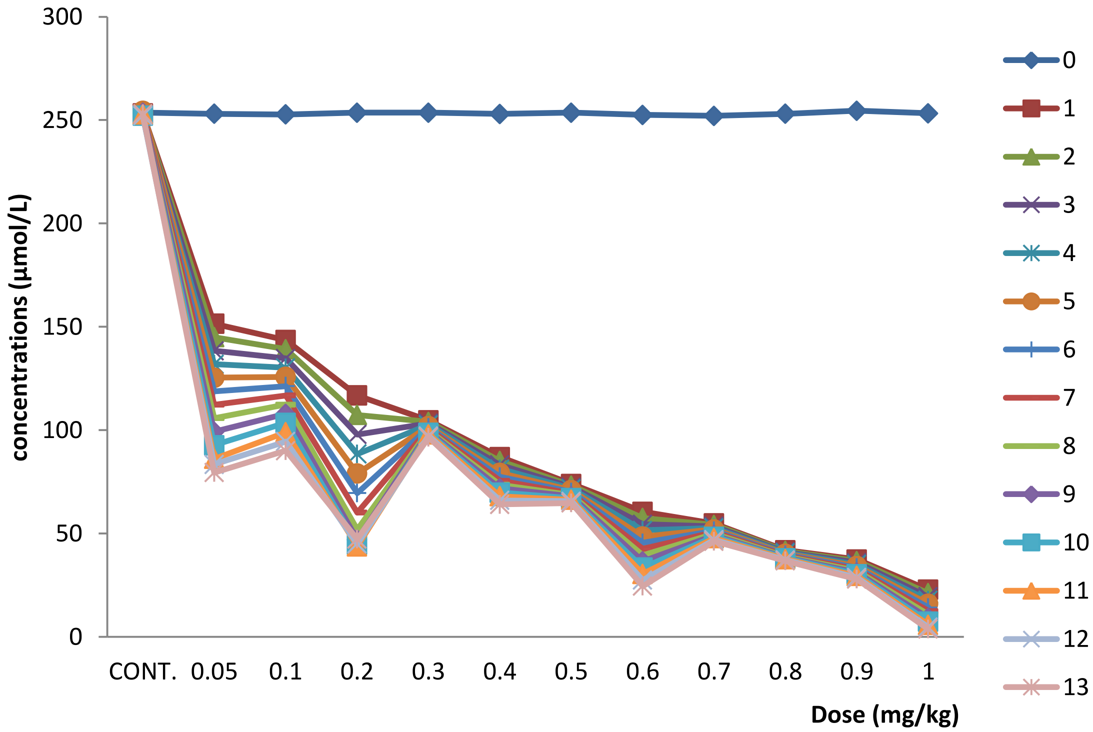

Fig. 70b; Graph of concentration against dose for total antioxidant capacity level.

- **NITRIC OXIDE**

There was significant increase in the nitric oxide level when compared with the control at $p \leq 0.05$ (fig 71a). At the onset of the experiment (week 1), the nitric oxide level of groups 4 (0.3mg/kg) to 11(1mg/kg) were high but as the weeks extends it decreases and week 13 shows the lowest level of nitric oxide. For groups 1(0.05mg/kg) to 4(0.3mg/kg), weeks 10-13 fell below the control and week 0 values (fig 71b). The result of nitric oxide showed that all the groups revealed the same pattern of behaviour over time (fig. 71a). In all instance, the group that received 0.05mg/kg of BPA showed higher nitric oxide level relative to the group that received 0.1mg/kg of BPA (fig. 71a). The groups that were administered 0.1mg/kg to 0.5mg/kg of BPA showed dose dependent increases in serum concentration of nitric oxide (fig. 71a), then those groups that were exposed to 0.6mg/kg to 1mg/kg showed dose dependent increases in the serum level of nitric oxide except the group that received 0.9mg/kg of BPA that showed a decrease in nitric oxide level relative to the group that received 0.8mg/kg of BPA (fig.71a). The maximum effect of BPA on nitric oxide level was revealed by the test group 1mg/kg BPA (fig. 71a and 71b).

As the duration of exposure increases, the concentration of nitric oxide at various doses decreases; the weekly profile reveal a time sensitive response to BPA (fig. 71b). For the test groups that received 0.05 to 0.2mg/kg in the tenth (10^{th}) to thirteenth (13^{th}) week of exposure showed a decrease in the serum nitric oxide concentration below that of the control and week 0 (fig.71b).

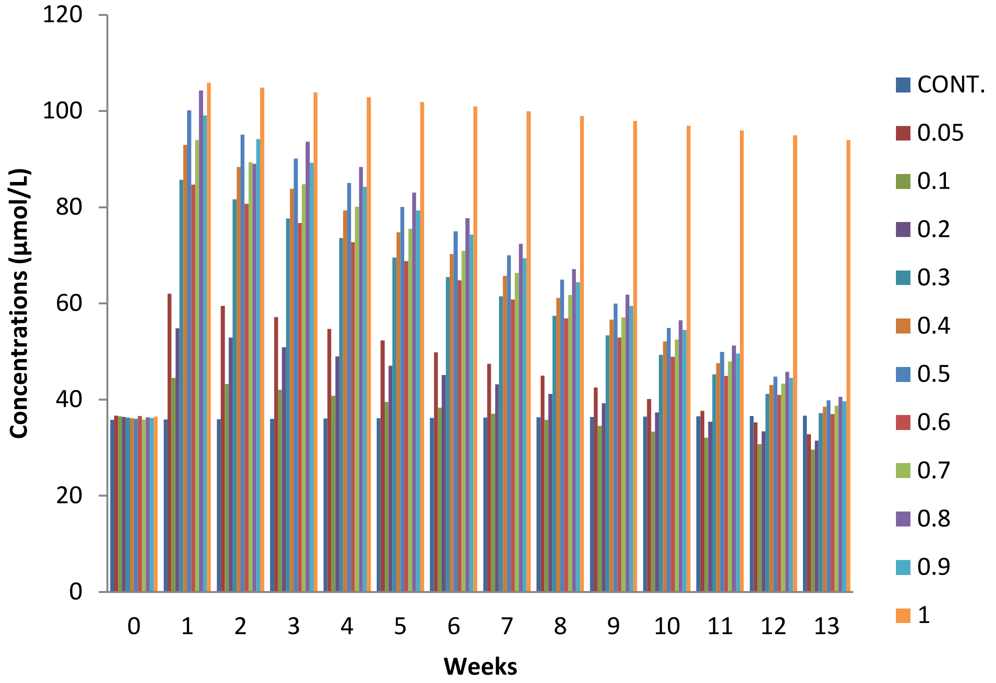

Fig. 71a; Bar chart of concentration against weeks (durations) for nitric oxide level.

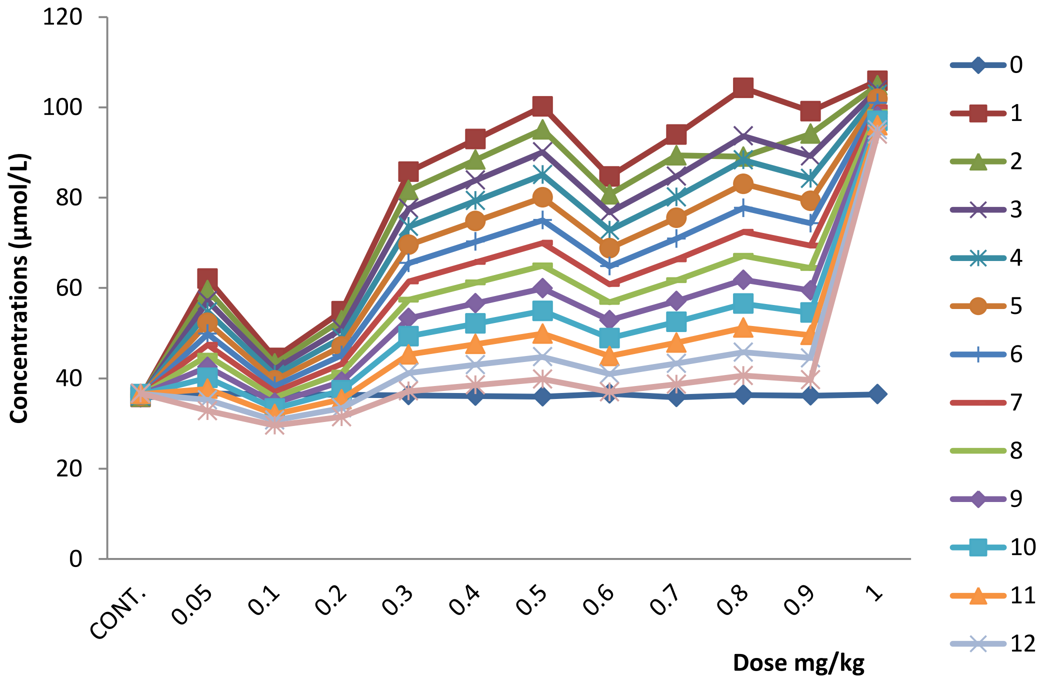

Fig. 71b;Graph of concentration against dose for nitric oxide level.

- **NITROTYROSINE**

There was significant increase in the nitrotyrosine level in all groups when compared with the control $p \leq 0.05$ (fig 72a). Groups 4 (0.3mg/kg) to 7(0.6mg/kg) did not show much variation in nitrotyrosine level during the period of exposure (fig 72b). Generally, the result nitrotyrosine revealed that after the exposure to BPA, the groups that were exposed to 0.4mg/kg to 1mg/kg showed dose dependent increases in the serum concentrations of nitrotyrosine (fig. 72a). For the test that was exposed to 0.05mg/kg of BPA showed lower nitrotyrosine level relative to that of 0.1mg/kg of BPA throughout the exposure period (fig.72a), while that of 0.2mg/kg test group showed lower level of nitrotyrosine relative to that of 0.3mg/kg BPA group (fig. 72a) during the first five (5) weeks of the study, while the reversed effect was observed in weeks 6 to 13 (fig. 72a). The serum nitrotyrosine level in response to exposure to BPA, showed a time dependent effect (fig 72b).

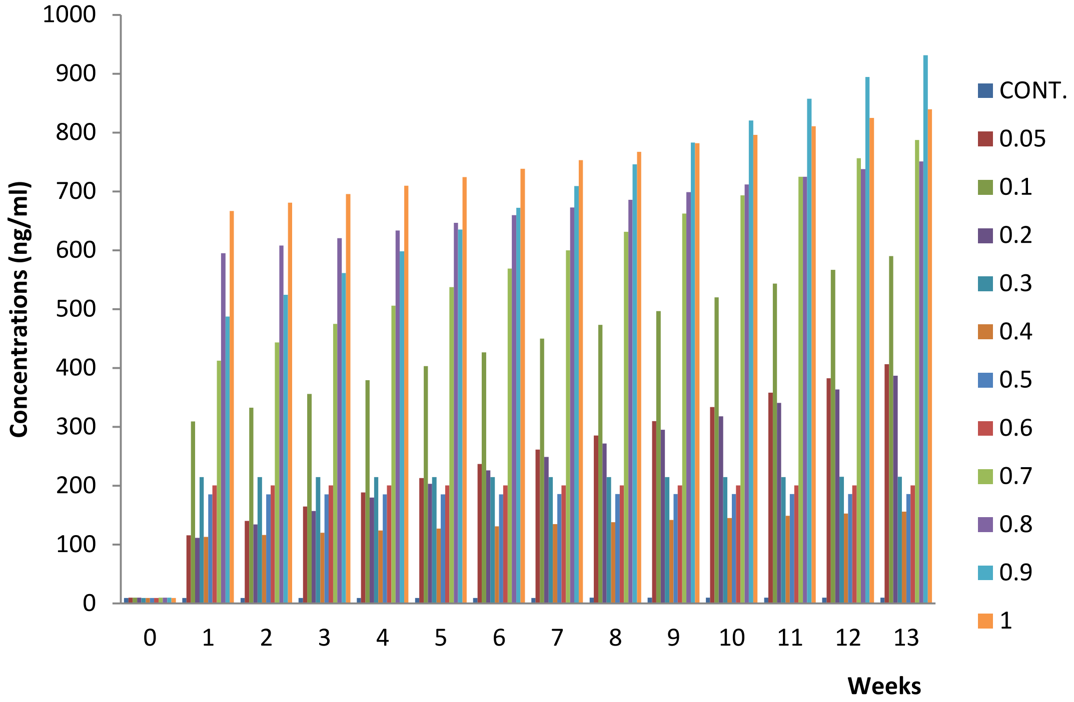

Fig.72a; Bar chart of concentration against weeks (durations) for nitrotyrosine level.

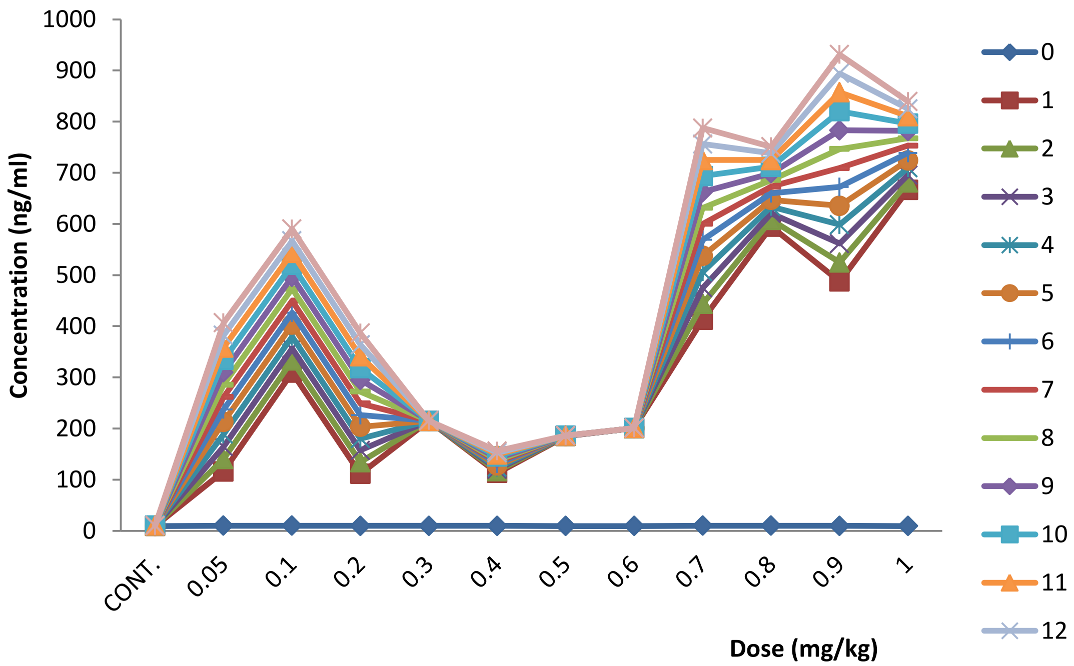

Fig.72b;Graph of concentration against dose for nitrotyrosine level.

- **PROTEIN CARBONYL**

The result of protein carbonyl level following exposure to BPA revealed that there were significant increases in the protein carbonyl level when compared with the control $p \leq 0.05$ (fig 73a). At group 5(0.4mg/kg), it was observed that all weeks showed peaked concentration of protein carbonyl, with week 13 having the highest peak, except at week 9, where 1mg/kg exposure group showed its peak effect (fig. 73a and 73b). The group exposed to 0.5mg/kg of BPA showed lower level of protein carbonyl relative to that of 0.4mg/kg exposure group, throughout the study duration (fig. 73a). Also, The group exposed to 0.9mg/kg of BPA showed lower level of protein carbonyl relative to that of 1mg/kg exposure group, throughout the study duration except in weeks 12 and 13 where the reversed effect was observed (fig. 73a). The significant ($p \leq 0.05$) increases are observed at groups 1(0.05mg/kg) to group 5 (0.4mg/kg), 9(0.8mg/kg) and 11(1mg/kg) (fig. 73b). Groups 7 (0.6mg/kg) and 10 (0.9mg/kg) showed no significant decreases in weeks 1 and 2 and in week 12 for group 7 (0.6mg/kg). The highest effect level of serum concentration of protein carbonyl observed for the exposure week by the 0.4mg/kg exposure group, with week 13 as the peak(fig 73b). Time sentivity effect was observed except at 0.5mg/kg and 0.8mg/kg exposure groups (fig.73b). The concentration of protein carbonyl dropped below that of control and week 0 in weeks 8 and 7, by the 0.1mg/kg and 0.2mg/kg exposure groups respectively (fig.73b). The concentrations of protein carbonyl, also dropped below that of control and week 0 in weeks 1, 2 and 12, by the 0.6mg/kg exposure group (fig.73b). The concentrations of protein carbonyl dropped below that of control and week 0 at all the weeks of exposure, by the 0.5mg/kg exposure group (fig.73b).

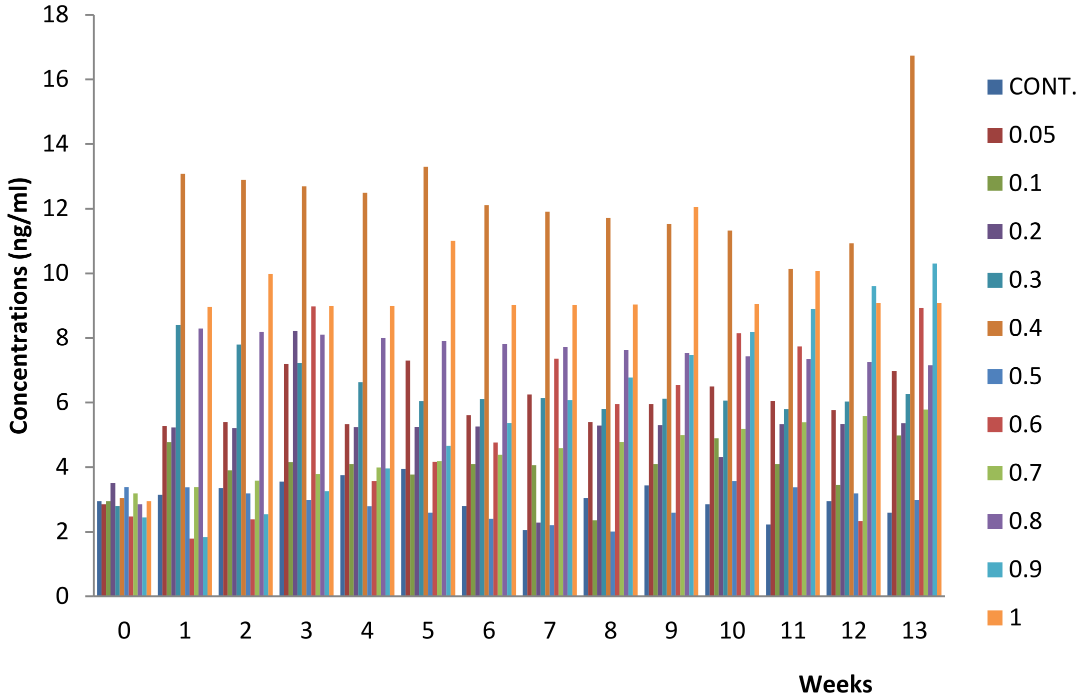

Fig 73a; Bar chart of concentration against weeks (durations) for protein carbonyl level.

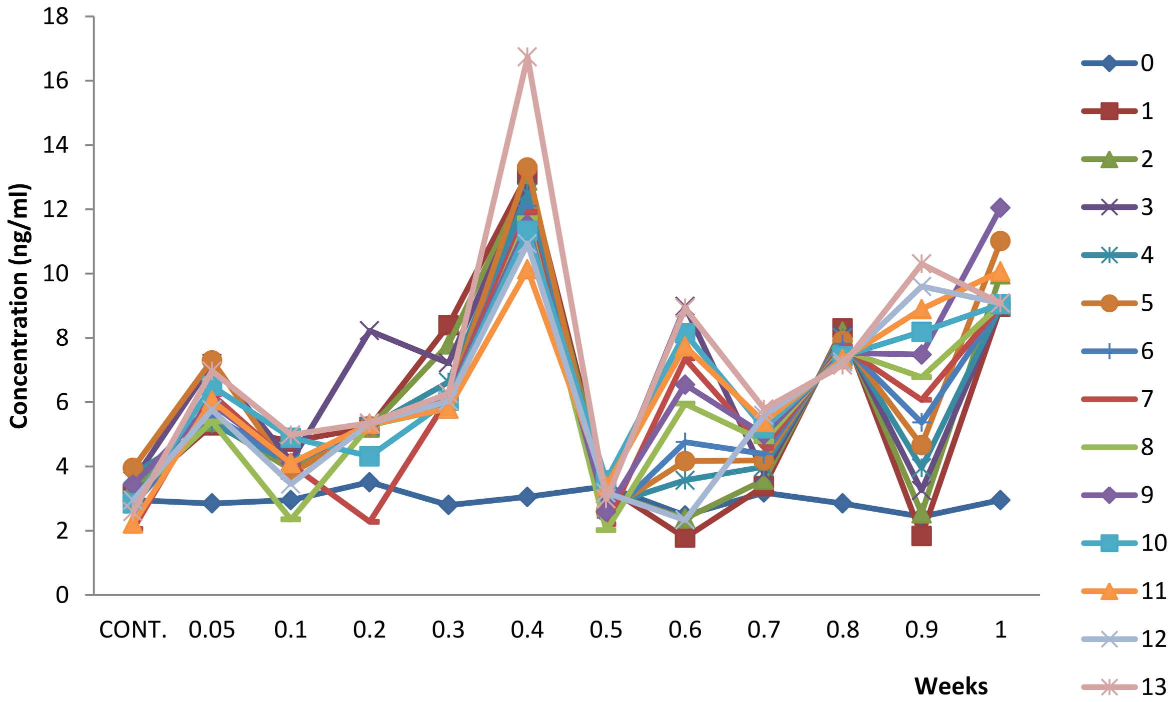

Fig 73b;Graph of concentration against dose for protein carbonyl level.

4.7.2 RESULT OF LIPID PEROXIDATION

- **MALONDIALDEHYDE (MDA)**

There was significant dose dependent increase in the malodialdehyde (MDA) level when compared with the control p≤0.05 (fig 74a). The malondialdehyde result revealed the same pattern of response to BPA exposure by the entire dose groups (fig. 74a). The groups exposed to 0.05mg/kg to 0.6mg/kg and 0.7mg/kg to 1mg/kg of BPA showed dose dependent increases in serum malondialdehyde concentration (fig. 74a). At weeks 1 to 13, the groups that showed the maximum effect of BPA exposure on malondialdehyde are those of 0.1mg/kg, 0.9mg/kg and 0.6mg/kg (74a and 74b). The test groups 0.05mg/kg to 0.3mg/kg showed no sensitivity to time of exposure while 0.4mg/kg to 1mg/kg showed mild sensitivity to time of exposure (fig 74b). In week 10, the test group of 0.6mg/kg showed its maximum effect (fig. 74b).

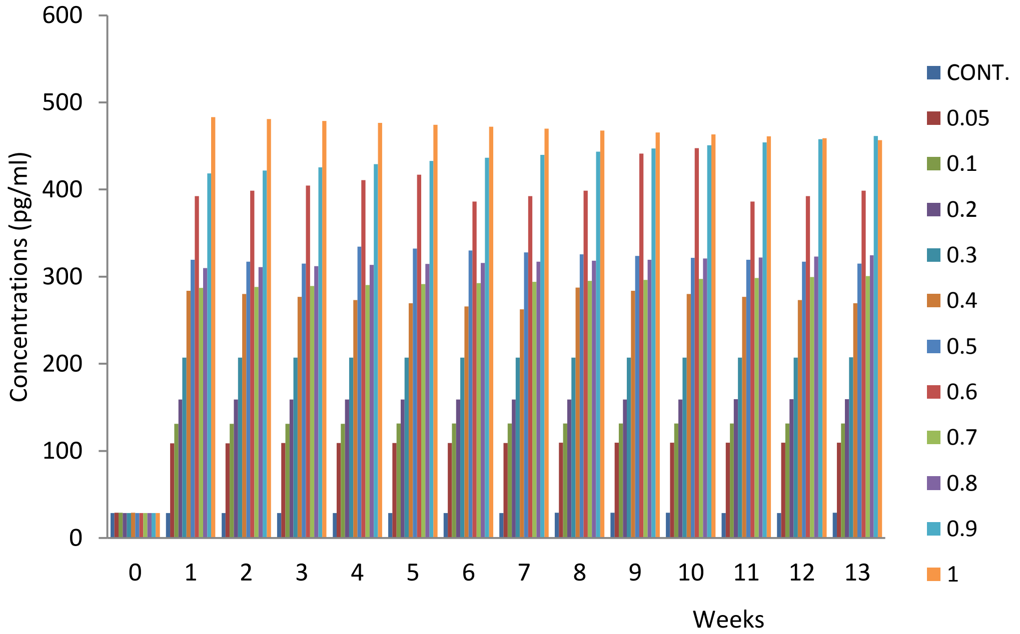

Fig.74a; Bar chart of concentration against weeks (durations) for malodialdehyde (MDA) level.

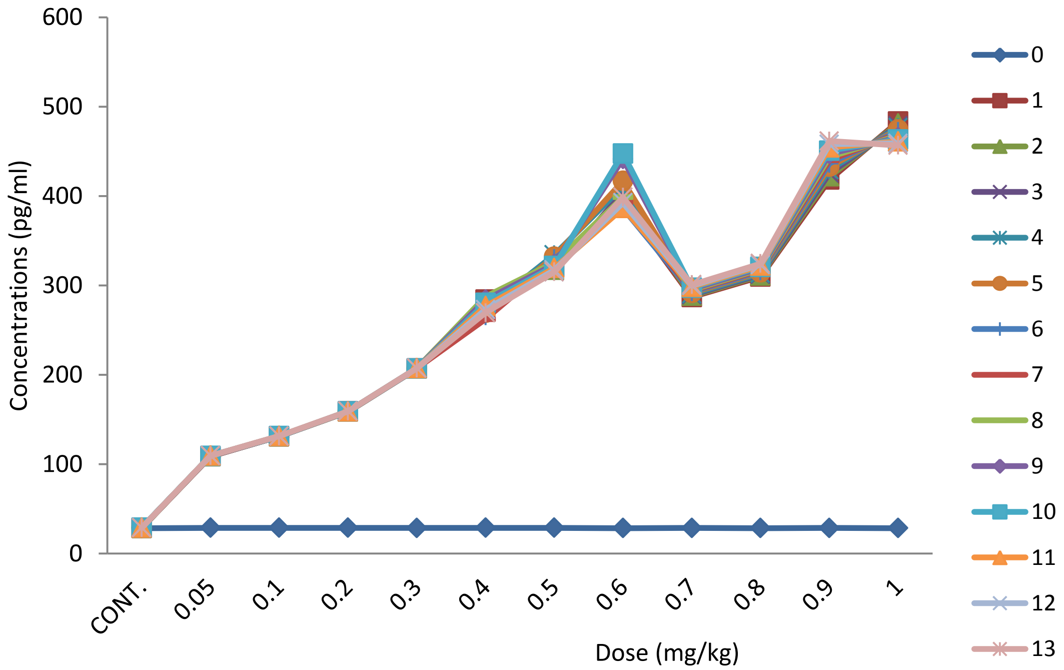

Fig.74b;Graph of concentration against dose for malodialdehyde (MDA) level.

- **LIPID HYDROPEROXIDE**

There was significant increase in the lipid hydroperoxide level when compared with the control $p \leq 0.05$ (fig 75a). The result lipid hydroperoxide revealed dose depndent increases by the test group that were administered 0.05mg/kg to 0.3mg/kg of BPA, but those of 0.3mg/kg showed a decrease in serum concentration of lipid hydroperoxide from the fourth (4^{th}) to the thirteenth (13^{th}) week of exposure relative to those of 0.2mg/kg test group throughout the exposure period of the study (fig.75a). The test groups exposed to 0.5mg/kg to 0.7mg/kg showed dose dependent increases from the first (1^{st}) to the nineth (9^{th}) week of exposure (fig. 75a). As the time of the study increases, the concentration of lipid hydroperoxide increases (fig. 75b). The Test group 0.8mg/kg showed no sensitivity to time of exposure (fig.75b).

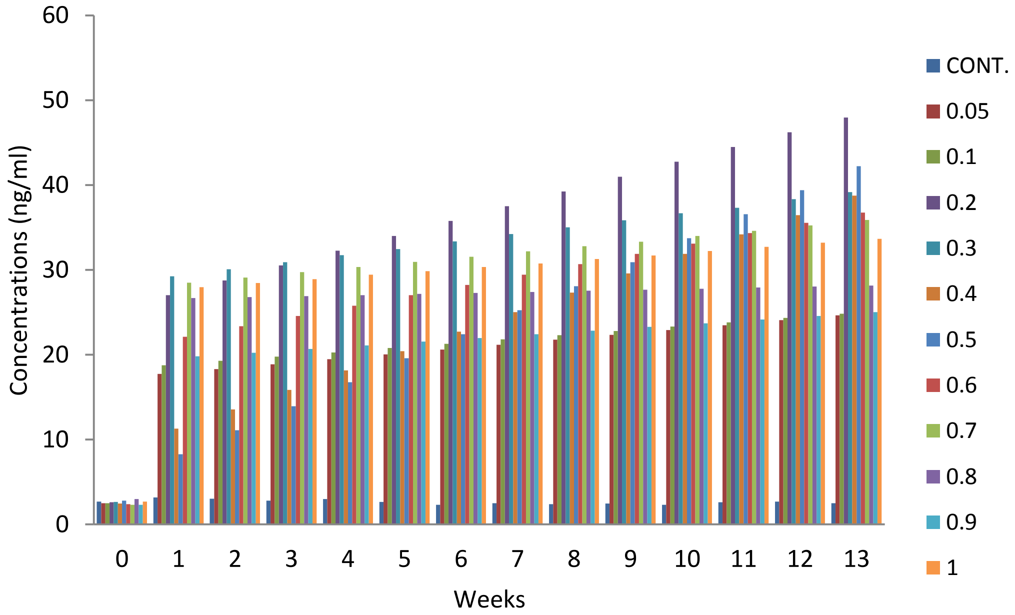

Fig 75a; Bar chart of concentration against weeks (durations) for lipid hydroperoxide level.

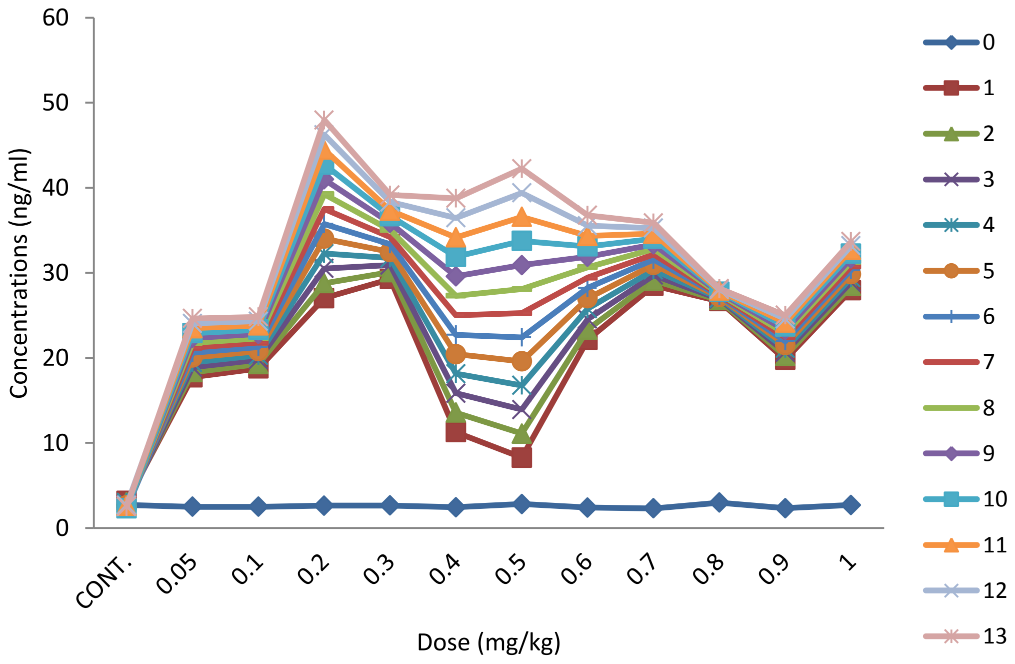

Fig 75b;Graph of concentration against dose for lipid hydroperoxide level.

- **8 – ISOPROSTANE**

There was significant increase in the 8-isoprostane level in all test groups when compared with the control at $p \leq 0.05$ (fig 76a). The weekly effect was mildly observed (fig 76b). The result of 8-isoprostane showed similar characteristic thrroughout the exposure period (fig. 76a). The test groups that were exposed to 0.05mg/kg to 0.3mg/kg, 0.4mg/kg to 0.7mg/kg and 0.8mg/kg to 1mg/kg of BPA revealed dose dependent increases in the serum concentration of 8-isoprostane (fig. 76a). The test group 0.4mg/kg, 0.5mg/kg and 0.8mg/kg showed no sensitivity to time of exposure (fig. 76b).

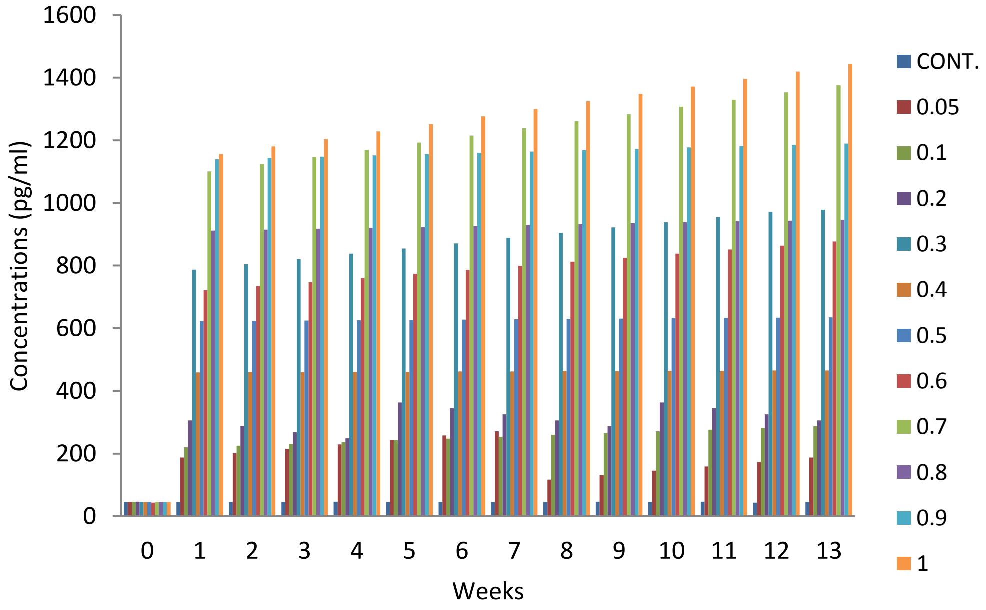

Fig 76a; Bar chart of concentration against weeks (durations) for 8-ISOPROSTANE level.

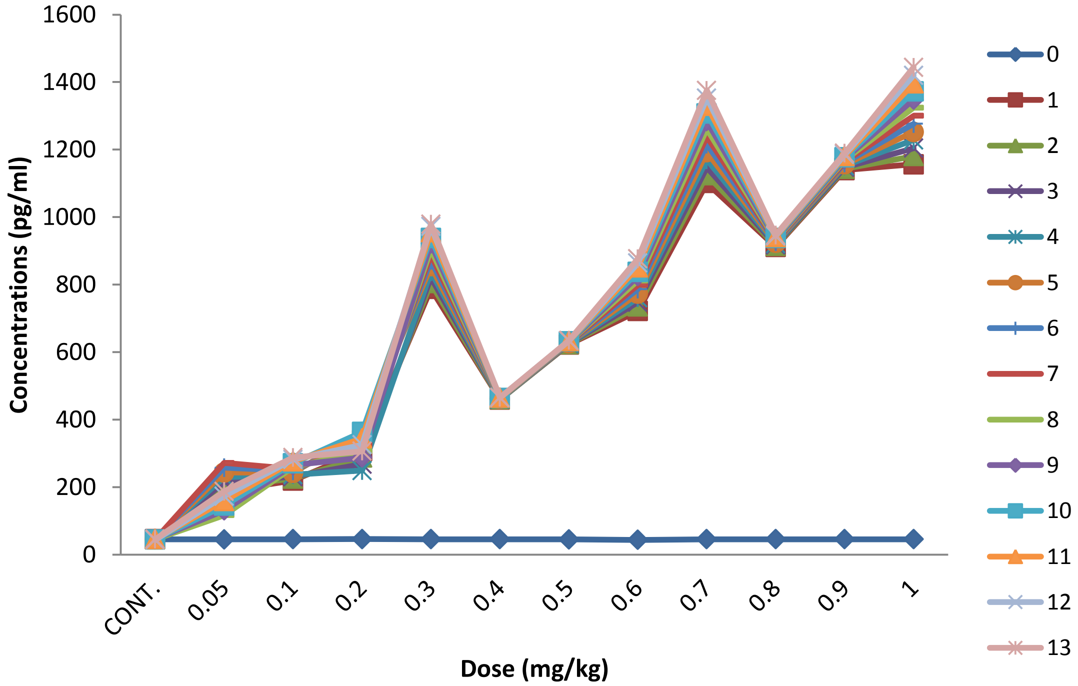

Fig 76b;Graph of concentration against dose for 8-ISOPROSTANE level.

- **HEXANOYLLYSINE**

There were significant increases in the hexanoylysine levels in all the exposed groups when compared with the control at $p \leq 0.05$ (fig 77a). The weekly effect was mildly observed (fig 77b).
The hexanoylysine result showed that the entire dose groups have similar pattern of response to BPA exposure over time (fig. 77a). There were dose dependent increase in the serum concentrations of hexanoylysine by the groups that were exposed to 0.05mg/kg to 0.2mg/kg of BPA, this was followed by dose dependent decreases in hexanoylysine level by the groups exposed to 0.3mg/kg to 0.5mg/kg (fig. 77a); then the test groups that were exposed to 0.6mg/kg to 1mg/kg of BPA showed dose dependent increases in hexanoylysine level (fig.. 77a).
The serum level of hexanoylysine appeared to be constant over time, as groups 0.05mg/kg to 0.6mg/kg showed no sensitivity to time of exposure (fig. 77b). The mild sensitivity to time was exhibited by the test groups 0.7mg/kg to 1mg/kg, and the increase by these groups were time dependent (fig. 77b) .

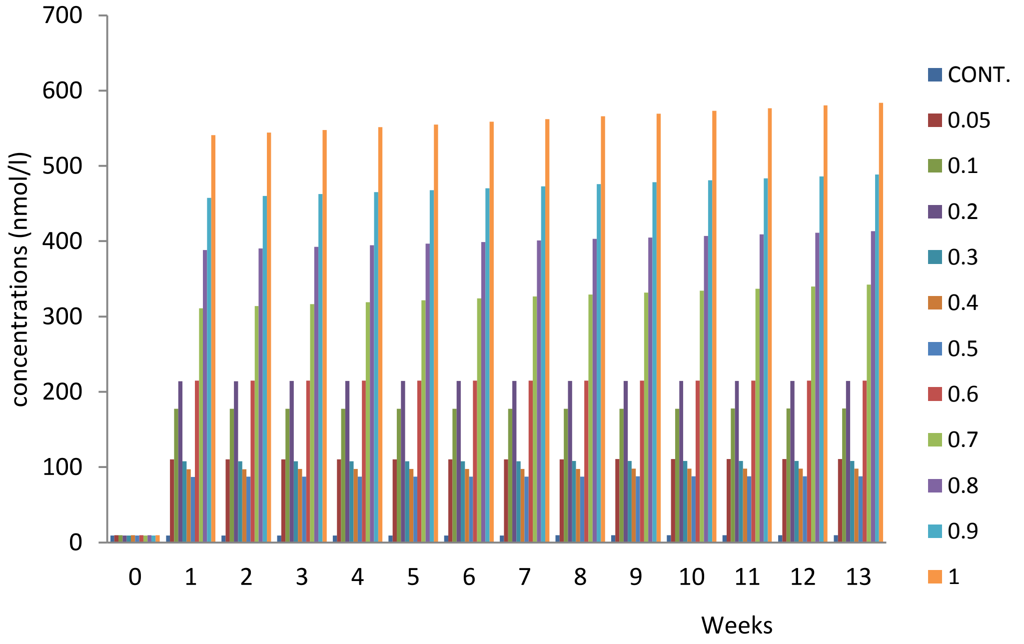

Fig. 77a; Bar chart of concentration against weeks (durations) for hexanoylysine level.

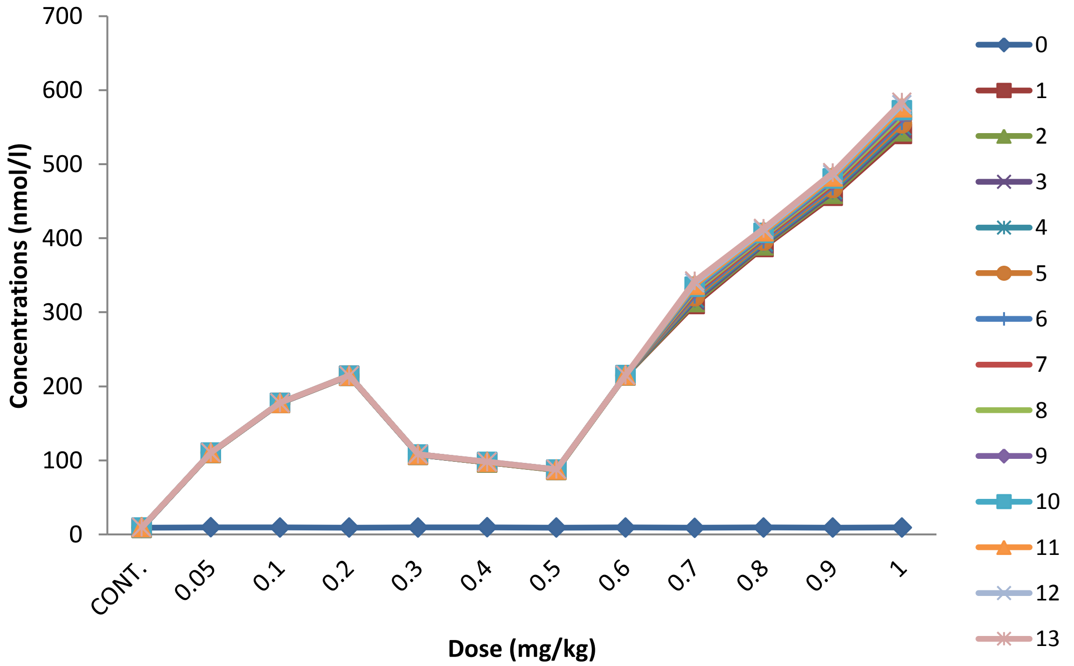

Fig. 77b;Graph of concentration against dose for hexanoylysine level.

- **OXIDIZED LIPID**
  - **OXIDISED PHOSPHOLIPID (OXPL-LDL)**

There was significant increase in the OXPL-LDL level when compared with the control $p \leq 0.05$ (fig 78a). The significance were observed at all weeks in all test groups except for group 6 (0.5mg/kg) at weeks 1-5; then group 1(0.05mg/kg) to 4(0.3mg/kg) shows significant increase only at weeks 9-13(fig 78b). During the first four (1-4) weeks of exposure to BPA, the groups that were administered 0.05mg/kg to 0.4mg/kg of BPA showed dose dependent increases of the oxidised phospholipid level (fig. 78a). The group that was exposed to 0.9mg/kg of BPA showed lower oxidised phospholipid level relative to that 1mg/kg at all time of exposure and also, the group that was exposed to 0.6mg/kg of BPA showed lower oxidised phospholipid level relative to that 0.7mg/kg at all time of exposure (fig.78a). The group that received 1mg/kg showed the highest level of oxidised phospholipid at all time of exposure (fig. 78a and 78b). In the first (1^{st}) and second (2^{nd}) weeks of exposure, the serum level of oxidised phospholipid decreased below that of control and week 0 for the groups exposed to 0.05mg/kg, 0.1mg/kg and 0.5mg/kg of BPA. (fig. 78b). The time sensitive effect observed in fig.78b, was mild at 0.4mg/kg and relatively time dependent effect was observed (fig. 78b).

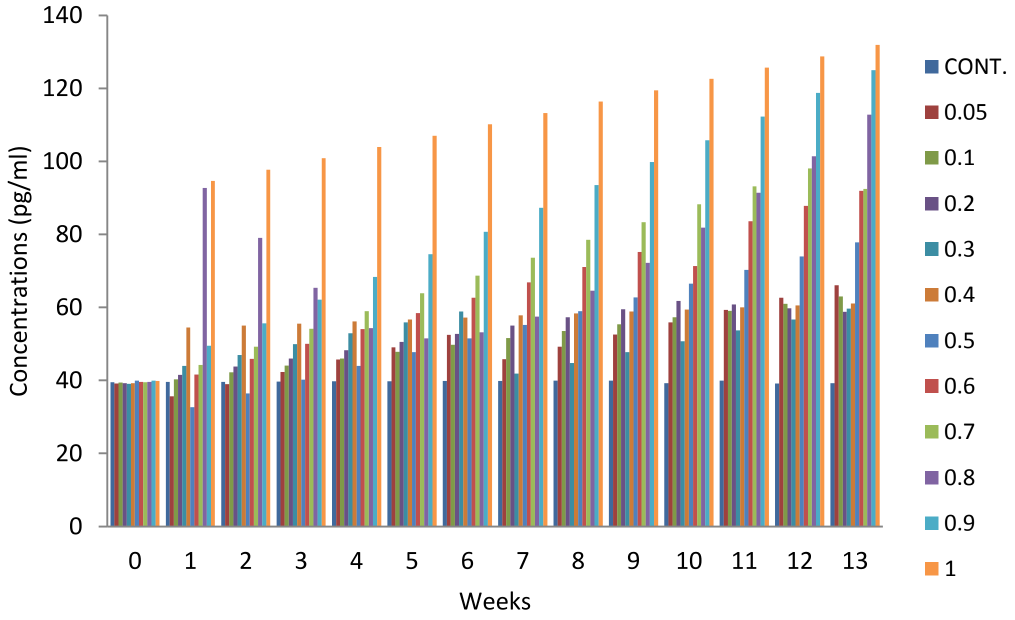

Fig. 78a; Bar chart of concentration against weeks (durations) for OXPL-LDL level.

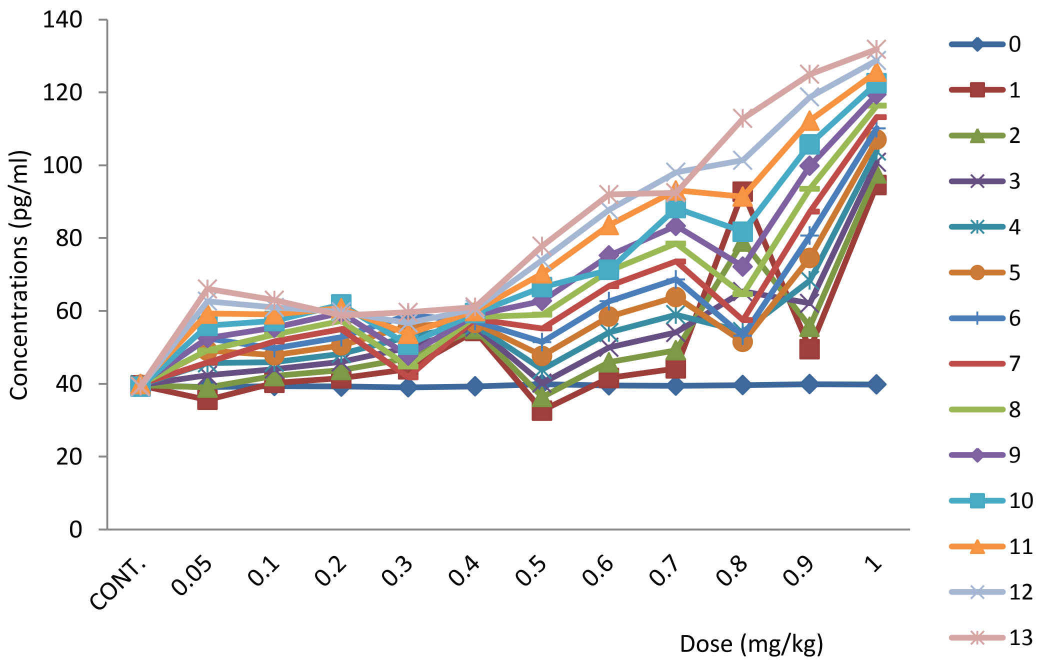

Fig. 78b;Graph of concentration against dose for OXPL-LDL level.

- **MDA-LDL**

There was significant increase in the MDA-LDL level when compared with the control at $p \leq 0.05$ (fig 79a) in groups 6(0.5mg/kg) and 11(1mg/kg). Group 7(0.6mg/kg) to 10(0.9mg/kg) shows significant increase only at weeks 8-13. There was no significant difference observed in groups 1(0.05mg/kg) to 4(0.3mg/kg) when compared with the control (fig 79b). The group that received 1mg/kg of BPA showed the highest level of MDA-LDL, which consistently increase in weeks 1 to 5, 6 to 11 and 12 to 13 with the highest level in week 5 of exposure (fig.79a). Another group that showed high level of MDA-LDL was the group that received 0.5mg/kg of BPA (fig. 79a). At all exposure time, the group exposed 0.6mg/kg of BPA showed gradual dose dependent increase except that in all instance the group that received 0.8mg/kg of BPA showed decrease in MDA-LDL relative to that of 0.7mg/kg. The study revealed a time sensitive effect (fig. 79b). The observed effect was time dependent except at the test group of 0.2mg/kg and 1mg/kg (fig.79b).

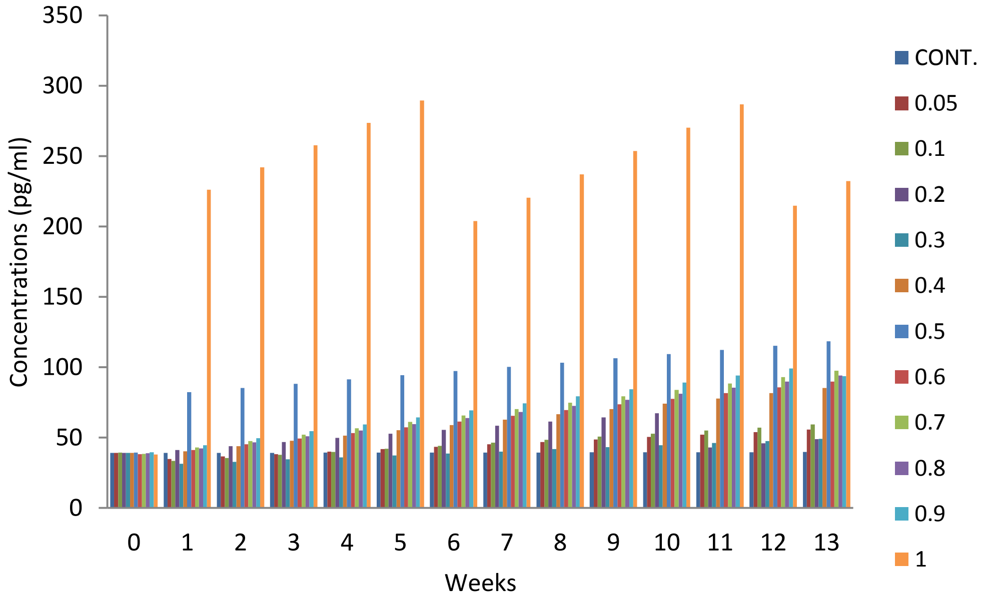

Fig. 79a; Bar chart of concentration against weeks (durations) for MDA-LDL level.

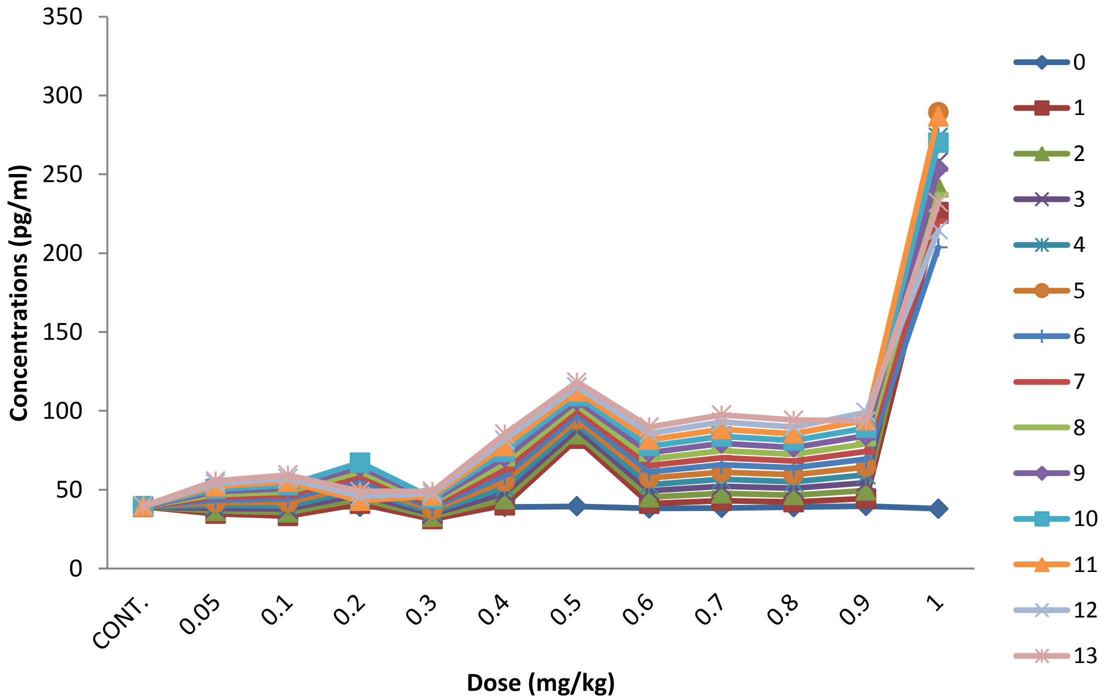

Fig. 79b;Graph of concentration against dose for MDA-LDL level.

- **CARBOXYMETHYLLYSINE MODIFIED LDL (CML-LDL)**

There were significant increases in the CML-LDL level in groups 2(0.1mg/kg), 6(0.5mg/kg), 9(0.8mg/kg) to11(1mg/kg), when compared with the control at $p \leq 0.05$ (fig 80a). In weeks 5 to 13 of exposure to BPA, the groups exposed to 0.2mg/kg to 0.5mg/kg showed dose dependent increase in carboxymethyllysine modified LDL concentration (fig. 80a). At group 3(0.2mg/kg) to 8(0.7mg/kg) in weeks 1 to 3, showed no significant decreases compard to the control and week 0; while weeks 7 to 13 showed significant increase (fig. 80b). At group 9 (0.8mg/kg), the concentration of carboxymethyllysine modified LDL decreases with time of exposure while other exposure groups showed increases in the carboxymethyllysine modified LDL level with time of exposure (fig. 80b) except for group 11 (1mg/kg) where there was no systematic response to the effect of BPA (fig 80b). The observed effect was time sensitive (fig. 80b).

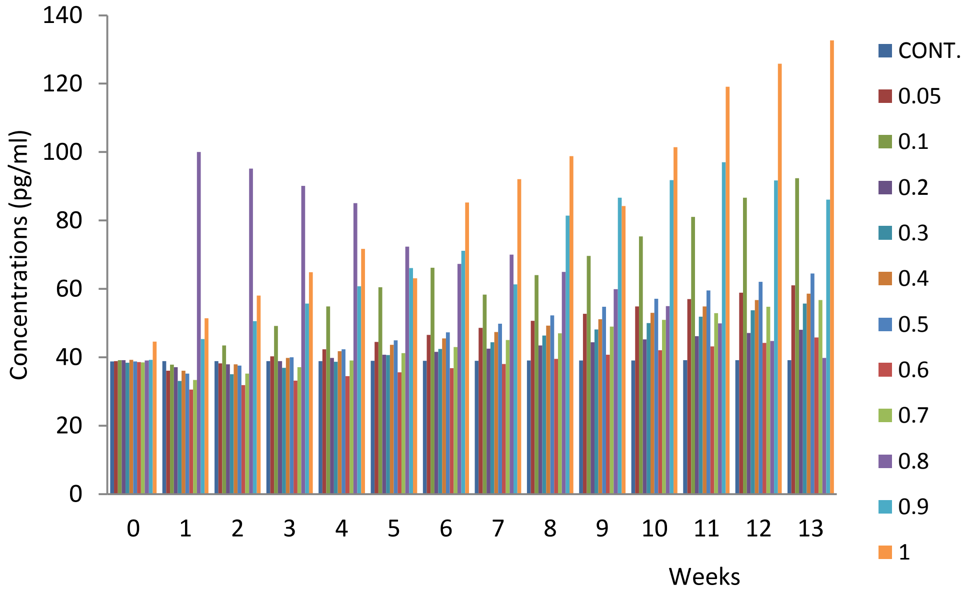

Fig 80a; Bar chart of concentration against weeks (durations) for CML-LDL level.

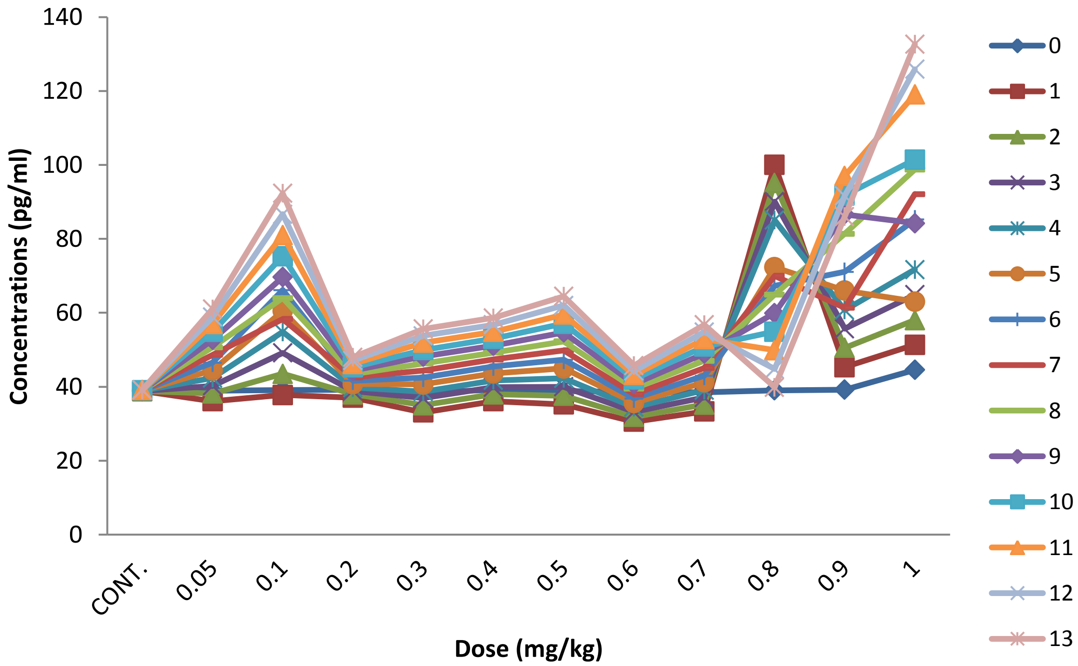

Fig 80b;Graph of concentration against dose for CML-LDL level.

➢ **4-HYDROXYNONENAL MODIFIED LDL (HNE – LDL)**

There was significant increase in the HNE-LDL level when compared with the control at $p \leq 0.05$. Initially, there was no significant difference observed for groups 1(0.05mg/kg) to 6(0.5mg/kg) in weeks 1 to 4. The increase was more pronounced in week 13 for the entire exposure groups (fig 81a). In weeks 1 to 11, the test groups that received 0.4mg/kg to 0.8mg/kg of BPA showed dose dependent increases in the serum concentration of HNE-LDL, except in weeks 1 to 6, where the group that received 0.8mg/kg showed a decrease in the HNE-LDL level relative to that of 0.7mg/kg (fig. 81a); also, it was observed that the test group exposed to 0.9mg/kg of BPA showed lower level of HNE-LDL relative to that of 1mg/kg group (fig. 81a). In weeks 12 to 13, the observed dose dependent effect extends to 1mg/kg with a slight decrease by the group that received 0.9mg/kg relative to that of 1mg/kg and the group that received 0.9mg/kg of BPA showed lower HNE-LDL level relative to that of 1mg/kg (fig.81a). The observed effect was time dependent except at test group 0.2mg/kg and time sensitive except at 0.7mg/kg of BPA exposure group (fig. 81b).

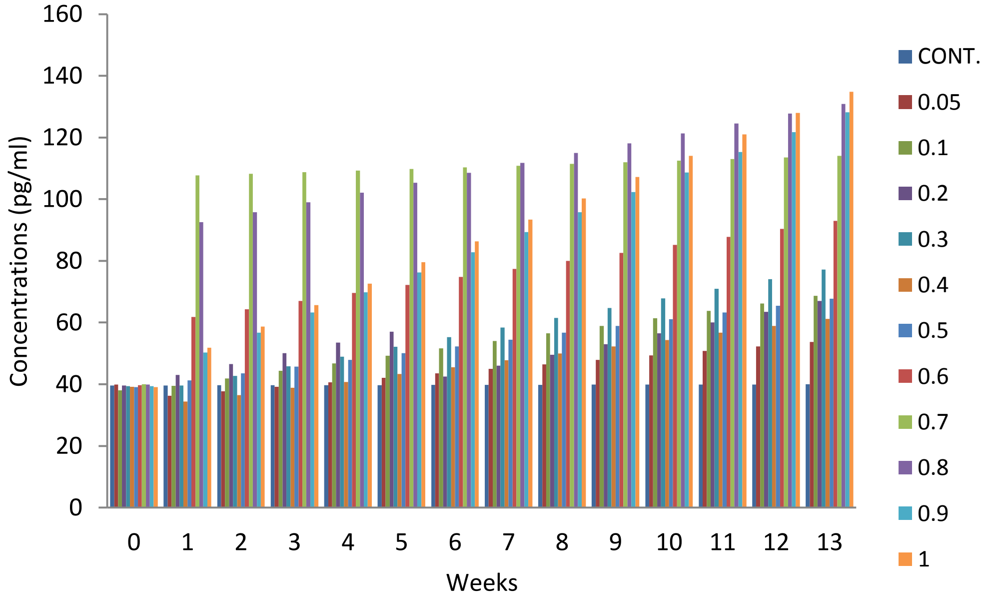

Fig. 81a; Bar chart of concentration against weeks (durations) for HNE-LDL level.

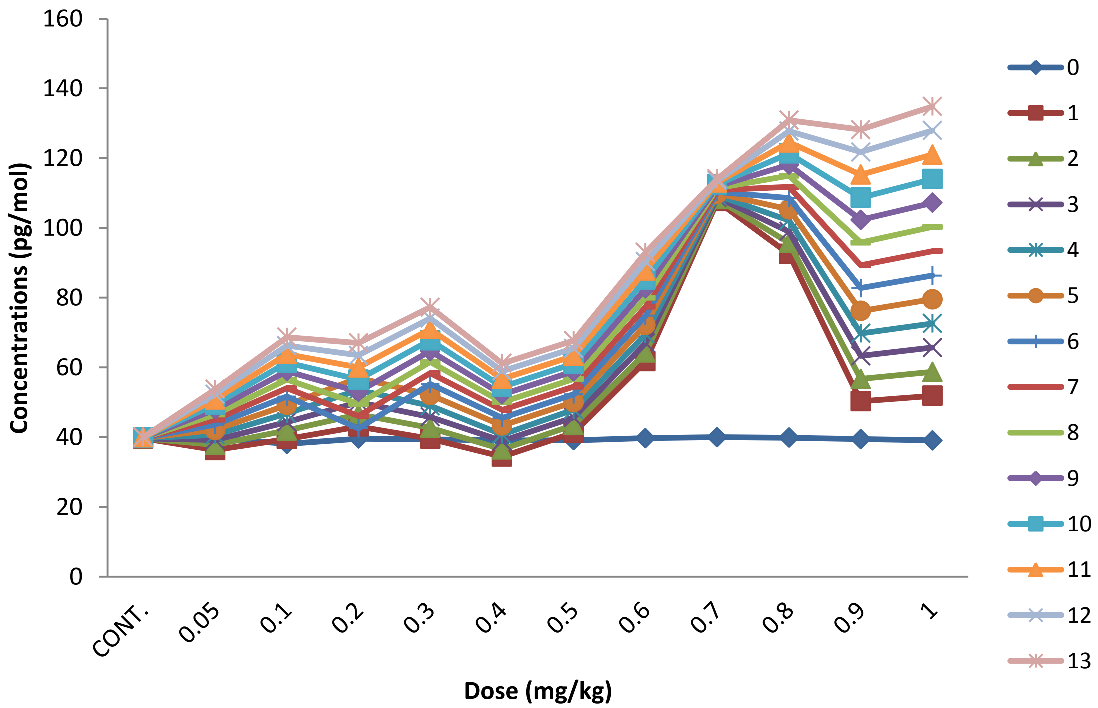

Fig. 81b;Graph of concentration against dose for HNE-LDL level.

4.7.3 RESULT OF PEROXIREDOXINE SYSTEM

- **PEROXIREDOXIN-1 (PRX-1)**

There was significant decrease in prx-1 activity, which was dose dependent across all the weeks at $p \leq 0.05$ when compared to the control (fig 82a). The result for peroxiredoxin-1 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg of BPA revealed dose dependent increases in peroxiredoxin-1 level (fig. 82a). The test groups exposed to 0.5mg/kg to 1mg/kg of BPA showed dose dependent decreases in serum level of peroxiredoxine-1 except that the test groups of 0.7mg/kg and 0.9mg/kg showed increased peroxiredoxine-1 levels relative to 0.8mg/kg and 0.9mg/kg respectively, in weeks 1,2, 10 to 13 (fig 82a). In all instances, the observed increases were lower that of the control (fig. 82a). The test groups of 0.05mg/kg to 0.1mg/kg also showed dose dependent decreases in peroxiredoxin-1 except in weeks 9 to 13, where the test group 0.1 showed higher peroxiredoxin-1 relative to those of 0.05mg/kg (fig. 82a). The test groups of 0.5mg/kg and 0.8mg/kg showed no time sensitivity in the change of peroxiredoxin-1 after the administration of BPA (fig 82b). As the time of exposure increase, the concentration of peroxiredoxine-1 decreases except that the reverse-time dependent effect was observed at the group that were administered 0.1mg/kg to 1mg/kg (fig. 82b).

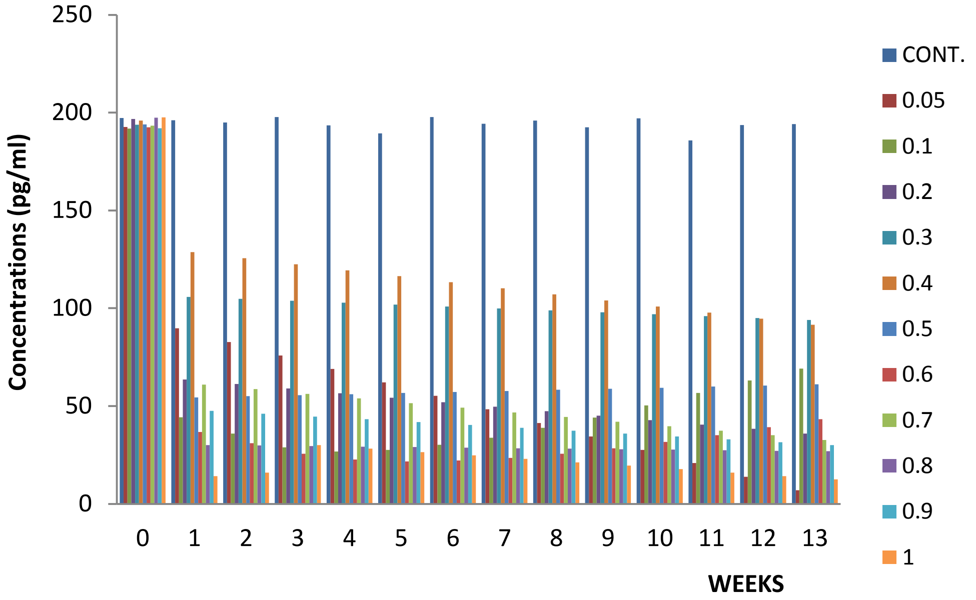

Fig. 82a; Bar chart of concentration against weeks (durations) for peroxiredoxin-1 (PRX-1).

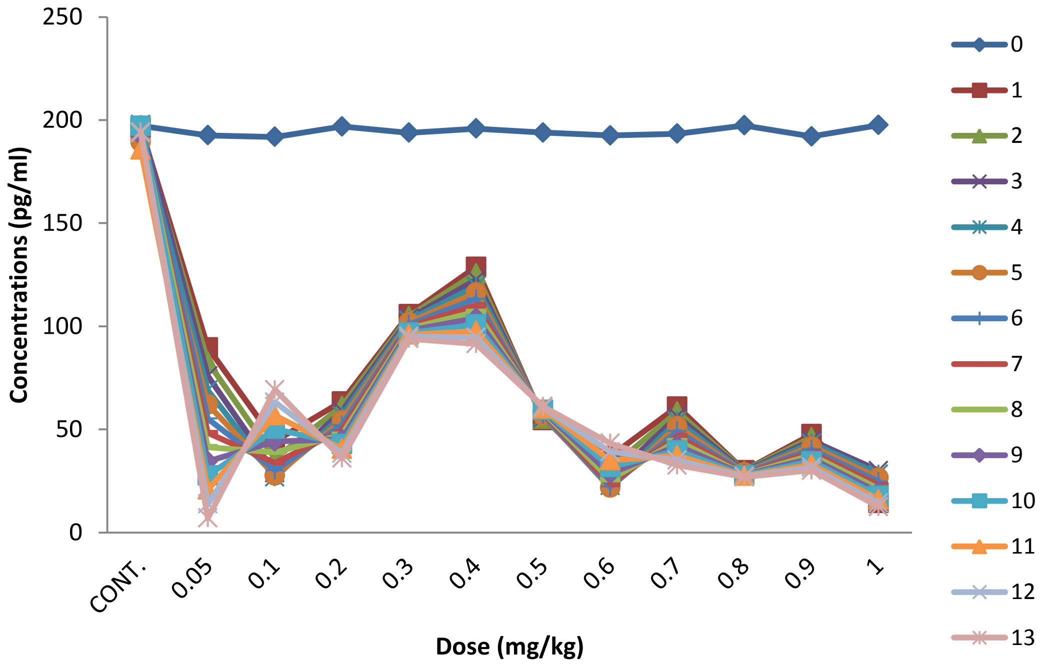

Fig. 82b; Graph for concentration against dose for peroxiredoxin-1 PRX-1.

- **PEROXIREDOXIN-2(PRX-2)**

Although, there appearto be a significant decrease in prx-2, when compared to the control group and week 0 at $p \leq 0.05$ (fig 83a), there was an initial increase at group 2 (0.1mg/kg), then a sharp decrease at group 3(0.2mg/kg), this was followed by a gradual dose dependent increase in prx-2 level at groups 4 (0.3mg/kg) to 11(1mg/kg) (fig 83b).

The result for peroxiredoxin-2 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg and 0.5mg/kg to 1mg/kg of BPA showed dose dependent increases in peroxiredoxin-2 level (fig. 83a). In all instances, the observed increases were lower than that of the control (fig. 83a). The test group of 0.05mg/kg to 0.1mg/kg also showed a dose dependent increase in peroxiredoxin-2 except at week 13 where the test group 0.1 showed lower peroxiredoxin-2 relative to those of 0.05mg/kg (fig. 83a).

The test group of 0.1mg/kg and 0.2mg/kg showed no time sensitivity in the peroxiredoxin-2 level after the administration of BPA (fig 83b). An inverse-time dependent effect was observed at the groups that were administered 0.3mg/kg to 1mg/kg (fig. 83b).

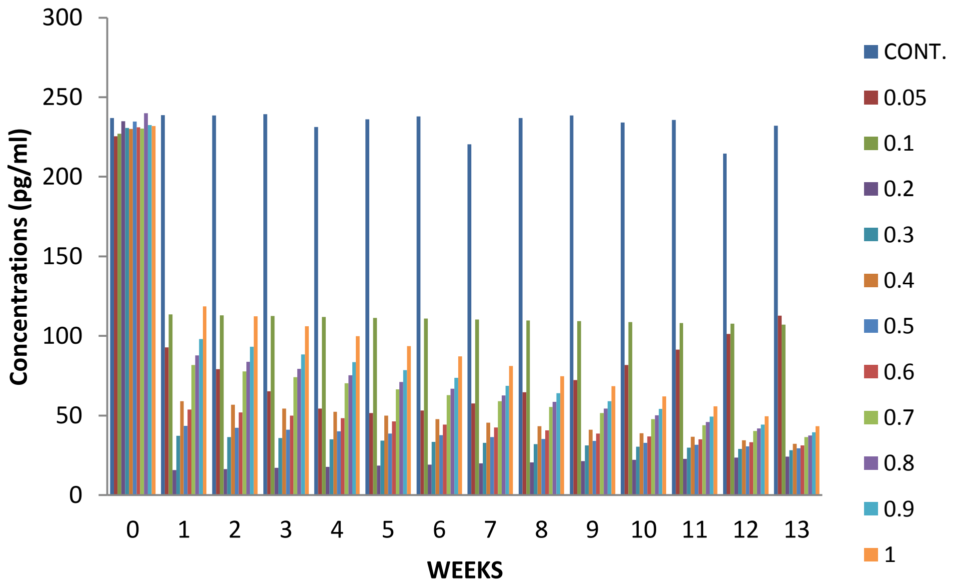

Fig. 83a; Bar chart of concentration against weeks (durations) for peroxiredoxin-2 (PRX-2).

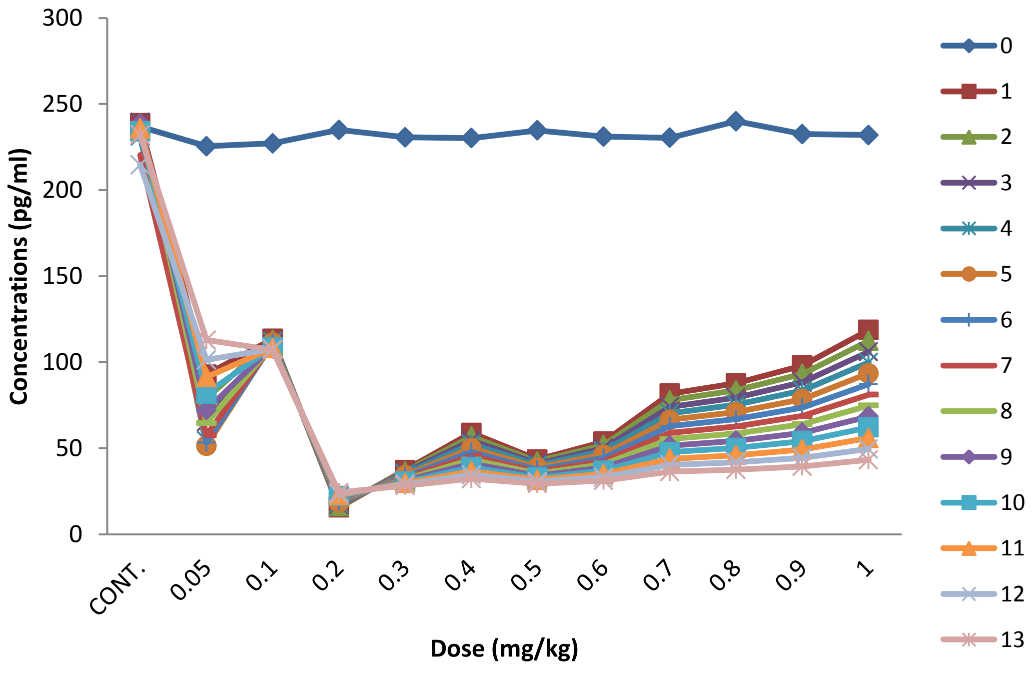

Fig. 83b; Graph for concentration against dose for peroxiredoxin-2 (PRX-2).

- **PEROXIREDOXIN-3(PRX-3)**

From the Graph below, it was observed that the prx-3 serum concentration significantly decreased in comparism to the control group and week 0 at $p \leq 0.05$ (fig 84a). There was an initial fluntuation at the onset of the experiment at groups 1 (0.05mg/kg) to 6 (0.5mg/kg), this was followed by a gradual dose dependent increase in serum prx-3 level at groups 7(0.6mg/kg) to 11(1mg/kg) (fig 84b). The result for peroxiredoxin-3 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg and 0.5mg/kg to 1mg/kg of BPA revealed dose dependent increases in peroxiredoxin-3 level (fig. 84a). In all instances, the observed increases were lower that of the control (fig. 84a). The test groups of 0.05mg/kg to 0.1mg/kg also showed dose dependent increases in peroxiredoxin-3 concentrations (fig. 84a).

The test groups of 0.1mg/kg and 0.2mg/kg showed no time sensitivity in peroxiredoxin-3 level after the administration of BPA (fig 84b). An inverse-time dependent effect was observed at the groups that were administered 0.3mg/kg to 1mg/kg (fig. 84b).

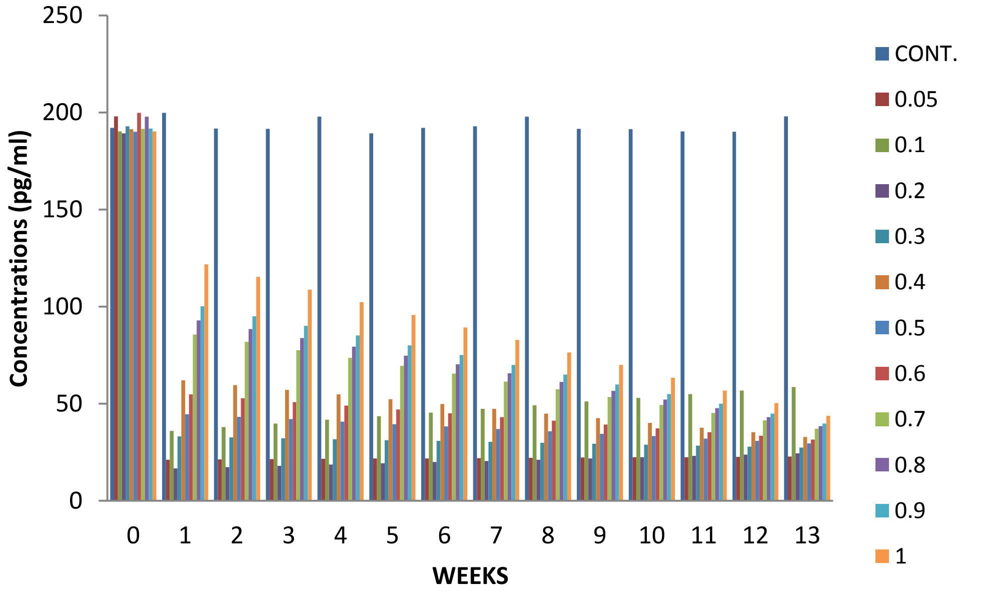

Fig 84a; Bar chart of concentration against weeks (durations) for peroxiredoxin-3 (PRX-3),

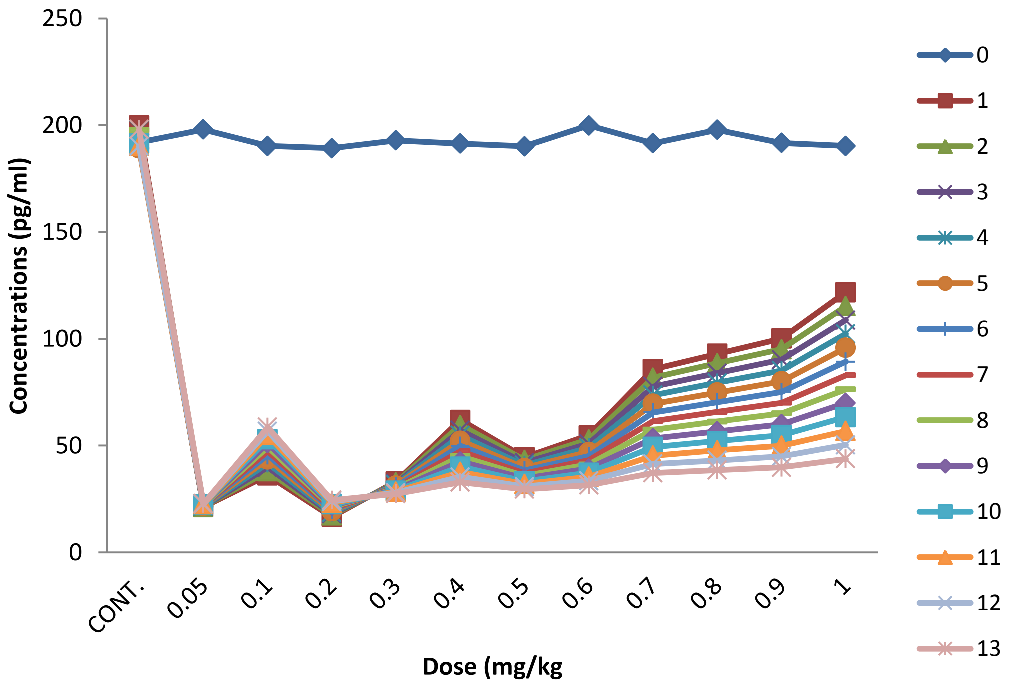

Fig 84b; Graph for concentration against dose for peroxiredoxin-3 (PRX-3),

- **PEROXIREDOXIN-4(PRX-4)**

From the Graph below, it was observed that the prx-4 serum concentration significantly decreased in comparism to the control group and week 0 at $p \leq 0.05$ (fig. 85a) and dose dependent increase in serum prx-4 concentrations (fig 85b). The result for peroxiredoxin-4 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg and 0.5mg/kg to 1mg/kg of BPA revealed dose dependent increases in peroxiredoxin-4 level (fig. 85a). In all instances, the observed increases were lower that of the control (fig. 85a). The test groups of 0.05mg/kg to 0.1mg/kg also showed dose dependent decreases in peroxiredoxin-4 level after the exposure to BPA in the first five (5) weeks of exposure and in weeks 6 to 13 a dose dependent increase in peroxiredoxin-4 was observed (fig. 85a).

The test group of 0.3mg/kg showed no time sensitivity in peroxiredoxin-4 levels after the administration of BPA (fig 85b). An inverse-time dependent effect was observed at the groups that were administered 0.3mg/kg to 1mg/kg (fig. 85b).

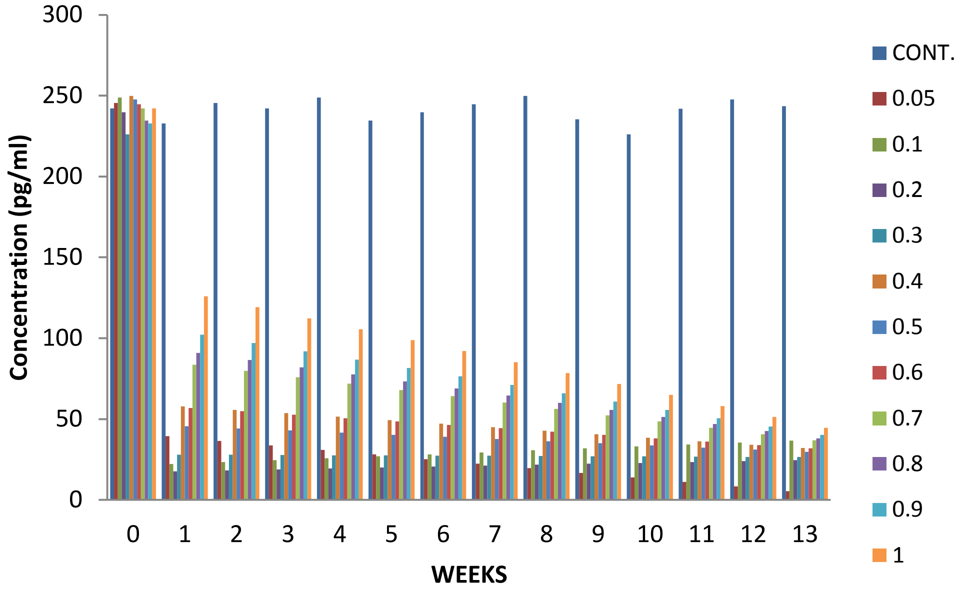

Fig. 85a; Bar chart of concentration against weeks (durations) for peroxiredoxin-4 (PRX-4).

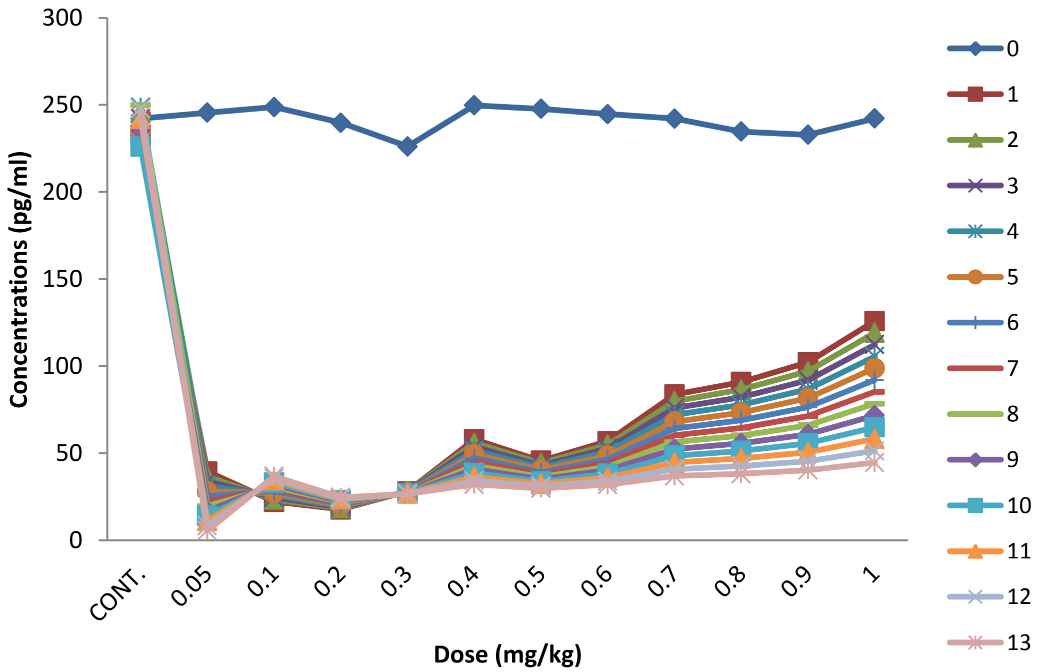

Fig. 85b; Graph for concentration against dose for peroxiredoxin-4 (PRX-4).

- **PEROXIREDOXIN-5(PRX-5)**

There was significant decrease in PRX-5 level when compared with the control at $p \leq 0.05$ (fig.86a), then there was a gradual rise to the peaks at group 11 (fig 67b). The result for peroxiredoxin-5 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg and 0.5mg/kg to 1mg/kg of BPA revealed dose dependent increases in peroxiredoxin-5 level (fig. 86a). In all instances, the observed increases were lower that of the control (fig. 86a). The test groups of 0.05mg/kg to 0.1mg/kg also showed dose dependent decreases in peroxiredoxin-5 level after the exposure to BPA in the first three (3) weeks of exposure and in weeks 4 to 13, dose dependent increases in peroxiredoxin-5 was observed (fig. 86a).

The test group of 0.2mg/kg showed no time sensitivity in peroxiredoxin-5 after the administration of BPA (fig 86b). An inverse-time dependent effect was observed at the groups that were administered 0.3mg/kg to 1mg/kg (fig. 86b).

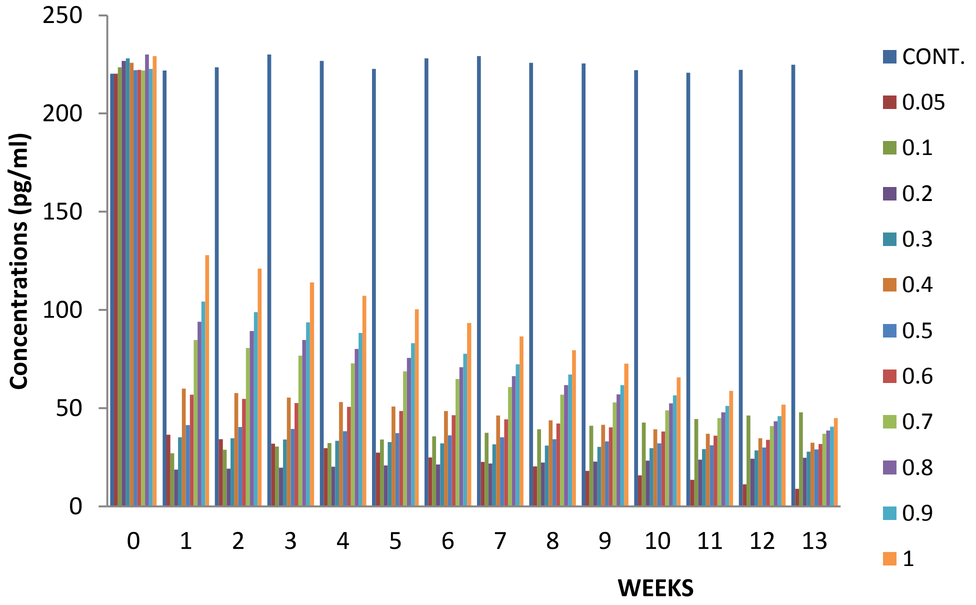

Fig. 86a; Bar chart of concentration against weeks (durations) for peroxiredoxin-5 (PRX-5) level.

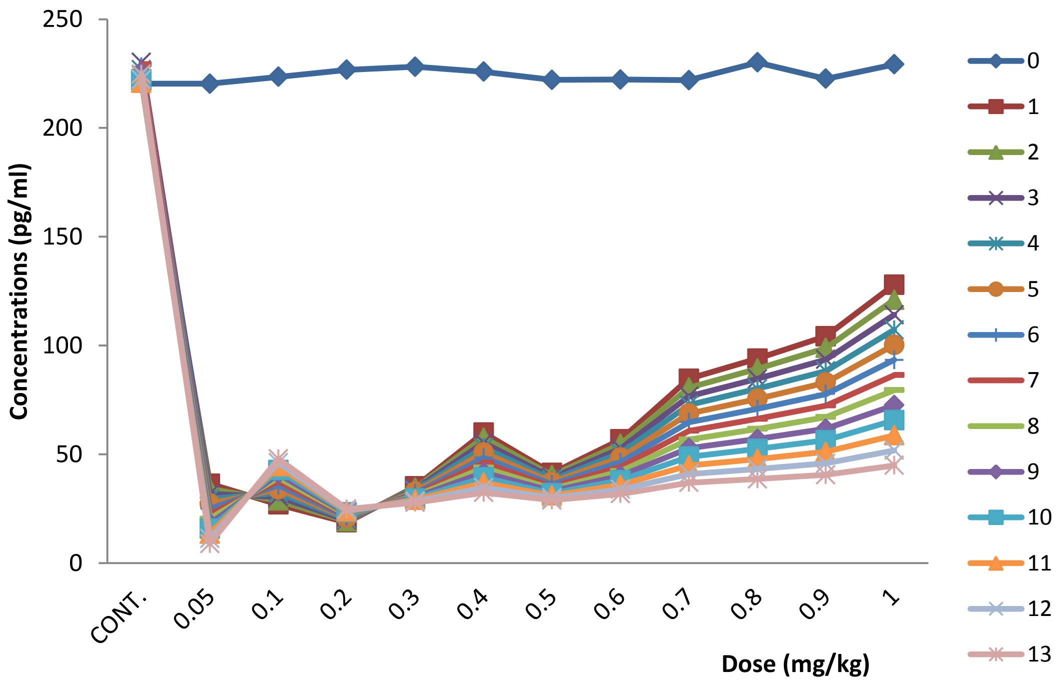

Fig. 86b;Graph of concentration against dose for peroxiredoxin-5 (PRX-5) level.

- **PEROXIREDOXIN-6(PRX-6)**

There was significant decrease in PRX-6 level when compared with the control at $p \leq 0.05$. Very low level of PRX-6 activities was observed compared to control (fig 87b). The result for peroxiredoxin-6 showed that following the exposure to graded doses of BPA, the test groups that were administered 0.2mg/kg to 0.4mg/kg and 0.6mg/kg to 1mg/kg of BPA increased peroxiredoxin-6 level as doses increases (fig. 87a). In all instances, the observed increases were lower that of the control (fig. 87a). The test groups of 0.05mg/kg to 0.1mg/kg also showed dose dependent decreases in peroxiredoxin-6 level after the exposure to BPA in the first two (2) weeks of exposure and in weeks 3 to 13, a dose dependent increase in peroxiredoxin-6 was observed (fig. 87a). The test group exposed to 0.5mg/kg of BPA showed increased level of prx-6 relative to those of 0.6mg/kg (fig. 87a).

The test group of 0.2mg/kg showed no time sensitivity in peroxiredoxin-6 level after the administration of BPA (fig 87b). An inverse-time dependent effect was observed at the groups that were administered 0.3mg/kg to 1mg/kg (fig. 87b).

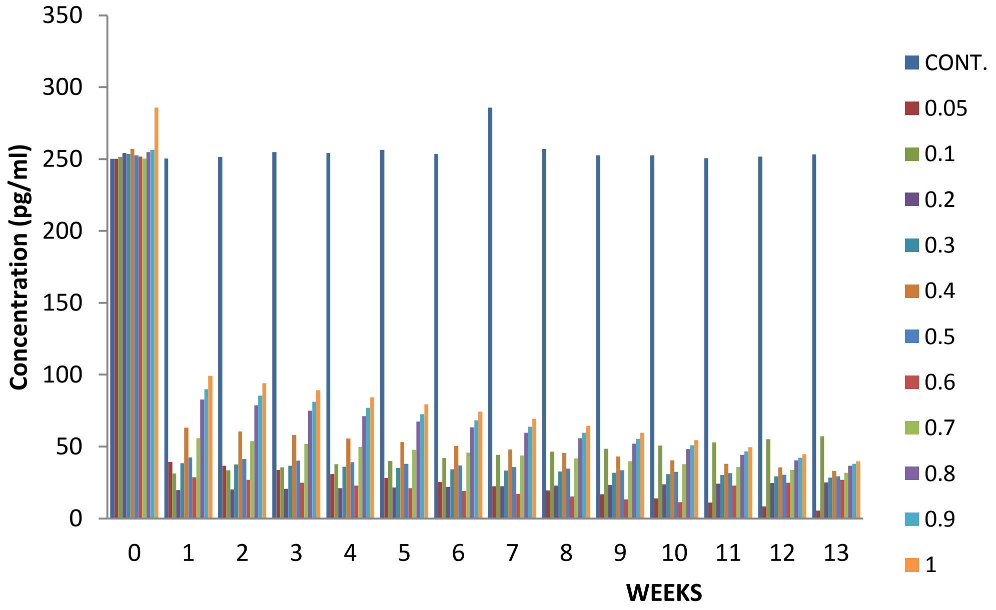

Fig. 87a; Bar chart of concentration against weeks (durations) for peroxiredoxin-6 (PRX-6) level.

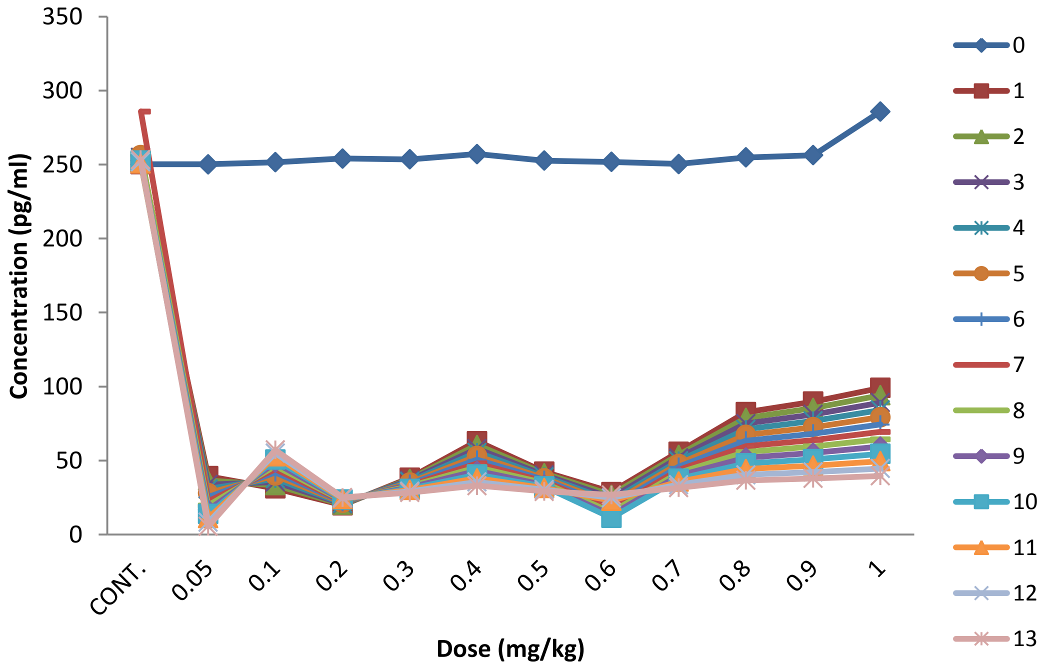

Fig. 87b;Graph of concentration against dose for peroxiredoxin-6 (PRX-6) level.

4.7.4 RESULT OF GLUTATHIONE SYSTEM

- **GLUTATHIONE REDUCTASE**

There was significant decrease in the glutathione reductase level of groups 3(0.2mg/kg) to 9(0.8mg/kg) when compared with the control and week 0 at $p \leq 0.05$. Group 1 (0.05mg/kg), and 10(0.9mg/kg) showed significant increases (fig 88a). Throughout the duration of the study, the test group that received 0.05mg/kg showed higher level of glutathione reductase relative to that of 0.1mg/kg (fig.88a). The groups exposed to 0.1mg/kg, 0.8mg/kg and 1mg/kg of BPA showed no sensitivity to time of exposure (fig.88b).

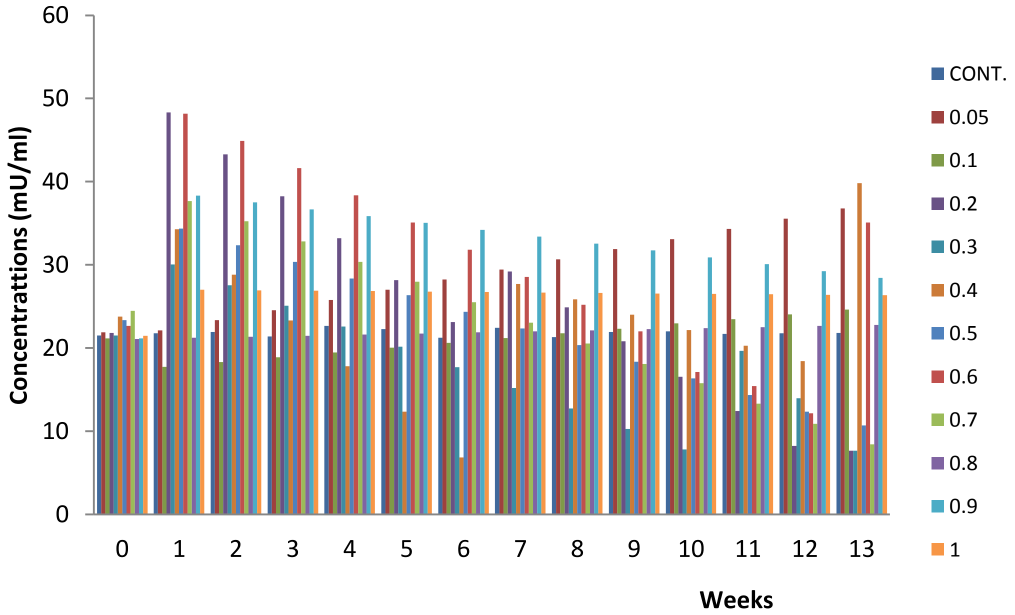

Fig. 88a; Bar chart of concentration against weeks (durations) for glutathione reductase level.

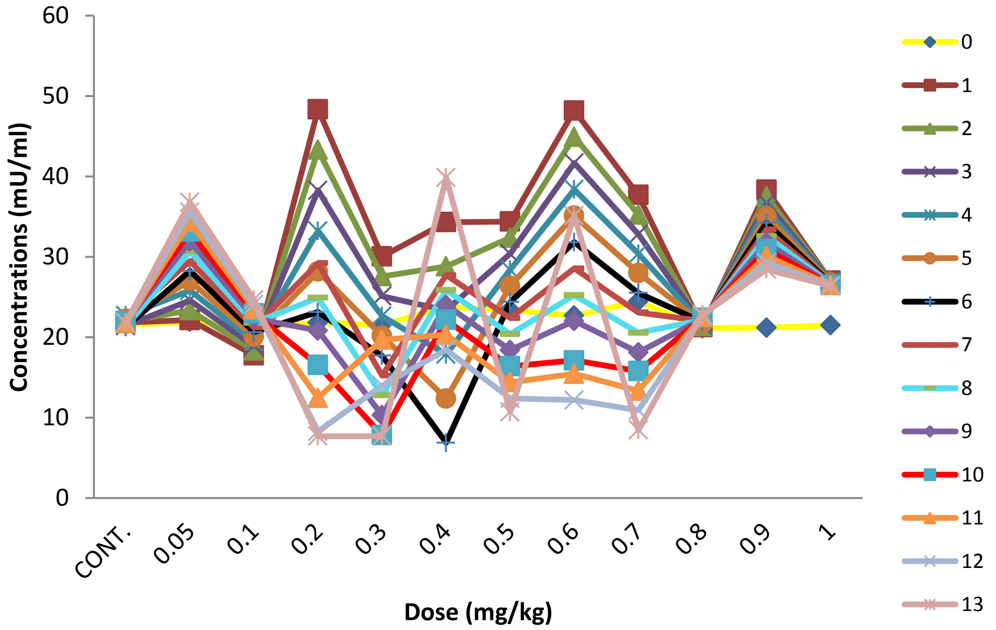

Fig. 88b;Graph of concentration against dose for glutathione reductase level.

- **GLUTATHIONE PEROXIDASE**

There was significant dose dependent decrease in the glutathione peroxidase level when compared with the control and week 0 at $p \leq 0.05$ (fig 89a). In weeks 1 to 6, the test group that was exposed to 0.05mg/kg of BPA showed higher level of glutathione peroxidase relative to that of 0.1mg/kg, but weeks 7 to 13, the reversed effects were observed (fig.89a). In weeks 1 to 10 and 12, the test group that was exposed to 0.04mg/kg of BPA showed higher level of glutathione peroxidase relative to that of 0.3mg/kg, but weeks 11 and 13, the reversed effects were observed (fig.89a). Throughout the exposure period, the test group that was exposed to 1mg/kg of BPA showed lower serum level of glutathione peroxidase relative to that of 0.9mg/kg (fig.89a). Only the group exposed to 0.05mg/kg of BPA showed a clear time sentivity effect and decreases with time of exposure (fig.89b). The groups exposed to 0.5mg/kg, 0.6mgg/kg, 0.8mg/kg and 0.9mg/kg showed no sentivity to time of exposure (fig. 89b).

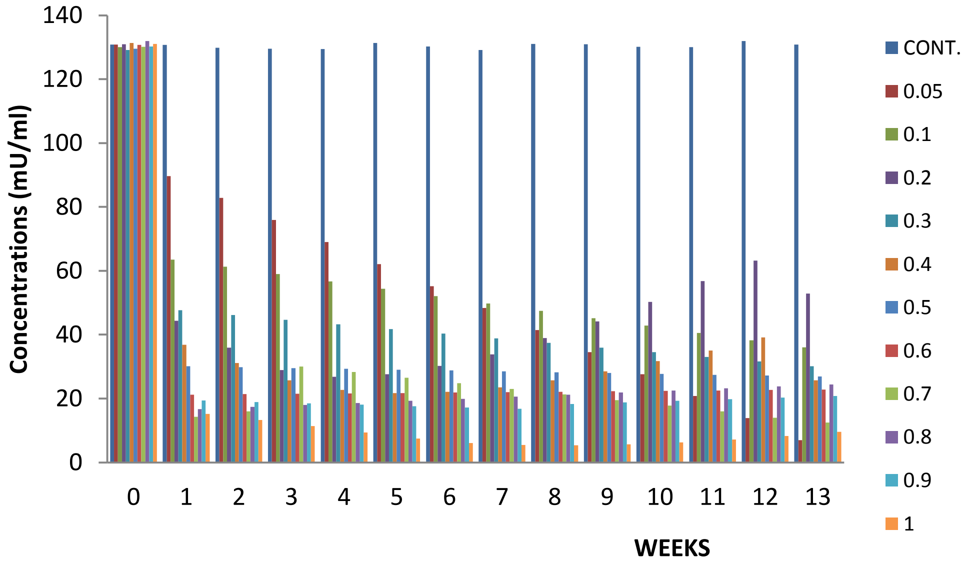

Fig 89a; Bar chart of concentration against weeks (durations) for glutathione peroxidase level.

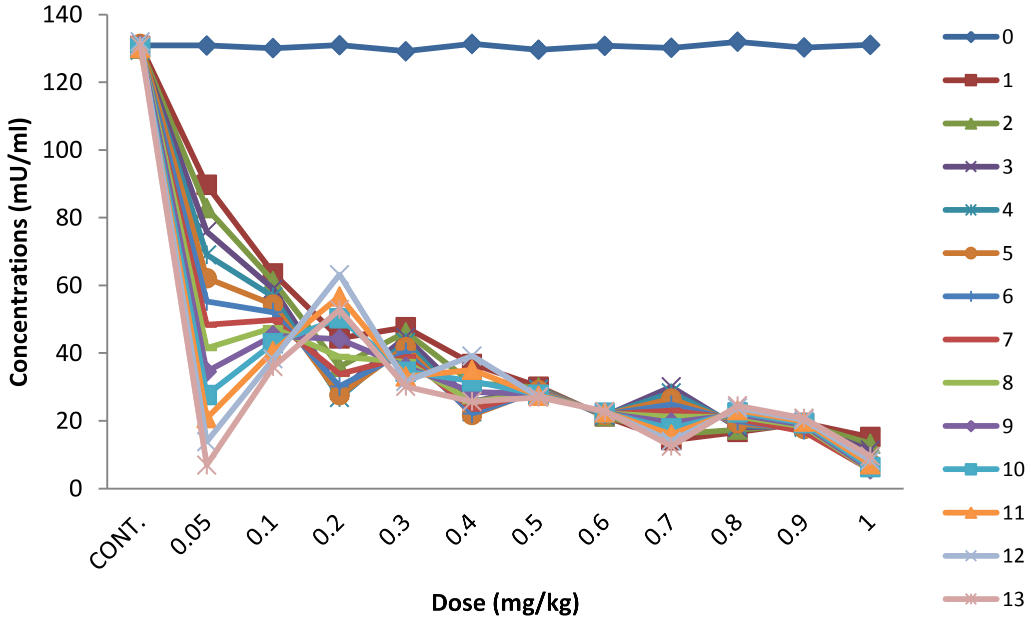

Fig 89b;Graph of concentration against dose for glutathione peroxidase level.

- **TOTAL GLUTATHIONE**

There was significant increase in the total glutathione level when compared with the control at $p \leq 0.05$, except for group 6(0.5mg/kg) (fig 90a). Total glutathione result showed that the groups that were administered 0.5mg/kg to 1mg/kg of BPA revealed dose dependent increases in total glutathione except in weeks 10 to 13, where the group exposed to 0.8mg/kg showed a decrease in total glutathione level relative to those exposed to 0.7mg/kg of BPA (fig. 90a). The groups exposed to 0.05mg/kg to 0.2mg/kg also showed dose dependent increases in total glutathione, which was consistent over time (fig. 90b). The test groups 0.3mg/kg, 0.9mg/kg and 1mg/kg BPA showed no sensitivity to time (fig. 90b).

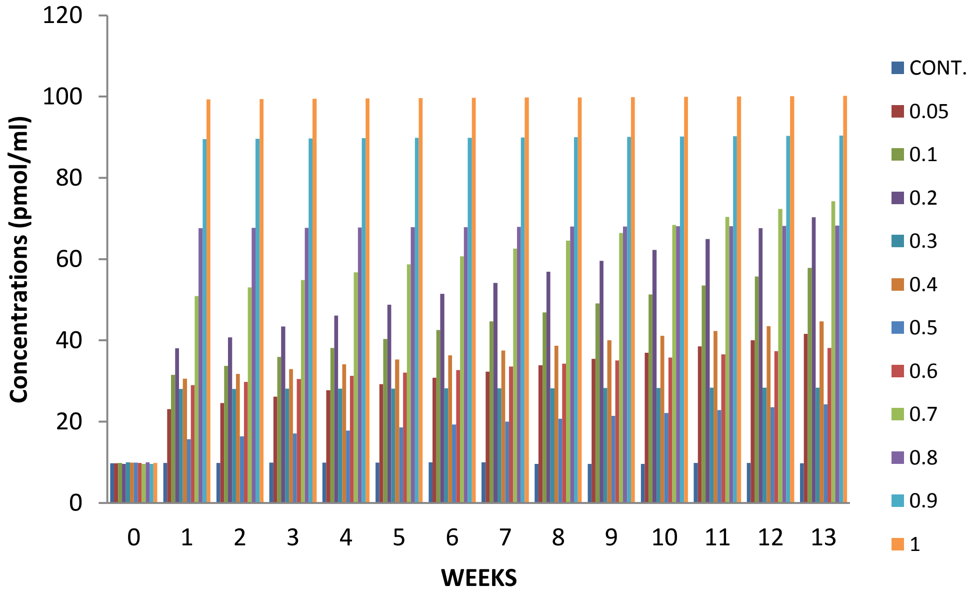

Fig. 90a; Bar chart of concentration against weeks (durations) for total glutathione level.

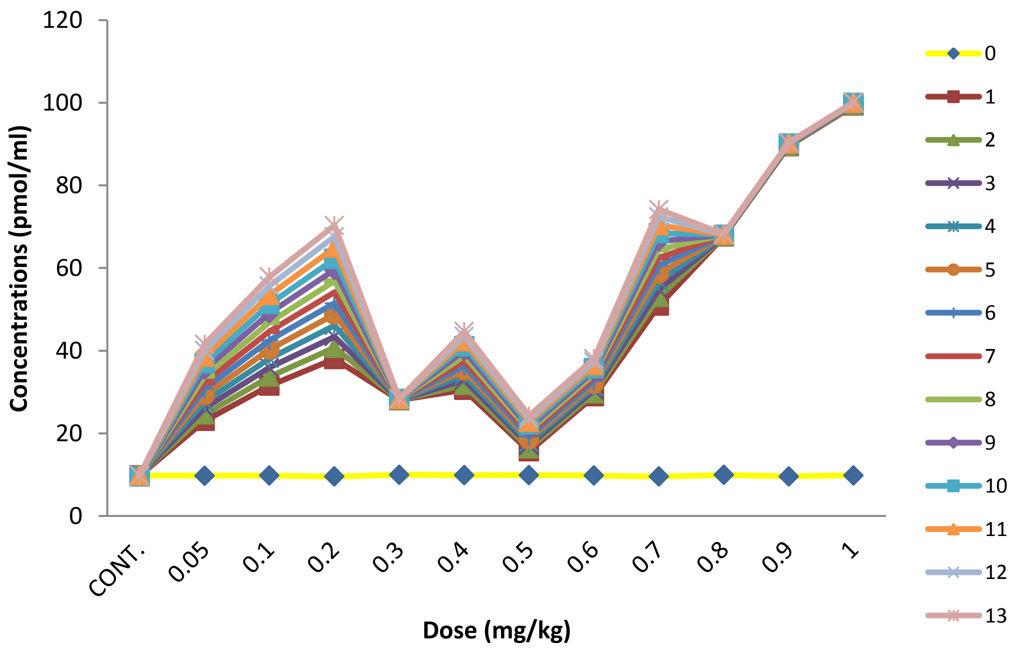

Fig. 90b;Graph of concentration against dose for total glutathione level.

- **OXIDISED GLUTATHIONE**

There was significant increase in the oxidised glutathione level when compared with the control at $p \leq 0.05$ (fig 91a). The result of oxidised glutathione level revealed that the test group that was administered 0.05mg/kg of BPA showed lower oxidised glutathione relative to that exposed to 0.1mg/kg of BPA, except in week 9 where the reversed was observed. (fig. 91a). In the first five weeks (1-5) of exposure, the test groups exposed to 0.2mg/kg to 0.9mg/kg showed dose dependent increases in the serum level of oxidised glutathione, with their 1mg/kg exposure group showing a decrease in oxidised glutathione level relative to that of 0.9mg/kg (fig.91a) , also in week 5, the 0.5mg/kg exposure group showed an increase in the oxidised glutathione level relative to that of 0.6mg/kg exposure group (fig. 91a). In weeks 6 to 13, the test groups that received 0.2mg/kg to 0.5mg/kg and 0.6mg/kg to 1mg/kg of BPA revealed dose dependent increases in the serum concentration of oxidised glutathione (fig.91a). The group exposed to 0.9mg/kg of BPA showed increases in the level of oxidised glutathione at the first five weeks (1-5) of exposure, while the exposure group of 1mg/kg showed the maximum level of oxidised glutathione in weeks 6 to 13 (fig. 91a). The test groups exposed to 0.2mg/kg to 0.4mg/kg and 0.6mg/kg to 0.9mg/kg showed no sensitivity to time of exposure (fig. 91b). The test groups exposed to 0.05mg/kg, 0.1mg/kg, 0.5mg/kg and 1mg/kg showed time sensitivity and time dependent effect except that the 0.05mg/kg exposure group showed peak in week 9 of the study (fig.91b).

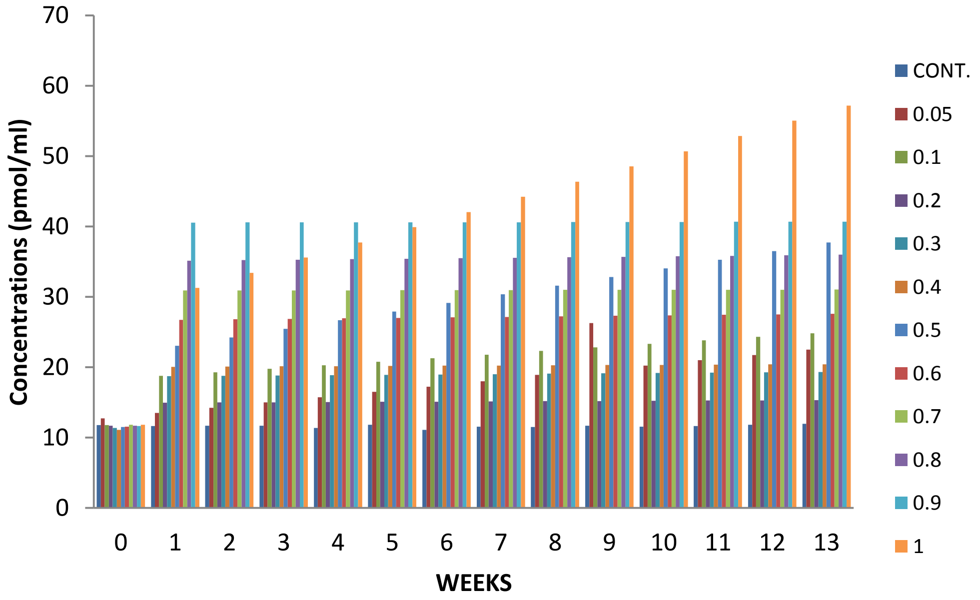

Fig. 91a; Bar chart of concentration against weeks (durations) for oxidised glutathione level.

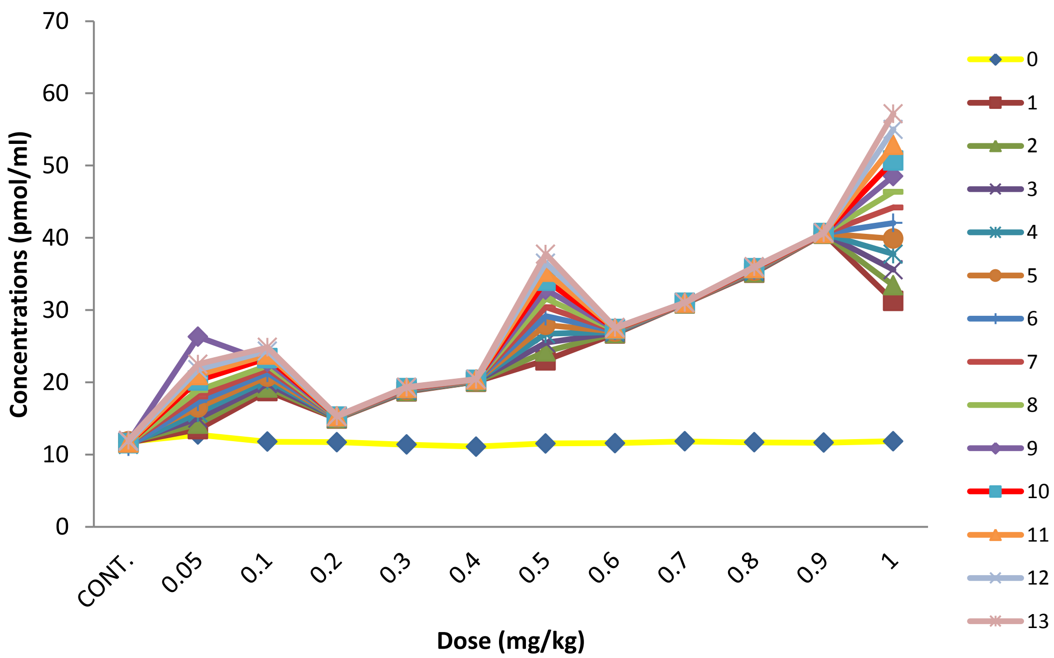

Fig. 91b;Graph of concentration against dose for oxidised glutathione level.

- **REDUCED GLUTATHIONE**

There was significant decrease in the reduced glutathione level of the entire exposure groups when compared with the control at $p \leq 0.05$, (fig 92a). The result of reduced glutathione revealed that the test group exposed to 0.05mg/kg of BPA showed lower serum level of reduced glutathione relative to those of 0.1mg/kg (fig. 92a). The groups that received 0.4mg/kg to 0.6mg/kg of BPA showed dose dependent decreases while the groups that received 0.7mg/kg to 1mg/kg of BPA showed dose dependent increases except in weeks 10 to 13, where those of 1mg/kg dropped relative to 0.9mg/kg exposure group (fig. 92a). The test groups that were exposed to 0.3mg/kg, 0.8mg/kg and 0.9mg/kg of BPA showed no sensitivity to time of exposure (fig. 92b). The test groups exposed to 0.05mg/kg to 0.2mg/kg and 0.4mg/kg to 0.7mg/kg showed increased reduced glutathione over time, While those of 1mg/kg showed a decrease in reduced glutathione over time (fig. 92b).

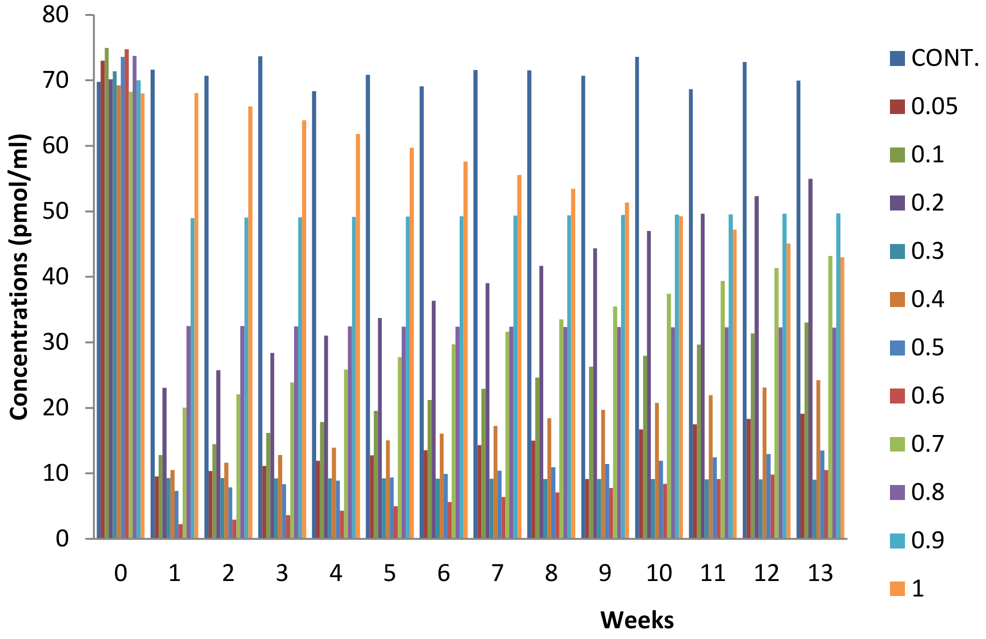

Fig. 92a; Bar chart of concentration against weeks (durations) for reduced glutathione level.

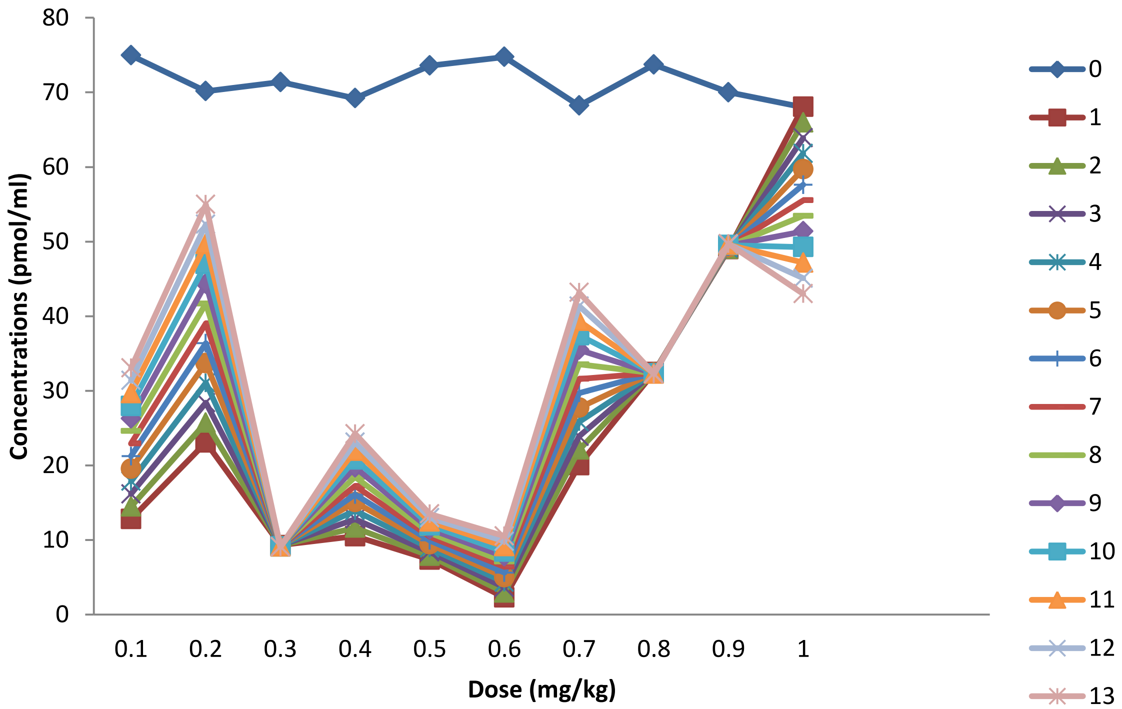

Fig. 92b;Graph of concentration against dose for reduced glutathione level.

4.7.5 RESULT OF THIOREDOXIN SYSTEM

- **THIOREDOXINE**

There was significant dose dependent and time dependent increase in the thioredoxin level when compared with the control at $p \leq 0.05$, except for group 11(1mg/kg) that was not time dependent (fig. 93a). Group 1(0.05mg/kg) showed no significant difference in the first five weeks (fig 93b). The group exposed to 0.05mg/kg of BPA showed a decreased in serum concentration of thioredoxin relative to that of 0.1mg/kg exposure group throughout the time of the study (fig. 93a). Also, the groups that were administered 0.7mg/kg to 1mg/kg of BPA, showed dose dependent increases in the level of thioredoxin throughtout the period of exposure (fig. 93a). In weeks 1 to 10 of the exposure, the group exposed to 0.7mg/kg of BPA showed a lower level of thioredoxin, while in weeks 11 to 13, the reversed effects were observed (fig. 93a). All the exposure group revealed a time sensitive effect and showed the highest concentration of thioredoxin in week 13 (fig.93b).

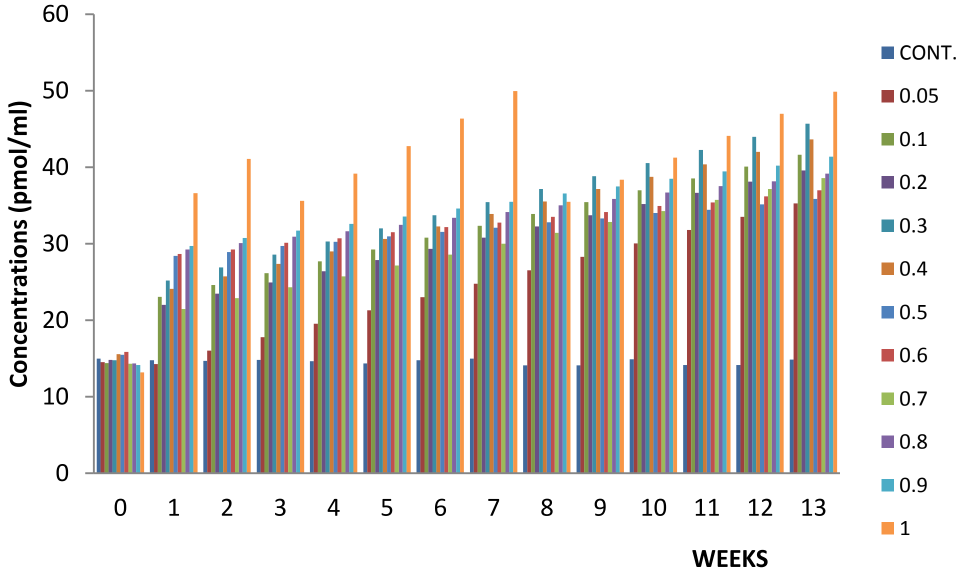

Fig. 93a; Bar chart of concentration against weeks (durations) for thioredoxin level.

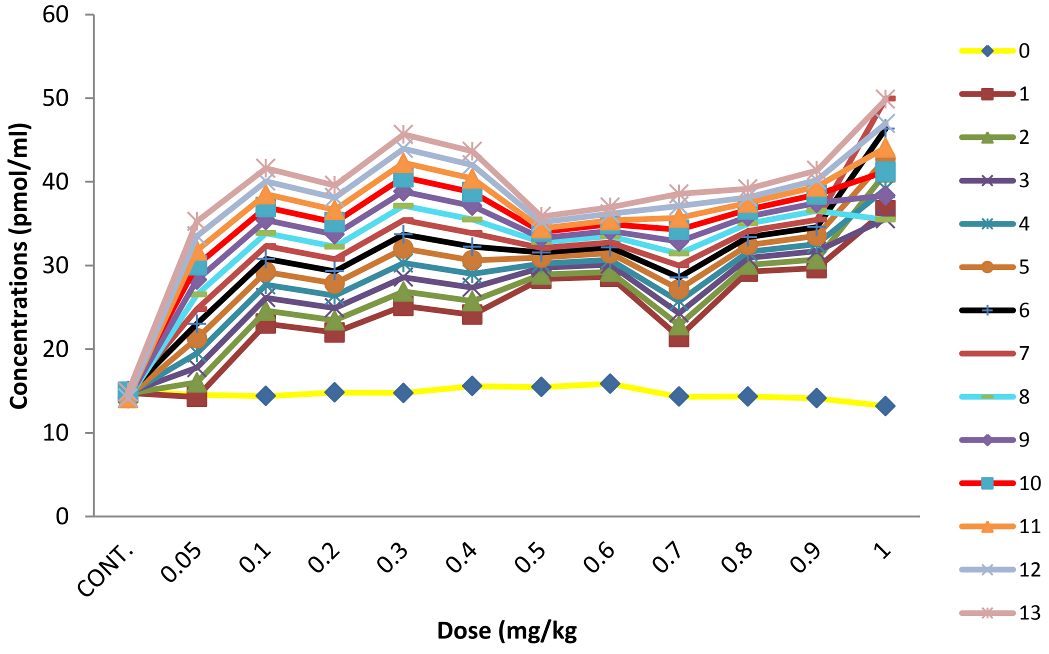

Fig. 93b;Graph of concentration against dose for thioredoxin level.

- **THIOREDOXINE REDUCTASE**

There was significant decrease in the thioredoxin reductase level when compared with the control at $p \leq 0.05$ (fig 94a). In weeks 9 to 12, a dose dependent decrease in serum concentration of thioredoxin reductase was shown by the groups exposed to 0.05mg/kg to 0.4mg/kg of BPA except in weeks 11 and 12, where the 0.3mg/kg exposure showed an increase in the thioredoxin reductase level relative to that of 0.2mg/kg exposure group (fig. 94a). Throughout the exposure duration, the group exposed to 0.4mg/kg of BPA showed a lower serum level of thioredoxin reductase relative to that of 0.3mg/kg exposure group (fig.94a). All the exposure group showed time sensitivity effect and a non time dependent effect (fig.94b).

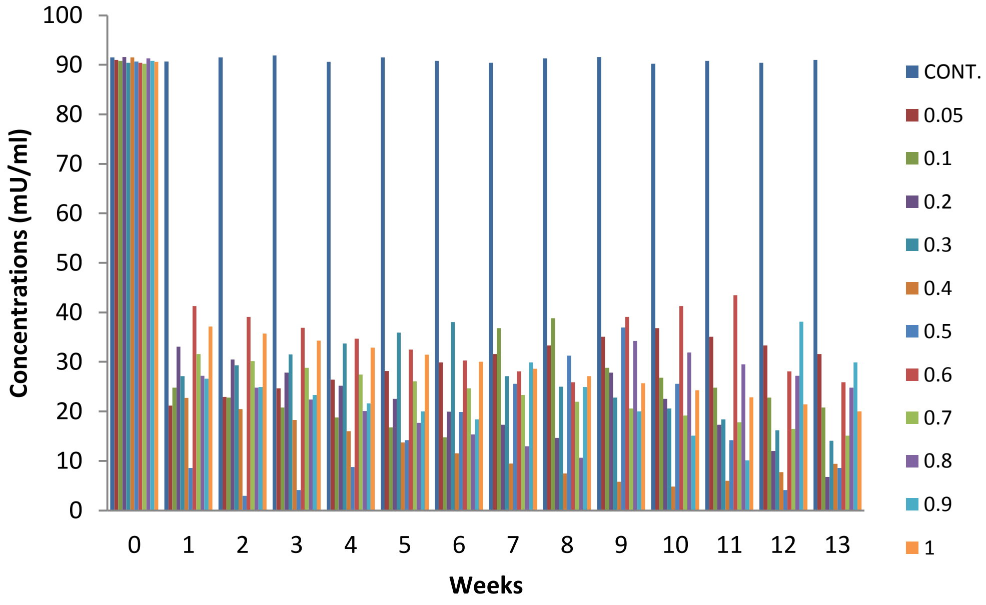

Fig. 94a; Bar chart of concentration against weeks (durations) for thioredoxin reductase level.

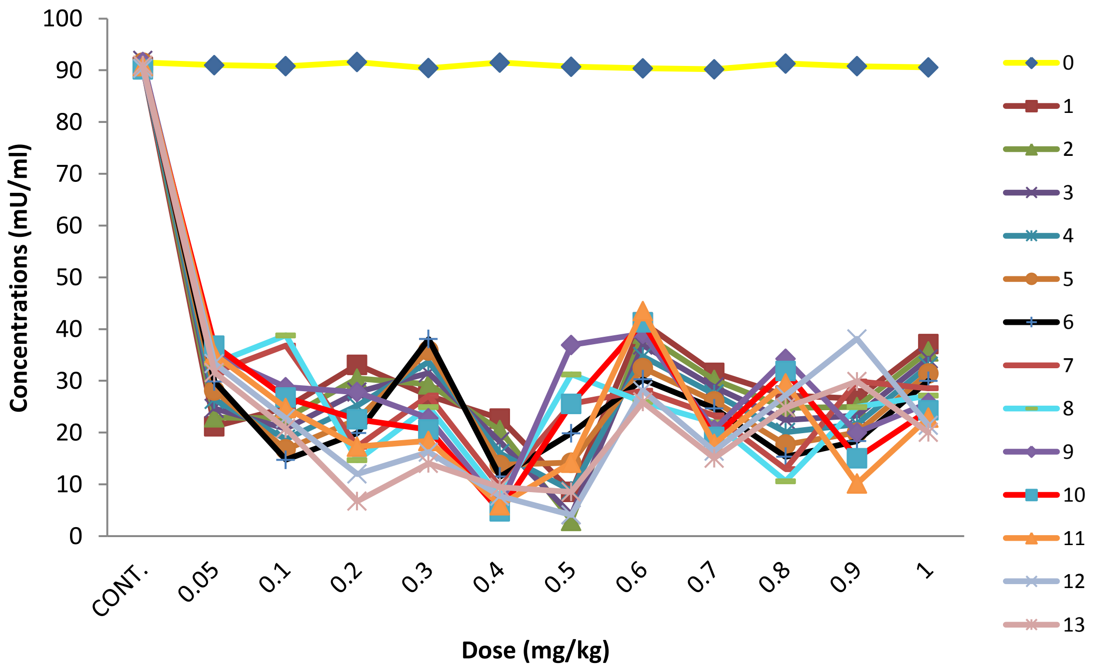

Fig. 94b;Graph of concentration against dose for thioredoxin reductase level.

CHAPTER FIVE

DISCUSSIONS AND CONCLUSION

5.1 DISCUSSION

All the bisphenol A treated animals showed treatment related clinical signs such as, soft stool, and frequent urination. Irregular movements and unrest tremor were observed in bisphenol A treated rats.

The result of the median lethal dose (LD_{50}) of Bisphenol A was obtained as shown on table 4, for all the methods used on both the male and female rats, after oral and subcutaneous administration. The results indicated that the LD_{50} of Bisphenol A obtained for the male rats were higher than those of the female species in all cases for the oral and subcutaneous route of administration. Comparing the effect of the method on the LD_{50} of the rats, it was observed that there is no much difference between the three methods used for both the male and the female rats for both the oral and subcutaneous route of BPA administration. The LD_{50} for male rats are 2828.43, 2825.00, 2800 mg/kg (BW) and that for female rats 2144.76, 2166.67, 2160 mg/kg (BW) for oral administration. Following subcutaneous administration, the LD_{50} for the male rats 244.949, 235.42, 240 mg/kg (BW) and that of the female rats 163.10, 170.00, 160 mg/kg (BW) using Lorke's, Karber's and Up and Down methods respectively.

The LD_{50} values of Bisphenol A found in some publications were different. Earlier reports recorded LD_{50} value for BPA as 4100 mg/kg (BW) by NTP (1982); 3300-4240 mg/kg (BW) by Morrissey *et al*., (1997) and Goodman *et al*., (2006); 3,250 mg/kg (BW) by Chapin *et al.*, (2008) and WHO, (2010); 3228 mg/Kg by Preethis *et al.*, (2014). LD_{50} value published in some Material Safety Data Sheet of Societies producing Bisphenol A as Sigma Aldrich was >2000mg/kg of Bisphenol A by oral route. Also, Oral LD_{50} values beyond 2 000 mg/kg are indicated in the rat and mouse, and dermal LD_{50} values above 2 000 mg/kg are evident in the rabbit.

The present data revealed an increase in body weight of animals at the onset of the experiment; this was followed by a gradual decrease in the weight as the duration of the research progresses. The decrease in body weight was without effect on the feeding. These results are consistent with those of other investigators who showed that BPA exposure increased body weight relative to controls (Rubin *et al.*, 2016; Miyawaki *et al.*, 2007). Several investigators confirmed that BPA has a role in weight gain and it was implicative to the development of obesity (Shankar *et al.*, 2013). Howdeshell *et al*., (1999), showed that BPA provoked an increase in body weight. Evidence showed an increase in body weight as BPA administration (Rubin *et al*., 2016). In another study, Miyawaki *et al*., (2007) showed increased body and adipose tissue weight. Exposure to BPA also results in accelerated body weight gain (Patisaul and Bateman, 2008). Yang *et al.,* (2016) showed increased body weight and fat mass in both male and female mice exposed to BPA. At higher dose, Hassan *et al.*, (2012), reported a decrease in the body weight. Another study showed a decrease in body weight in the group treated with ≤466 mg/kg/day BPA (Yamasaki *et al.*, 2002). Another, 90-days studies in rats, Til *et al.*, (1978), showed a decrease in body weight gain and minor changes in organ weight, without any effects on food consumption (Tyl *et al.*, 2008).

In vitro studies pointed out that BPA stimulates increase in lipid accumulation in the differentiating adipocytes and upregulates the expression of adipocyte proteins through the activation of glucocorticoid receptor. Interestingly, it is known that BPA inhibits adiponectin release from adipose tissue, an important adipokine that protects from the metabolic syndromes

(Ben-jonathan *et al.*, 2009). This suggests that BPA can cause an alteration in adipogenesis, suggesting that BPA could be a potential environmental obesogen. Another possible explanation of enhanced weight gain in BPA-exposed animals is an increase in food intake as the estrogenic action of BPA have been shown to have effect on neuronal circuits that control appetite, thereby inducing hunger and excessive food consumption at lower doses.

The relationship between Bisphenol-A (BPA) exposure and reproductive hormone levels among the female rats were studied as shown in figures 21 to 29. The result obtained showed increase in levels of estrogen, estrone, prolactin, progesterone, testosterone, leuteinizing hormone and androstenedione, while estrial and follicle stimulating hormone were decreased.
Maohua *et al.,* (2015), study that there was showed a significant positive association between urine BPA level and serum Prolatin, E2 and Progesterone concentrations with decrease in FSH. BPA has been known to be associated with an increase in serum PRL levels (Peretz *et al.,* 2014), Increased testosterone synthesis and increased estradiol levels, (Zhou *et al.,* 2008). Similarly, treatment with BPA induced hyperprolactinemia in rats (Goloubkova *et al.,* 2010). Maohua *et al.,* (2015) reported increased progesterone level in occupationally exposed female workers, Tohei *et al.,* (2001) reported that plasma concentrations of LH were increased significantly and Zhou *et al.,* (2013) observed that the serum AD concentration in BPA exposed workers was lower than that in unexposed workers, and that the serum BPA concentration was inversely associated with the serum AD level. Also, Maohua *et al.,* (2015) showed the associations between BPA, low FSH and high LH. Zhai *et al.,* (2014), sugests that BPA exposure is found to be linked to higher PRL level among adult females. In another study, BPA exposed women showed luteinizing hormone excess (Hampton 2013). Kandaraki *et al.,* (2011) reported an association between serum BPA levels, increased testosterone and androstenedione levels in women. In rodent studies, perinatal (Xi *et al.,* 2011) and postnatal (Fernández *et al.,* 2010; Tan, *et al.,* 2013) low dose BPA exposure increased serum estradiol levels. Further, low doses of BPA increased testosterone and progesterone levels (Fernández *et al.*, 2010; Tan *et al.*, 2013) and decreased serum follicle stimulating hormone (FSH) (Fenichel *et al*., 2013). Galloway *et al.*, (2010) found that higher daily BPA excretion was associated with a higher total testosterone concentration. Takeuchi and Tsutsumi (2012) also found significant positive correlations between serum BPA concentrations, total testosterone and free testosterone (FT) levels. Zhou *et al.,* (2013) study observed inverse associations between serum BPA concentrations, serum androgen (AD) and FT levels. The serum BPA concentration was positively associated with the serum sex hormone binding globulin (SHBG) level. Vitku *et al.,* (2015) observed increased E2 and E1 in the plasma.

The above study suggests that BPA exposure may alter the reproductive hormones and thus implies that BPA can alter steroid hormone pathways. Exposure to BPA was evident in impaired steroidogenesis and decrease in the number of steroid receptors (Peretz *et al.,* 2014). Low dose BPA exposure led to disruption of estrous cyclicity (Adewale *et al.,* 2009). The data from this study indicate that BPA exposure adversely alters sex hormone levels; these data strongly suggest that BPA might have negative effects on the gonadal function through changes in steroid synthesizing cells and enzymes, which then might affect steroid synthesis and circulating steroid levels. The effects could also be as a result of altered expression of steroidogenic enzymes as suggested by Nanjappa *et al.,* (2012). Another possible explanation is that the metabolism of E2 could be also protracted by the interaction of BPA with enzymes involved in steroid conjugation such as estrogen glucuronidase or sulfotransferase. This is of concern because they are considered in pathologic condition that may lead to ovulatory problems. Again, there is also evidence that

BPA interfere with receptor ligand binding for most sex hormones such estrogen, LH etc, as reported by Rajakumar *et al.,* (2015). BPA act as an androgen antagonist that interrupts normal androgen receptor binding activity and thereby alters the interaction between androgen receptor and androgen (Wetherill *et al.,* 2007). BPA has been reported to interfere with the function of leydig cells (Akingbemi *et al.,* 2004).
Furthermore, BPA competes with sex steroids for sex hormone binding globulin (SHBG). BPA simulate estrogen, and thus stimulates increase in the level of serum prolactin, because estrogen has a direct role in stimulating prolactin gene expression (Ehrlich *et al.,* 2013), which promote prolactin synthesis and release (Rajakumar *et al.,* 2015). BPA activate PRL gene through the estrogen response element near the prolactin gene 5′ end of enhancer after it has bound with the receptor as though it was estrogen (E2). Interestingly, BPA also bind to membrane estrogen receptor (mER), and these membrane-bound receptors are capable of nongenomic steroid actions (Peretz *et al.,* 2014). The pituitary cells in which mER are found and are expressed, respond to low levels of BPA exposure by causing a calcium flux which leads to PRL release (Mok-Lin *et al.,* 2010). BPA can also induce prolactin gene expression and cell proliferation in primary anterior pituitary cells (Zhai *et al.*, 2014). Although epidemiological studies on BPA suggests that both low and high doses of BPA increase plasma estradiol, testosterone, and corticotropin releasing hormone levels due to an increase in mRNA expression of corticotrophin releasing hormone and activation of protein kinase C (Tan *et al.,* 2013). The mechanism underpinning the ability of BPA to exert these effects may be found in the fact that BPA alters the expression of steroidogenic enzymes (Horstman *et al.*, 2012; Nakamura *et al.,* 2010) and alters follicle-stimulating hormone levels, which are required to stimulate steroidogenesis (Qiu *et al*., 2013).

Suggestively, BPA has effect on sex hormone such estrogenic and androgenic effects, and BPA exposure is implicative in a variety of adverse effects on reproductive system including altered steroidogenesis, and altered ovarian steroidogenic gene. As a powerful endocrine disruptor, it affects the activity of pituitary lactotroph cells, and cause estrogen responsiveness, induces hyperprolactinemia. BPA may stimulates cell proliferation, stimulates uterine, vaginal, and mammary growth and differentiation *in vivo*. The present study has added to the evidences that BPA is also disruptive to women hormone homeostasis. Together, these data raised concerns about the potentially deleterious impact of BPA on human reproductive health. Public awareness of on these deleterious effects should be strengthened and protection against BPA exposure should be promoted.

In this research, it was observed that the thyroxine stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4) level were higher than that of the control, while the bound thyroxine was low compared to the control. The free triiodothyronine (FT3) and total triiodothyronine (TT3) were initially low at group 1 but at the other doses it was on the increase. The bound triidothyronine were lower when compared to that of the control. There was increasing evidence that exposure to the synthetic compounds BPA, can impair normal thyroid function. BPA alters bound circulating and tissue thyroid hormone concentrations using animal experiments, and BPA were observed to alter thyroid hormone status by epidemiological investigations.

Epidemiologic studies have revealed an association between BPA exposure and altered thyroid hormones such as increased free triiodothyronine (FT3) (Wang *et al.,* 2013), increased thyroid function (Wang *et al.,* 2013), altered total T4 and TSH (Meeker and Ferguson 2011), altered FT4 and TSH levels (Sriphrapradang *et al.,* 2013), and reduced bound T4 (Chevrier *et al.,* 2013).

Almudena *et al.,* (2015) showed that BPA induced an increase in free T3 levels while Du *et al.,* (2014) revealed a decrease in serum bound T4 level. Zoeller *et al.*, (2005) reported plasma free T4 increase.

BPA elicits its effect on the thyroid system as proposed: for thyroxine (T4) BPA inhibits the activity on the thyroid peroxidase (TPO) enzyme of the thyroid follicles, a key enzyme involved in the synthesis of T3 and T4 (Sheng *et al.,* 2012). BPA may have an effect on the thyroid receptor β physiology (TRβ), by repressing the transcription of TRβ in cells and having an antagonistic role on TRβ, thereby interfering with the negative feed-back that the thyroid hormones have on TSH release in the pituitary (Zoeller *et al*., 2005). Again, BPA alteration of thyroid hormones could be due to its ability to bind competitively with thyroid hormone transport proteins and induce UDP-glucuronosyl transferase activity which amplified biliary excretion of thyroxine (Zhang *et al.,* 2016), and also BPA may cause direct damage to the thyroid gland (Du *et al.,* 2014). The observed increase in estrogen trigers decreases in serum bound T4, as proposed by Zhai *et al.,* (2014).

For triiodothyronine (T3), the effect of exposure to BPA could be explained by an increase of free T3 synthesis in the thyroid follicles and a higher T4 deiodination in the liver. BPA up-regulate genes involved in the synthesis of thyroid hormones in the thyroid follicle (Gentilcore *et al*., 2013), that gives support to the hypothesis that BPA could trigger an increase of T3 synthesis. Also, considering the similar structure of BPA and T3, BPA could impair thyroid hormone action by inhibiting T_3 binding to the TR and by suppressing its transcriptional activity. The BPA may silence or inhibit the expression of a number of genes that govern normal thyroid development.

BPA was observed to have a direct effect on thyroid follicular cell and leads to an altered expression of the genes involved in thyroid hormones synthesis. Other potential mechanisms for the effect of BPA on thyroid hormones include inhibiting thyroid hormone pathways (Heimeier *et al.,* 2009) and thyroid hormone receptor (TR) transcription suppression (Sheng *et al.,* 2012), competitive binding with thyroid hormone for the thyroid plasma transporter such as Transthyretin (Ishihara *et al*., 2003). Also, BPA act as an agonist or antagonist of thyroid hormone receptor because of its structural similarity to thyroid hormone. BPA inhibits thyroperoxidase activity, and accordingly block thyroid-induced metamorphism (Iwamuro *et al.,* 2013). At receptor level BPA bind to thyroid hormone receptor as a ligand and act as antagonist thereby, inhibiting TR-mediated transcriptional activity (Sun *et al.,* 2009; Freitas *et al.,* 2010). BPA displace TH from serum binding proteins such as thyroxin-binding globulin (TBG), transthyretin (TTR), and albumin, because it can displace TH from these binding proteins, it can cause a very rapid decrease in the bound serum hormone levels (Du *et al.,* 2014). Zhai *et al.,* (2014) suggested another explanation to these results, is the enhanced T4 glucoronization in the liver and therefore excretion of T4 into the bile, decreasing the amount of bound T4 in plasma. Another explanation could be an increase of T4 synthesis in the thyroid gland, driven by higher TSH levels induced after endocrine disruptor exposure as observed in the study.

Hence given that thyroid hormone receptors are expressed ubiquitously and abundantly in various organs, BPA may perturb thyroid hormone action throughout the body tissue.Therefore, BPA can interfere with thyroid hormone functions and homeostasis by inhibiting hormone synthesis, altering serum transport proteins, and increasing catabolism of thyroid hormones (VanderBerg *et al.,* 2013). Suggestively, the exposure of BPA may alter thyroid functions, leading to thyroid abnormalities, such as subclinical hypothyroidism and goitre. The risk for developing thyroid nodules and obesity, as suggested that altered thyroid function may be one of the reasons for the relationship between BPA and obesity (Hatch *et al.,* 2010).

In summary, BPA is a potent inhibitor of thyroid hormone, which directs development. BPA alters a subset of important genes controlled by T3 that contribute to proper development, represents a serious risk to human development through disruption of TH signaling pathways. The study showed that BPA interferes with thyroid hormone activity. This research provides additional evidence into the fact that BPA interferes primarily with thyroid hormone level in addition to estrogen signals.

In this study, it was observed treatment with BPA activates interlukins. The only interleukins that were suppressed at the low dose level are interleukins 30, 31 and 32 but were activated by the high doses of BPA. The interleukins activated includes those interleukins involved in acute and chronic inflammation, and also those that are proinflatory and anti-inflammatory interleukins

BPA exposure promotes cytokine inflammatory shifts. The shift of cytokines causes the breakdown of self-tolerance (Moudgil and Choubey 2011). BPA impact the differentiation processes of the cytokines which can cause unintended activation of the immune system in the absence of any pathological conditions, thus promoting inappropriate polarization of T cells and cytokine profiles and shifting the immune system into an overzealous immunological state (Pisapia *et al.,* 2012). BPA can trigger allergic responses, changes to inflammatory modulators (Rao *et al.,* 2014), dysregulation of inflammatory cytokines, both pro-inflammatory and anti-inflammatory, leading to the formation of a microenvironment that may promote disordered cell growth (Fischer *et al.,* 2016).

BPA exposure activates the signaling pathways that have been shown to be the exact signaling pathways of molecular processes in immune disease pathophysiology (Wu and Mohan 2009), such as estrogenic signaling pathway and hapten-activating signaling pathways which specifically disrupt immune signaling pathways found in immune disease. Also, BPA exposure is responsible for a long-term effect on mast cell-mediated production of pro-inflammatory mediators, mast cell dysregulation, and subsequent stimulation of chronic pulmonary inflammation (O'Brien *et al.,* 2014).

Exposure to environmental BPA pollutants could be one of the risk factors responsible for the development of inflammatory diseases such as asthma, emphysema, and bronchitis (Rudyk *et al.,* 2012). The hyperprolactinemia observed earlier, has an association with immunity; increased prolactin levels are associated with production of anti-DNA antibodies, islet cell antibodies, thyroglobulin antibodies, thyroid peroxidase antibodies, adrenocortical antibodies, and transglutaminase antibodies (de Bellis *et al.,* 2012). High Prolactin promotes increased cytokine activity and immunoglobin production. It also interferes with B cell tolerance and has autoimmune promoting effects (Shelly *et al.,* 2012). These correlations strongly suggest that BPA may promote autoimmune pathophysiology by increasing prolactin release that then promotes immune-stimulating activity. Higher BPA levels identified in women is characterized by, increased prevalence of hepatic steatosis, and more severe hyperandrogenism as observed earlier (Tarantino *et al.,* 2013). BPA also appears to directly affect immune cell signaling pathways and thus immune responses (Alizadeh *et al.,* 2006). BPA stimulates cell proliferation and induced expression of estrogen responsiveness. BPA induced splenocyte proliferation, and hyperstimulation of cellular immunity (Youn *et al.,* 2002). BPA impaired release of inflammatory cytokines from macrophages in general after exposure to BPA (Bodin *et al.,* 2014). Additionally, BPA may promote immunological shifts linking BPA's potential role to abnormal antigen-presenting cell responses (Byun *et al.,* 2005). It was suggested that BPA exacerbates preexisting immune diseases and that continued exposure to endocrine disruptors may potentiate the incidence and severity of the immune diseases (Yurino *et al.,* 2004).

Of note, some previous studies are in consistent with the findings of the present study; Administration of BPA elicited an inflammatory response by enhanced expression of IL-18 and increase IL-4 production (Lee *et al.,* 2003). Abedelhaffeza *et al.,* (2017) revealed an overexpression of IL-18. Savastano *et al*., (2015) revealed that pro-inflammatory cytokine IL-6 levels were significantly high. The strong correlation between BPA and IL-6 levels supports the pathogenetic involvement of BPA in increasing visceral adiposity and determining chronic inflammation. Yang *et al.,* (2016) showed that IL-6, and IL-1, were all significantly increased in response to BPA treatment at the highest dosage. Yang *et al.,* (2016) study showed increased circulating levels of IL-6, and IL-1, these reports suggest a critical role for BPA in chronic inflammation. Bodin *et al.,* (2015) observed increased release of IL-6, IL-10, and IL-4 in the BPA exposure groups compared to control. Fischer *et al.,* (2016) revealed significant difference in the expression interleukin 1 (IL-1) gene family (IL-1β and IL-1rn), and interleukin 2 gene family (IL-7 receptor). Yazdani *et al.,* (2016), showed the up-regulation of IL-1beta. Liu *et al.,* (2016) showed increased pro-inflammation cytokines and interleukin-6 (IL-6) production, and change in anti-inflammation cytokines interleukin-10 (IL-10). BPA was reported to enhance IL-4 production. Hongchuan *et al.,* (2010) revealed enhanced production of both IL-10 and IL-12 and showed high IL-5/IFN-γ, IL-10/IFN-γ and IL-13/IFN-γ ratios. Exposure to BPA significantly promoted antigen-stimulated production of IL-4, IL-10, and IL-13 (Ellis *et al.,* 2013).

The heighted inflammatory response upon exposure to BPA could be mediated through its ability to activate extracellular regulated protein kinases (ERK) and nuclear factor _B (NF-_B) signaling cascades with a subsequent increased expression of pro-inflammatory cytokines (Liu *et al.,* 2014). In addition, the enhanced oxidative stress observed with BPA exposure may activate NF-_B mediated transcription of pro-inflammatory mediators (Teppala *et al.,* 2012). As earlier stated, BPA effected cytokines expression through estrogen receptor α/β (ERα/β)-dependent mechanism with the evidence of ERα/β antagonist reversed the expression of cytokines. BPA has hapten and estrogenic activity, both of which play roles in activating hyperactive immune responses that may occur in immune pathophysiology.The stimulatory effects of BPA observed in this study may be at least in part explained by its estrogen-like action (Uemura *et al.,* 2008). The relative binding affinity of BPA to ER-α or ER-β has been known, indicating that BPA should activate these receptors (Fenichel et al 2013). It is conceivable that: (i) BPA binds differently within the ligand-binding domain of ER-α or ER-β and recruits dissimilar coregulators; (Safe *et al.,* 2002) or (ii) BPA elicits rapid responses by binding to membrane-anchored ERs, (Wan *et al.,* 2010; Hongchuan *et al.,* 2010)

Although this study showed a statistically significant difference in circulating interleukins, it is among the study that reports the evidence of the association between BPA and serum levels of interleukins, as markers of inflammation in rats. This evidence further supports the hypothesis that BPA environmental exposure leads to the derangement of inflammatory pathways.

The findings of this research showed that hydroperoxidase and myeloperoxidase were decreased when compared with the control group, while hydrogen peroxides were higher than the control group. Present study results are in accordance with Chen and Schopfer (2012) who reported that BPA showed its toxicity by increasing H_2O_2. Chitra *et al.,* (2013) and Sravani, *et al.,* (2016), showed increased levels of H_2O_2. Kabuto *et al*., (2014) and Sravani, *et al.,* (2016), observed over production of H_2O_2 radicals which can only be effectively detoxified by increased glutathione peroxidase activity. Circulating myeloperoxidase levels were low in animal studies (Chettimada *et al.,* 2012). Previous studies in rats (Kunitomo *et al.,* 2008) and mice (Kumar *et al.,* 2014) were able to show a low measure of myeloperoxidase.

The result of this experiment showed that there was decrease in superoxide dismutase (SOD), catalase, total antioxidant capacity (TAC) and increase in nitric oxide, nitrotyrosine and protein carbonyl levels. BPA induced reactive oxygen specis (ROS) production and significantly increase oxidative stress, which is accompanied by marked alterations in TAC (Fridovich, 2007). The decrease in catalase (CAT) activity increased the toxic effect of the free radicals formed from the BPA effect. Nitric oxide (NO) is a highly diffusible free radical. NO as a potent oxidant and nitrating agent is capable of attacking and modifying proteins, lipids, and DNA as well as depleting antioxidant defenses (Sayed-Ahmed *et al*., 2010). It could be postulated that the activation in NO levels induced by BPA administration can lead to the reduction in the rate and force of cardiac contractions (Pant *et al.,* (2011). Because BPA caused induction of free radicals in the hepatic tissue, in consequence, it leads to disruption in the antioxidant defense system. It was found that BPA disturbs the balance of the mitochondrial antioxidant-pro-oxidant status through reduction of the activities of mitochondrial respiratory chain enzymes, which may cause mitochondrial dysfunction and increased ROS generation (Khan *et al.,* 2016). Additionally, it could be mediated through the ability of BPA to stimulate the polymorphism of oxidative stress related genes (Kim 2016). The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage, because CAT have been considered the primary scavengers of H_2O_2 (Veiga-Lopez *et al*., 2015); SOD can catalyse decomposition of superoxide radicals to produce H_2O_2. However, in absence of adequate CAT activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and may contribute to the oxidative stress of BPA, Which indicated liver tissues damage (Kabuto *et al.,* 2014). High dose of BPA not only increases the free radical formation but also decreases its ability to detoxify reactive oxygen species (Hassan *et al.,* 2012). The formation of superoxide radicals together with NO can form peroxynitrite induced by high doses of BPA causes tissue damage leading to an increase in the levels of NO (Hassan *et al.,* 2012). Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins by either the a-amidation pathway or by oxidation of glutamyl side chains, leading to formation of a peptide in which the N-terminal amino acid is blocked by an α-ketoacyl derivative (Escorbar *et al.,* 2013). In addition, CO groups may be introduced into proteins by secondary reaction of the nucleophilic side chains of Cys, His, and Lys residues, with aldehydes (4-hydroxy-2-nonenal, malondialdehyde) produced during lipid peroxidation or with reactive carbonyl derivatives (ketoamines, ketoaldehydes, deoxyosones) generated as a consequence of the reaction of reducing sugars, or their oxidation products with lysine residues of proteins proteins (glycation and glycoxidation reactions), with the eventual formation of the advanced glycation/lipoxidation end products, that is, glycoxidation products, such as carboxymethyllysine and lipoxidation products, such as malondialdehyde-lysine and 4-hydroxy-nonenal-protein adduct. Protein carbonyl content is actually the most general indicator and by far the most commonly used marker of protein oxidation and accumulation of protein carbonyls has been observed in several human diseases including Alzheimer’s disease (AD), diabetes, inflammatory bowel disease (IBD), and arthritis, just for citing a few. With regard to

nitrotyrosine, oxidized tyrosine moieties have been used to study pathways involved in oxidative stress after BPA exposure (Mitra *et al.,* 2014). Increased protein tyrosine nitration occurs during states that lead to high oxidant rates, such as inflammation. Importantly, several studies have shown that both oxidized tyrosines are associated with a proinflammatory state, such as atherosclerosis (Pennathur and Heinecke 2014), diabetes (Pennathur *et al.,* 2015), lupus (Smith *et al.,* 2014), and rheumatoid arthritis (Vivekananda *et al.,* 2013).
Oxidative and nitrosative stress result from an imbalance between oxidants and antioxidants. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to oxidative damage in the form of protein oxidation. Protein oxidation leads to protein function loss, cellular dysfunction, and ultimately cell death. Tyrosine nitration is one specific form of protein oxidation that is associated with Alzheimer's disease (AD) (Escorbar *et al.,* 2013). Nitric oxide (NO) reacting with the superoxide anion forms the product, peroxynitrite, known to lead to nitration of tyrosine (3-NT) residues. Nitration of proteins results in the inactivation of several important mammalian proteins such as superoxide dismutase, actin, tyrosine hydroxylase and likely interfere with tyrosine phosphorylation-mediated cell signaling due to steric effects. Oxidative stress could also stimulate additional damage via the over expression of inducible (i) and neuronal (n) specific NO synthase leading to increased levels of NO. NO and superoxide anion react at diffusion controlled rates to produce peroxynitrite, an extremely strong oxidant that causes oxidative damage to lipids, DNA, carbohydrates and proteins, particularly the amino acids cysteine, methionine, tryptophane, phenylalanine and especially tyrosine (Mitra *et al.,* 2014). Peroxynitrite can nitrate tyrosine at the 3-position that, by steric effects, could prevent the phosphorylation of the OH moiety on tyrosine residues, thereby rendering that protein dysfunctional and potentially leading to cell death.

Nitration of proteins may lead to irreversible damage to the proteins and also affect the energy status of neurons by inactivating key enzymes. This widespread occurrence of oxidative alterations not only decreases or eliminates the normal functions of these macromolecules, but also may activate an inflammatory response that is; the complement cascade, cytokines, acute phase reactants and proteases in system. Increased levels of protein carbonyls and protein-bound HNE were reported suggests the build up of oxidative stress (Mitra *et al.,* 2014).Targets of oxidative stress include phospholipid membranes, proteins, and nucleic acids. As such, increased systemic oxidative stress can lead to irreversible changes in these molecules, as well as in mitochondria (Zorov *et al.,* 2006). Elevated nitrotyrosine during pregnancy were found in perinatal asphyxia (Badham *et al.,* 2010), and chronic hypoxia (Escorbar *et al.,* 2013). An increase in nitrotyrosine was evident in both maternal and fetal sheep plasma in response to low BPA exposure. The low BPA dose induces postnatal systemic nitrosative stress in the adult. Importantly, the systemic nitrosative stress evident demonstrates that BPA exposure can disrupt oxidative stress pathways. The finding that BPA increased nitrotyrosine in adipose tissue of adult sheep and rats points to tissue-specific programming effects as well (Nishimura *et al.,* 2014). The oxidation of amino acid residues, the subsequent formation of protein aggregates by cross-linking and the production of protein fragments may result in the loss of activity and inactivation of enzymes and metabolic pathways, finally ending up with cell death.

In accordance with the findings of this work, Hassan *et al.,* (2012) also repoeted decrease in SOD. Ansoumane *et al.,* (2015) reported decrease in the activities of antioxidant enzymes, namely, CAT, and SOD after exposure to BPA. Wu *et al.,* (2011) showed significant decrease in SOD level. Hassan *et al.,* (2012) showed a decrease in CAT activity. Eid *et al.,* (2015) also demonstrated a decrease in the activities of antioxidant enzymes such as SOD, and CAT, elevated levels of NO and a decrease in total antioxidant capacity. Aboul Ezz, *et al.,* (2015) revealed a decrease the catalase activity. Abedelhaffeza *et al.,* (2017) observed decreased SOD activities and TAC after BPA administration. Chitra *et al.*, (2013), showed that the activities of superoxide dismutase, catalase, were decreased. Umbilical cord levels of nitrotyrosine were found to be high (Weber *et al.,* 2014). There is a positive correlation between maternal nitrotyrosine levels with cord nitrotyrosine levels (Weber *et al.,* 2014) that maternal oxidative stress, specifically nitrotyrosine, could be used as a predictor of fetal oxidative stress.

The reduction in the activity of catalase could be due to the depletion of the enzyme in attempting to eliminate the hydrogen peroxide generated after the exposure to BPA. This may also be due to enzyme inactivation caused by excess ROS production in mitochondria and microsomes (Aboul Ezz (2015). The decrease in SOD activities were accompanied by the decrease in CAT activity. The possible explanation for this observation is that the metabolism of BPA mainly occurs in the liver. Hence it is glucuronidated by liver microsomes (Yokota *et al.*, 1999), and that glucuronidation was mediated by UGT2B1, an isoform of UGT in rat liver. The metabolites of BPA produced by microsomal Cytochrome P_{450} showed the enhanced estrogenic activity such as DNA adduct formation with BPA metabolites (Ansoumane *et al.,* 2015). Meanwhile, the mechanisms by which SOD can lead to increased cell death have been reported (Fang *et al.,* 2013). Also another mechanism for SOD induced apoptosis is through its ability to activate p53 by production of H_2O_2 (Veiga-Lopez *et al.,* 2015). The reduction in catalase which can be due to the exhaustion of the enzyme in attempting to eliminate the hydrogen peroxide generated after the exposure to BPA. This could also be due to enzyme inactivation caused by excess ROS production in mitochondria and microsomes (Pigeolet *et al.,* 1990). It was found that BPA disturbs the balance of the mitochondrial antioxidant-pro oxidant status through reduction of the activities of mitochondrial respiratory chain enzymes, which can cause mitochondrial dysfunction and increased ROS generation (Khan *et al.*, 2016). Additionally, it could be mediated through the ability of BPA to stimulate the polymorphism of oxidative stress related genes (Kim 2016). The selective elevation in nitrotyrosine, evidenced in the current study suggests that BPA may enhance reactive nitrogen species through mechanisms that do not use myeloperoxidase such as peroxynitrite formation through direct reaction between superoxide and nitric oxide or through endothelial nitric oxide synthase uncoupling. Indeed, generation of nitrotyrosine by such mechanisms has been postulated in vasculopathy associated with diabetes, cardiovascular disease, and Fabry's disease (Shu *et al.,* 2014). Although some proteins and tyrosine residues are known to be preferentially nitrated (Fang *et al.,* 2013).

In the current study, BPA increased ROS production as assessed by the measurement of the end products of lipid peroxidation. The concentration of MDA, lipid hydroperoxide, 8-isoprostane and hexanoyllysine were high compared to the control. The oxidized lipids such as OXPL-LDL, MDA-LDL, CML-LDL, and HNE-LDL were high. These results are in agreement with the previous studies which demonstrated that BPA administration increased MDA levels in the tissue (Kabuto *et al*., 2014) while Eid *et al*., (2015) demonstrated that BPA caused elevated levels of MDA, while Nandi *et al.,* (2005) reported that MDA concentration increased with the time. Hassan *et al.,* (2012) showed increased TBARS levels which cause lipid peroxidation in the liver. Abedelhaffeza *et al.,* (2017) showed an increase in lipid peroxidation as evidenced by the enhanced MDA levels. Korkmaz *et al.,* (2010) showed an increase in TBARS level. Ansoumane *et al.,* (2015) showed increased lipid peroxidation (LPO) and lipid hydroperoxide. Sravani *et al.,* (2016) observed increase in TBARS and levels of lipid hydroperoxides and 8-iso-prostaglandin, the major biochemical markers of ROS generation, have been shown to be elevated in the plasma and pericardial fluid and are also positively correlated to its severity (Prysyazhna *et al.,* 2012).

BPA administration induced a state of oxidative stress in rats as evident from the increase in MDA levels after exposure to BPA. It was reported that BPA increases the generation of reactive oxygen species (ROS) and induced hepatic damage and mitochondrial dysfunction (Moon *et al*., 2012; Asahi *et al*., 2010). It is evident that BPA has an adverse effect on the generation of ROS and reduction of antioxidant defenses, aggravating a state of oxidative stress. It is clear that pathological conditions may occur after prolonged exposure to BPA even at extremely low levels which raises the demands for prohibiting the use of BPA in plastic industries. Lipid hydroperoxide is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation and excessive damage of cellular macromolecules (protein, lipids, and nucleic acids) and major contributor to the toxicity of contaminants (Fenichel *et al.,* 2012). The increase in Lipid hydroperoxide reported here can be the result of increased production of free radicals and a decrease in antioxidant status. The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage (Veiga-Lopez *et al.,* 2015). Increased lipid peroxidation indicates an increased oxygen free radical generation. BPA induced ROS production and significantly compromises mitochondrial function (Aboul Ezz *et al.,* 2015). It has been reported that BPA exposure results in augmentation of oxidative stress, with increased levels of lipid peroxidation and reactive oxygen species and depletion of the antioxidant defense system (Eid *et al*., 2015). Lipids such as phospholipid and other types of lipids found in cells especially as components of plasma membrane and organelle membranes are another major target for the generated ROS, free radicals and Oxidative stress (Shu *et al.,* 2014). Lipid oxidation derived products assayed as oxidized lipid, the study showed that the serum levels of the four (4) oxidized lipid products were high. They includes oxidized phospholipid LDL, MDA-LDL, CML-LDL and HNE- LDL. The consequences of these findings are impaired membrane functions, inactivate membrane bound receptors and enzyme and increase cellular membrane permeability, leading to the opening of the mitochondrial permeability trasition pore that may cause the release of pro apoptotic molecules to the cytosol (Ansoumane *et al.*, 2015). In addition to the oxidized lipids, depletion of reduced glutathione (GSH) also facilitates the opening of the mitochondrial permeability trasition pore. These molecules are usually more reactive than the initial molecules they were formed from and present a range of pathological effects, including increasing vascular permeability, Low Density Lipoprotein (LDL) oxidation and enhancing oxidative stress (Aboul Ezz *et al.,* 2015). Free radical oxidation is responsible for the degradation of fatty acids and their esters in biological membranes and lipoproteins and plays an important role in a wide range of pathological events. The damage on cell membrane architecture is induced by lipid peroxidation of membranes fatty acid (Shu *et al.,* 2014). Lipid hydroperoxides are important non radical intermediates of lipid peroxidation. They present several biological activities such as cytoxicity and are relatively easily decomposed to reactive radical species that can propagate the oxidative stress (Nandi *et al.,* 2005). The oxidative damage to lipoproteins and especially LDL, is known to play a role in a number of diseases, such as cardiovascular diseases, arthritis, dementia and the metabolic syndrome. High circulating oxidized LDL concentrations have been linked to an increased cardiovascular disease risk (Ansoumane *et al.,* 2015). Oxidized LDLs promote endothelial cell damage, are chemotactic for leukocytes and may be endocytosed in an uncontrolled manner by macrophages(Aboul Ezz *et al.,* 2015).

Oxidative damages to proteins, lipids, or DNA may all be seriously deleterious and may be concomitant. However, proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often catalysts rather than stoichiometric mediators; hence, the effect of damage to one molecule is greater than stoichiometric. ROS leading to protein oxidation include radical species such as superoxide, hydroxyl, hydroperoxyl, and nonradical species such as hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$), and peroxynitrite (ONOO_) (Nandi *et al.*, 2005). Carbonyl (CO) groups are produced on protein side chains especially of Pro, Arg, Lys, and Thr; when they are oxidised. These moieties are chemically stable.

All the peroxiredoxin analysed were significantly decreased when compared with the control at $p \leq 0.05$. Rahman *et al.*, (2015) revealed that BPA induced the down-regulation peroxiredoxin-5. Also, Park *et al.*, (2012) recently have identified PRDX5. In another, Schumacher (2013), showed that BPA induced the decrease in peroxiredoxin-5. Under BPA exposure, Rahman *et al.*, (2015), found that various concentrations of BPA, down regulated peroxiredoxin-5. Altered expression of peroxiredoxin-5 has been reported in breast carcinoma (Noh 2001), hepatocellular carcinoma (Choi *et al.*, 2007), and mesothelioma cells (Kinnula *et al.*, 2002), Neo *et al.*, (2013) showed Peroxiredoxin-1 depletion.

Peroxiredoxins are an ubiquitous family of antioxidant enzymes that also control cytokine-induced peroxide levels and thereby mediate signal transduction in mammalian cells (Rhee *et al.*, 2005). It has been described as a sensor of peroxide. Interestingly, peroxiredoxins forms disulfide complexes with other proteins when cardiac muscle is exposed to oxidant conditions (Burgoyne *et al.*, 2012b). Peroxiredoxin could be hypothesized to have a role in directly promoting PKG dimerization once it has been oxidized by peroxide. The observation that peroxide oxidizes NADPH suggests that NADPH oxidation could be a factor in peroxide promoting PKG dimerization. This could occur through peroxiredoxin-thioredoxin-thioredoxin reductase pathway and glutathione peroxidase-glutathione reductase pathways of peroxide metabolism consuming NADPH (Rudyk *et al.*, 2012). Rahman *et al.*, (2015) showed that PRDX5 are antioxidant enzymes that are predominantly expressed in response to stress. In somatic cells, PRDX3, and PRDX5 are responsible for consumption of approximately 99.9% of mitochondrial H_2O_2 (Cox *et al.*, 2010). Peroxiredoxin (Prx) exhibit peroxidase activity, which is dependent on reduced forms of thioredoxin and glutathione (GSH).

The findings of these results showed an increase in glutathione reductase, total glutathione and oxidized glutathione (GSSH), but glutathione peroxidase and reduced glutathione (GSH) were decreased after BPA exposure. In concordance with our results, Hassan *et al.*, (2012) also reported a significant decrease in reduced glutathione (GSH), Wu *et al.*, (2011), reported significant decrease in the levels of GSH in BPA group. Ansoumane *et al.*, (2015) showed that BPA administration causes a decrease in the activities of antioxidant enzymes, namely, glutathione peroxidase. Korkmaz *et al.*, (2010) reported the decrease in GSH. Hassan *et al.*, (2012) showed decreased in glutathione peroxidase. Also, Chitra *et al.*, (2013), showed that the activities of glutathione peroxidase were decreased significantly, Eid *et al.*, (2015), demonstrated that BPA caused decrease in the activities of antioxidant enzymes glutathione peroxidase. Aboul Ezz *et al.*, (2015) revealed that BPA administration induced a state of oxidative stress by decrease in GSH levels.

Glutathione provides a first line of defence against (ROS), as it can scavenge free radicals and reduce H_2O_2 ((Burgoyne *et al.*, 2012a). The depletion of GSH and GSH/GSSG ratio in blood has been reported to be a good marker in hypertension (Vaziri *et al.,* 2000; Husain 2002). Hassan *et al.,* (2012) also showed a significant downregulation in the GST gene expression levels. A number of compounds lead to induce activity and expressions of GST isoenzymes (Fenichel *et al.*, 2013).

GSH acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as glutathione peroxidase and glutathione reductase. The reduction of GSH shows the failure of primary antioxidant system to act against free radicals observed earlier in the endogenous antioxidant etc. The decrease in activities of the antioxidant enzymes might predispose the liver to increased free radical damage, because glutathione peroxidase have been considered the primary scavengers of H_2O_2 (Veiga-Lopez *et al.*, 2015); However, in absence of adequate glutathione peroxidase activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals and can contribute to the oxidative stress of BPA. Decreased GSH concentration indicates an increased generation of ROS, which cause lipid peroxidation in the liver as observed earlier in the study discussion. Also, GSH is consumed during the conversion of hydrogen peroxide into hydrogen oxide. The peroxide is readily converted to the hydroxyl radical which can be involved in the observed decrease in GSH levels as GSH itself is also a general hydroxy-radical scavenger (Nandi *et al.,* 2005).

Glutathione system is essential for cell survival and provides the major antioxidant defence mechanism in cells. Glutathione system directly scavenges diverse free radicals such as superoxide anions, hydroxyl radicals, nitric oxide and carbon radicals. Glutathione system catalytically detoxifies hydroperoxides, and lipid peroxides. Its depletion increases the cellular susceptility to apoptosis. The study revealed depletion of reduced glutathione (GSH), glutathione peroxidase (GlnPrx) and accumulations of oxidized glutathione (GSSH), glutathione reductase and total glutathione. Glutathione system is necessary for maintaining immune mediated T- cell activation and phagocytosis, and in cellular and antibody mediated cytotoxicity (Hassan *et al.,* (2012). It is required to maintain a normal balance between the T- helper cell 1 and T- helper cell 2 cytokine response profile. The glutathiones especially the GSH levels, in concert with antigen presenting cell determine the fate of Th-1 and Th-2 pattern of response (Fenichel *et al.,* 2013). Th-1 response is characterised by the production of IL- 2, 12 and Th-2 by IL-4, 10 production, and also upregulation of a number of antibody response. Depletion of glutathione (GSH) is capable of completely alteration of the T- cell activation and enhancement of hypersentivity response. The accumulation of GSSH due to oxidative stress resulting from exposure to BPA, is directly toxic to cells (Nandi *et al.,* 2005). The accumulation of GSSH toxicity to cells is by the activation of the SAPK/MAPK pathway, thereby inducing cellular toxicity and then apoptosis.

The GSH thiol group of cysteine quenches free radicals and ROS generated in cell, by donating a reducing equivalent (H+ + e-). Its depletion could be a cause of exposure to environmental toxin (BPA), chronic liver condition/damage and inflammation as a result of induced oxidative stress by BPA exposure (Hassan *et al.,* (2012). GSH depletion either induce or potentiate apoptosis and facilitates/potentiates the response to other cellular death stimulus (Sharma *et al.,* 2004). Low/depleted GSH impacts on cellular function is evident in the accumulation of hydroperoxide, lipid peroxides, hydroxyl radical, carbon radicals and nitric oxide (NO), as mostly observed in this study. The accumulated/increased nitric oxide levels may produce nitrosative and oxidative stress and cellular damage which may induce autophagic cell death in GSH depleted cell (Hassan *et al.*, (2012). Low levels of GSH are associated with chronic exposure to chemical toxin, macular degeneration, neurodegeneration etc. A change in GSSH- to -GSH ratio is highly indicative of high levels of oxidative stress radicals. A shift in the ratio towards the oxidized pool tend to induce glutathioylation of the cysteine located in the DNA binding site of c-jun as well as produce a disulfide bond between cysteines proximal to the leucine zipper motif. The impact of these

changes is the capacity to induce a direct and reversible control of transcriptional regulation through these stress kinase pathways as earlier stated.
Glutathione peroxidase in concert with catalase and superoxide dismutase function to protect the cell from damage due to ROS. Glutathione peroxidase detoxifies peroxides with GSH acting as an electron donor in the reduction reaction; producing GSSH as an end product (Sharma *et al.,* 2004). This might be contributing to the pool/accumulation of GSSH observed by the study. The reduction of GSSH is catalysed by glutathione reductase in a process that requires NADPH (Fenichel *et al.,* 2013). Despite the high level of Glutathione reductase, much of the GSSH were not reduced to GSH, this could also account for the low level of GSH observed in the study. Depleted Glutathione peroxidase, catalase and superoxide dismutase as revealed by the study tends to increase the vulnerability of cells and cellular organelle to damage by oxidative stress induced by high levels of hydrogen peroxide and lipid hydroperoxide.

This finding is supported by various studies demonstrating that reduced glutathione is reduced in the tissues by oxidative stress. Therefore, the result confirmed that the exposure to BPA causes oxidative stress by disturbing the balance between ROS and antioxidant defenses system in liver. GST protects cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes (Sharma *et al.,* 2004). Glutathione-S-transferase catalyses the conjugation of GSH with several compounds produced *in vivo* during oxidative stress (Hassan *et al.,* (2012). The consumption of GSH during the generation of glutathione-S-conjugates by GSTs thus lowering the level of total intracellular glutathione after prolonged treatment.

From the result obtained, it was observed that the thioredoxin-1 was high and thioredoxin reductase decreases when compared with the control.The findings of this research are consistent with Neo *et al.,* (2013), who observed a decrease of thioredoxin-1 (Trx-1), and Trx reductase-1 (TrxR-1) depletion in BPA treatment group.

Systems controlling protein thiol redox in the cytosolic region such as thioredoxin-1 (Trx-1) and glutaredoxin could be hypothesized to have a role in promoting PKG1α dimerization under circumstances in which cytosolic NADPH oxidizes because thioredoxin reductase-1 and glutathione reductase utilize NADPH to maintain Trx-1 and glutathione in their reduced forms (Chae *et al.,* 2012, Neo *et al.,* 2010). Evidence has recently been reported, based on the actions of an inhibitor of thioredoxin reductase, that thioredoxin and thioredoxin reductase function to redox cycle the dimerized form of PKG1α back to the reduced monomeric form of PKG (Burgoyne *et al.,* 2012b; Rudyk *et al.,* 2012). Since thioredoxin reductase-1 utilizes NADPH to maintain Trx-1 in its reduced state (Forman *et al.,* 2004), this could occur through peroxiredoxin-thioredoxin-thioredoxin reductase and/or glutathione peroxidase-glutathione reductase pathways of peroxide metabolism consuming NADPH. Recent reports of the enhancement of this process by an inhibitor of thioredoxin reductase are consistent with thioredoxin and thioredoxin reductase normally functioning to reduce the dimerized form of PKG1α back to its monomeric form (Burgoyne *et al.,* 2012b, Rudyk *et al.,* 2012). In response to oxidative stress due to exposure to BPA, the thioredoxin system induced the autophosphorylation and activation of cell death signal regulating kinase 1 (ASK-1), which caused phosphorylation and activation of JNK and p38 both involved in apoptosis initiation. The thioredoxin reductase may be possibly inactivated by radicals such as H_2O_2 Neo *et al.,* (2013). Thioredoxin is a dithiol hydrogen donor cofactor; it is reduced by electron from NADPH via thioredoxin reductase (Fenichel *et al.,* 2013). In the study low level of thioredoxin reductase might impact cellular and enzyme functions, in that much of the thioredoxin are not reduced to its active form, in consequence there may be impaired DNA synthesis and impaired protein repair by sulfoxide reduction methionine sulfoxide due to inability of thioredoxin to donate hydrogen for ribonucleotide reductase and methionine sulfoxide reductase respectively (Rudyk *et al.,* 2012). Again, inactivation of peroxiredoxins that also catalyse the reduction of H_2O_2 leading to oxidative stress development and apoptosis induction (Neo *et al.,* (2013). In immunomodulation and inflammation, extracellular thioredoxin act as a co-cytokine and chemokine and a truncated form stimulates eosinophiles (Rudyk *et al.,* 2012). The stimulated/activated eosinophile triggers the release of interleukin 3, 4, 5, 9, 13, 16 and 25. Other consequences include induction of signals for apoptosis, alterations of transcriptional factors, enhance hyperoxia, impair implation and impair establishment of pregnancies leading to high rate of infertility.

In summary, living organisms are constantly exposed to BPA which triggers the formation of free radicals and reactive oxygen species that led to oxidative stress capable of modifing biomolecules (nucleic acids, proteins, carbohydrates and polyunsaturated lipids) and to exerting an important role in the development of tissue damage (Burgoyne *et al.,* 2012a). Cells pocessed antioxidant systems such as the glutathione, thioredoxin, peroxiredoxin and the endogenous antioxidant systems, that control the ROS, free radical and oxidative stress states and thereby control/enhance survival of the cells. An increase in free radicals levels such as the one observed in this study, where H_2O_2, Lipid peroxide etc are increased, may lead to decreases in different cellular defence systems, and if the elicited damage responsible for the observed increase in free radical levels is irreversible, it may lead to cell death (Neo *et al.,* (2013). The observed decrease in the antioxidant systems assayed can increase cellular oxidative stress which is a known inducer of transcription factors, that cause changes in the thiol- disulfide status in glutathione and thioredoxin, affecting critical cysteine in enzymes, enzyme receptors, protein and transport proteins utilizing them. Moreso, these changes can trigger increased modification of amino acids via nitrosylation and carboxylation, as the study revealed; increased level of nitrotyrosine, protein carbony and hexanoyllysine, may differentially affect protein funtions.

5.2 CONCLUSION

The median lethal dose (LD_{50}) of Bisphenol A obtained from all the methods (Lorke's, Karber's and Up and Down methods) showed no comparative difference while in all instances the female rats showed higher LD_{50} than the male counterpart.

BPA triggers higher weight gain at lower doses compared to the high doses. This is suggesting that BPA can cause an alteration in adipogenesis, proving that BPA could be a potential new environmental obesogen at lower doses.

BPA is disruptive to women hormone homeostasis. The present study is evident that BPA exposure alters the reproductive hormones and this implies that BPA can alter steroid hormone pathways. In response to sex hormones alteration due to exposure to BPA, BPA can impair implation and impair establishment of pregnancies leading to high rate of infertility. As a powerful endocrine disruptor, it cause estrogen responsiveness, induces hyperprolactinemia. BPA can stimulates cell proliferation, stimulates uterine, vaginal, and mammary growth and differentiation *in vivo*. Public awareness of on these deleterious effects should be strengthened and protection against BPA exposure should be promoted.

BPA is a potent inhibitor of thyroid hormone; the study showed that BPA interferes with thyroid hormone activity and functions which can presents a serious risk to human development through disruption of TH signaling pathways, leading to thyroid abnormalities, such as subclinical hypothyroidism, the risk of developing thyroid nodules and obesity.

BPA exposure did induced systemic alterations in the immune system, seen as altered interleukin response. BPA treatment induced and stimulates a shift from the normal of interleukin profiles and hyperstimulate the cellular immune system, affecting its functionality, which in turn promotes pathogenesis of the immune system that potentiate immunity with allergic and immune diseases. BPA exposure leads to immune disfunction through immune dysregulation of the interleukin profile, this indicates that BPA has potential to induce a disruptive shift and exaggerate cellular immune responses. BPA exacerbates pre-existing immune diseases and continued exposure to BPA can potentiate the incidence and severity of the immune diseases.

Administration of BPA elicited an inflammatory response. The circulating levels of inflammation markers were increased in response to BPA exposure. Inflammatory diseases such as athsma, are becoming increasingly prevalent worldwide and exposure to BPA could be one of the risk factors responsible for the development of such diseases.

BPA as an endocrine disorderly chemical released in environment, deplets the antioxidants of the system thereby enhancing cellular damage resulting from interaction between lipid, protein and DNA molecules and ROS. Regardless of the presence of this antioxidant system, an over or unbalanced production of ROS due to contact with the chemical may have been implicated in a number of clinical disorders.With the growing epidemic of disease worldwide and the extensive use of consumer goods containing BPA, there is risk of BPA acting as a potential triggering compound in diseases. Many of the mechanisms known to exist in bisease pathophysiology also appear to exist with immune reactivity from BPA exposure. In addition, severe oxidative stress resulting from exposure to BPA could lead to DNA damage and mutation of tumor suppressor genes. In consequence, the observed down regulation of these antioxidant defences can lead to increase ROS level, cellular oxidative stress, alterations of the redox status and cellular damage, and these can trigger high risks/chance of developing pathologies. A shift in the Oxidative stress biomarkers can cause cell transition from quiescent to proliferative status, growth arrest and cell death activation depending on the duration and extent of the biomarkers imbalance.

This study demonstrated that dose of BPA not only increases the free radical formation but also decreases its ability to detoxify ROS. Secondly, results indicated that BPA affected inflammatory responses via modulating of interleukins expression, and provided a new insight into the link between exposure to BPA and human health. The measurement of serum biomarkers for each treatment provides a good opportunity to evaluate the effects of various BPA concentrations and its physiological disposition, on system function and damage over time.

5.3 RECOMMENDATION

Further, the pathways involved in the increased activity of the biomarkers examined should be elucidated. Investigation needs to be conducted correlating immune disease development to BPA exposure. Additionally, the impact of BPA exposure on those already suffering from immune diseases needs to be investigated further based on potential overlapping pathophysiology.

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APPENDIX 1

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON WEIGHT

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WEIGHT	Between Groups	1247368.488	11	113397.135	295.478	.000
	Within Groups	640137.150	1668	383.775		
	Total	1887505.638	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX II

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON SEX HORMONES

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
	Between Groups	4264733.793	11	387703.072	425.412	.000
ESTROGEN	Within Groups	1520148.494	1668	911.360		
	Total	5784882.287	1679			
	Between Groups	3071421.881	11	279220.171	308.170	.000
ESTRONE	Within Groups	1511304.142	1668	906.058		
	Total	4582726.022	1679			
	Between Groups	17964.882	11	1633.171	249.211	.000
ESTRIOL	Within Groups	10930.994	1668	6.553		
	Total	28895.876	1679			
	Between Groups	91380.526	11	8307.321	328.639	.000
PROLACTINE	Within Groups	42163.654	1668	25.278		
	Total	133544.180	1679			
	Between Groups	26741.939	11	2431.085	252.924	.000
PROGESTRONE	Within Groups	16032.684	1668	9.612		
	Total	42774.623	1679			
	Between Groups	11476.180	11	1043.289	549.019	.000
TESTOSTERONE	Within Groups	3169.665	1668	1.900		
	Total	14645.845	1679			
	Between Groups	6765296.663	11	615026.969	358.398	.000
LH	Within Groups	2862365.182	1668	1716.046		
	Total	9627661.846	1679			
FSH	Between Groups	399783.488	11	36343.953	142.166	.000

	Within Groups	426413.679	1668	255.644		
	Total	826197.168	1679			
ANDROSTENEDIONE	Between Groups	8364911.756	11	760446.523	399.671	.000
	Within Groups	3173675.308	1668	1902.683		
	Total	11538587.064	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX III

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON THYROID HORMONES

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TSH	Between Groups	250513.482	11	22773.953	617.941	.000
	Within Groups	61473.384	1668	36.855		
	Total	311986.866	1679			
TT4	Between Groups	192287.575	11	17480.689	452.330	.000
	Within Groups	64461.353	1668	38.646		
	Total	256748.927	1679			
FT4	Between Groups	199323.666	11	18120.333	510.774	.000
	Within Groups	59174.351	1668	35.476		
	Total	258498.017	1679			
TT3	Between Groups	66315.143	11	6028.649	505.281	.000
	Within Groups	19901.376	1668	11.931		
	Total	86216.519	1679			
FT3	Between Groups	38321.095	11	3483.736	521.237	.000
	Within Groups	11148.223	1668	6.684		
	Total	49469.319	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX IV

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON INTERLEUKINS

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
IL1	Between Groups	12483492.112	11	1134862.919	246.403	.000
	Within Groups	7682333.885	1668	4605.716		
	Total	20165825.996	1679			
IL2	Between Groups	23199957.913	11	2109087.083	379.221	.000
	Within Groups	9276810.136	1668	5561.637		
	Total	32476768.049	1679			
IL3	Between Groups	21147439.355	11	1922494.487	290.914	.000
	Within Groups	11022914.295	1668	6608.462		
	Total	32170353.650	1679			
IL4	Between Groups	22486780.918	11	2044252.811	301.161	.000
	Within Groups	11322224.756	1668	6787.905		
	Total	33809005.675	1679			
IL5	Between Groups	21473914.799	11	1952174.073	312.647	.000
	Within Groups	10415023.328	1668	6244.019		
	Total	31888938.127	1679			
IL6	Between Groups	21637563.334	11	1967051.212	291.304	.000
	Within Groups	11263299.569	1668	6752.578		
	Total	32900862.902	1679			
IL7	Between Groups	20444170.769	11	1858560.979	282.669	.000
	Within Groups	10967161.560	1668	6575.037		
	Total	31411332.329	1679			
IL8	Between Groups	21523199.691	11	1956654.517	300.731	.000

	Within Groups	10852563.367	1668	6506.333		
	Total	32375763.059	1679			
	Between Groups	21011430.700	11	1910130.064	296.481	.000
IL9	Within Groups	10746386.920	1668	6442.678		
	Total	31757817.620	1679			
	Between Groups	9130348.385	11	830031.671	502.395	.000
IL10	Within Groups	2755787.717	1668	1652.151		
	Total	11886136.103	1679			
IL11	Between Groups	10748991.978	11	977181.089	328.365	.000
	Within Groups	4963806.636	1668	2975.903		

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
IL11	Total	15712798.614	1679			
	Between Groups	12372199.994	11	1124745.454	163.269	.000
IL12	Within Groups	11490681.848	1668	6888.898		
	Total	23862881.842	1679			
	Between Groups	7715510.575	11	701410.052	105.585	.000
IL13	Within Groups	11080641.122	1668	6643.070		
	Total	18796151.697	1679			
	Between Groups	3101410.060	11	281946.369	81.212	.000
IL14	Within Groups	5790877.855	1668	3471.749		
	Total	8892287.915	1679			
	Between Groups	3072487.021	11	279317.002	301.929	.000
IL15	Within Groups	1543079.437	1668	925.108		
	Total	4615566.457	1679			
IL16	Between Groups	4340221.379	11	394565.580	410.443	.000

	Within Groups	1603475.700	1668	961.316		
	Total	5943697.079	1679			
	Between Groups	1867057.159	11	169732.469	328.027	.000
IL17	Within Groups	863081.857	1668	517.435		
	Total	2730139.016	1679			
	Between Groups	4359157.129	11	396287.012	97.711	.000
IL18	Within Groups	6764901.275	1668	4055.696		
	Total	11124058.404	1679			
	Between Groups	2313028.089	11	210275.281	283.362	.000
IL21	Within Groups	1237778.311	1668	742.073		
	Total	3550806.399	1679			
	Between Groups	2327614.763	11	211601.342	270.926	.000
IL22	Within Groups	1302760.345	1668	781.031		
	Total	3630375.108	1679			
	Between Groups	2915158.561	11	265014.415	395.140	.000
IL23	Within Groups	1118701.097	1668	670.684		
	Total	4033859.658	1679			
IL25	Between Groups	3165347.767	11	287758.888	477.272	.000

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
IL25	Within Groups	1005676.912	1668	602.924		
	Total	4171024.679	1679			
	Between Groups	3467564.892	11	315233.172	485.962	.000
IL26	Within Groups	1081995.811	1668	648.679		
	Total	4549560.703	1679			
IL27	Between Groups	2346771.688	11	213342.881	335.504	.000

	Within Groups	1060660.243	1668	635.887		
	Total	3407431.931	1679			
	Between Groups	597106.278	11	54282.389	273.068	.000
IL30	Within Groups	331577.302	1668	198.787		
	Total	928683.580	1679			
	Between Groups	1090157.048	11	99105.186	363.232	.000
IL31	Within Groups	455101.357	1668	272.843		
	Total	1545258.404	1679			
	Between Groups	932467.916	11	84769.811	309.193	.000
IL32	Within Groups	457307.199	1668	274.165		
	Total	1389775.115	1679			
	Between Groups	1219260.867	11	110841.897	300.312	.000
IL33	Within Groups	615641.045	1668	369.089		
	Total	1834901.912	1679			

*. The mean difference is significant at the 0.05 level

APPENDIX IV

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON INFLAMMATORY BIOMAKERS

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
	Between Groups	17907.552	11	1627.959	256.314	.000
HPX	Within Groups	10594.168	1668	6.351		
	Total	28501.719	1679			
	Between Groups	5694499.647	11	517681.786	478.147	.000
HPEROXIDE	Within Groups	1805917.287	1668	1082.684		
	Total	7500416.934	1679			
	Between Groups	1258721.849	11	114429.259	436.664	.000
MYELOPEROXIDASE	Within Groups	437104.953	1668	262.053		
	Total	1695826.802	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX VI

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON ENDOGENOUS ANTIOXIDANT

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
	Between Groups	181396.703	11	16490.609	116.164	.000
SOD	Within Groups	236789.805	1668	141.960		
	Total	418186.508	1679			
	Between Groups	309157.742	11	28105.249	111.623	.000
CATALASE	Within Groups	419980.852	1668	251.787		
	Total	729138.594	1679			
	Between Groups	5278882.255	11	479898.387	184.505	.000
TAC	Within Groups	4338471.368	1668	2601.002		
	Total	9617353.624	1679			
	Between Groups	424764.750	11	38614.977	157.552	.000
NO	Within Groups	408815.620	1668	245.093		
	Total	833580.370	1679			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	85844872.999	11	7804079.364	447.408	.000
NITROTYROSINE	Within Groups	29094736.092	1668	17442.887		
	Total	114939609.091	1679			
	Between Groups	8621.905	11	783.810	205.316	.000
PROTEINCARBOMYL	Within Groups	6367.706	1668	3.818		
	Total	14989.611	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX VII

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON LIPID PEROXIDATION

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MDA	Between Groups	25560392.617	11	2323672.056	465.171	.000
	Within Groups	8332165.609	1668	4995.303		
	Total	33892558.226	1679			
LHPEROXIDE	Between Groups	105508.032	11	9591.639	139.753	.000
	Within Groups	114479.741	1668	68.633		
	Total	219987.773	1679			
OXPLLDL	Between Groups	542843.618	11	49349.420	120.393	.000
	Within Groups	683717.919	1668	409.903		
	Total	1226561.537	1679			
MDALDL	Between Groups	4800903.526	11	436445.775	1674.605	.000
	Within Groups	434724.269	1668	260.626		
	Total	5235627.795	1679			
CMLLDL	Between Groups	333847.519	11	30349.774	112.473	.000
	Within Groups	450092.317	1668	269.840		
	Total	783939.836	1679			
HNELDL	Between Groups	885975.868	11	80543.261	271.286	.000
	Within Groups	495219.847	1668	296.894		
	Total	1381195.715	1679			
ISOPROSTANE	Between Groups	248136268.088	11	22557842.553	552.807	.000
	Within Groups	68064366.944	1668	40805.975		
	Total	316200635.031	1679			

	Between Groups	39459530.960	11	3587230.087	690.710	.000
HEXANOLLYSINE	Within Groups	8662829.553	1668	5193.543		
	Total	48122360.512	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX VIII

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON PEROXIREDOXIN SYSTEM

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
	Between Groups	3213027.791	11	292093.436	170.841	.000
PRX1	Within Groups	2851836.367	1668	1709.734		
	Total	6064864.157	1679			
	Between Groups	4227613.072	11	384328.461	166.743	.000
PRX2	Within Groups	3844595.291	1668	2304.913		
	Total	8072208.363	1679			
	Between Groups	2827548.897	11	257049.900	129.945	.000
PRX3	Within Groups	3299532.540	1668	1978.137		
	Total	6127081.437	1679			
	Between Groups	4749179.937	11	431743.631	161.685	.000
PRX4	Within Groups	4454020.963	1668	2670.276		
	Total	9203200.900	1679			
	Between Groups	4001249.140	11	363749.922	153.716	.000
PRX5	Within Groups	3947117.660	1668	2366.377		
	Total	7948366.800	1679			
	Between Groups	5394162.293	11	490378.390	165.770	.000
PRX6	Within Groups	4934266.838	1668	2958.194		
	Total	10328429.131	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX IX

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON GLUTATHIONE SYSTEM

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
GLRX	Between Groups	33137.394	11	3012.490	35.682	.000
	Within Groups	140822.286	1668	84.426		
	Total	173959.680	1679			
GLPX	Between Groups	1338862.696	11	121714.791	142.838	.000
	Within Groups	1421327.776	1668	852.115		
	Total	2760190.473	1679			
TGL	Between Groups	1001024.700	11	91002.245	513.441	.000
	Within Groups	295636.025	1668	177.240		
	Total	1296660.725	1679			
OXGL	Between Groups	140042.923	11	12731.175	330.213	.000
	Within Groups	64308.829	1668	38.554		
	Total	204351.753	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX X

STATISTICAL ANALYSIS FOR EFFECT OF BPA ON THIOREDOXIN SYSTEM

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
THIOREDOXIN	Between Groups	59416.084	11	5401.462	91.314	.000
	Within Groups	98666.038	1668	59.152		
	Total	158082.123	1679			
THIOREDOXINREDUCTASE	Between Groups	547126.196	11	49738.745	138.501	.000
	Within Groups	599017.161	1668	359.123		
	Total	1146143.357	1679			

*. The mean difference is significant at the 0.05 level.

APPENDIX XI

REAGENTS

All the reagents used were already prepared and commercially obtained as kits and are here listed:

PRDX-1
PRDX-2
PRDX-3
PRDX-4
PRDX-5
PRDX-6

Lifespan Bioscience Inc, Seatle, WA, US

IL-1B
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-10
IL-17
IL-18
IL-21
IL-22
IL-26
IL- 30
Catalase
SOD
NO

Abcam United Kingdom

Nitrotyrosine
Lipid hydroperoxide (LPO)
Lipid peroxidation (MDA)
8-Isoprostane

OXI-Select, Minneapolis, USA

Protein Carbomoyl
CML-LDL
MDA-LDL
HNE-LDL
OXPL-LDL
Hydrogen Peroxide
Hydrogen Peroxidase
Myeloperoxidase

OXI-Select, Minneapolis, USA

Items	Supplier
Glutathione peroxidase Glutathione reductase Glutathione (GSSH/GSH) Prolactin Estrone	Sigma- Aldrich, St. Louis, USA
IL-14 IL-19 IL-25 IL-28 IL-29 IL-31 IL-32 IL-33	Quantikine ELISA, Minneapolis, USA
Hexanoyl-lysine Estradiol (Estrogen)	Eagle Biosciences Inc, Nashua NH
Total Antioxidant Capacity (TAC)	Oxford Biomedical Research, Oxford, USA:
LH FSH TSH	Phoenix Pharmaceuticals, Burligame, CA,
Thioredoxin and Thioredoxin reductase	IMCO Corporation Ltd, Stochholm, Sweden
Thyroxine (T4) Progesterone Testestorone Estriol	Enzo Life Sciences Inc, Boulevard Farmingale, NY

Triiodithyroxine (T3) Androstenedione	Diagnostic automation/Cortez Diagnostics Inc, Calabasas, CA

IL-8 IL-9 IL-11 IL-12 IL-13 IL- 15 IL-16 IL-20 IL-23 IL-24 IL-27 IL-30	Cloud-Clone Corp/ USCN Life Science Inc., Houston TX, USA

APPENDIX XII

FEMALE SEX HORMONE

Estrogen

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	48.66 ±0.001	46.87±0.05	49.27±0.00	49.99±0.005	49.90±0.00	47.84±0.00	47.67±0.00	48.00±0.00	48.41±0.00	47.66±0.00	48.37±0.00	48.81±0.00
1	48.05	46.65±0.03	51.70±0.00	52.77±0.004	54.43±0.00	48.17±0.00	52.76±0.005	93.97±0.00	101.21±0.005	182.59±0.00	204.29±0.00	261.03±0.005
2	47.31±0.00	53.95±0.02	52.60±0.005	57.30±0.005	57.42±0.005	50.27±0.00	55.91±0.00	99.88±0.005	103.40±0.005	180.39±0.005	200.18±0.005	257.56±0.00
3	48.07±0.00	53.29±0.005	54.63±0.005	63.63±0.005	58.35±0.00	54.51±0.00	59.46±0.00	105.84±0.005	109.59±0.005	178.20±0.00	196.08±0.00	254.08±0.005
4	47.83±0.00	57.09±0.00	63.95±0.005	69.69±0.005	69.53±0.005	61.02±0.00	66.11±0.005	109.64±0.005	112.28±0.005	171.50±0.005	191.97±0.005	250.61±0.00
5	48.09±0.00	58.00±0.005	64.77±0.00	79.08±0.005	69.29±0.00	64.91±0.00	67.9±0.0055	112.81±0.005	115.96±0.00	164.81±0.00	187.87±0.00	247.13±0.005
6	46.85±0.00	61.53±0.00	69.54±0.005	82.94±0.00	71.88±0.00	67.60±0.005	72.36±0.00	117.92±0.00	121.28±0.005	158.11±0.005	183.76±0.005	243.66±0.00
7	47.2±0.00	61.45±0.004	76.48±0.00	92.64±0.005	73.73±0.007	71.75±0.00	75.59±0.005	124.10±0.005	127.90±0.005	155.92±0.00	179.66±0.00	240.18±0.005
8	45.87±0.00	67.54±0.005	78.42±0.00	99.80±0.003	79.76±0.00	75.99±0.005	78.38±0.00	129.07±0.005	131.86±0.00	153.72±0.005	175.55±0.005	236.71±0.00
9	47.13±0.00	76.20±0.00	81.84±0.005	97.51±0.009	79.83±0.00	78.77±0.00	84.31±0.005	131.43±0.005	137.95±0.00	142.53±0.00	171.45±0.00	233.23±0.005
10	49.39±0.00	82.65±0.00	92.06±0.005	106.41±0.004	84.58±0.00	82.52±0.00	88.30±0.001	135.41±0.005	141.17±0.005	140.33±0.005	167.34±0.005	229.76±0.00
11	48.15±0.00	86.11±0.008	95.04±0.00	110.04±0.004	86.49±0.005	85.25±0.008	89.61±0.005	140.83±0.005	143.27±0.005	129.14±0.00	163.24±0.00	226.28±0.005
12	49.91±0.00	88.57±0.005	94.91±0.00	118.62±0.005	91.65±0.00	88.55±0.005	91.54±0.005	143.55±0.005	148.39±0.005	131.44±0.005	159.13±0.005	222.81±0.00
13	47.57±0.00	105.55±0.005	110.25±0.00	129.77±0.00	98.29±0.00	99.56±0.00	102.20±0.005	149.65±0.00	153.80±0.005	133.75±0.00	155.03±0.00	219.33±0.005

Estrone

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	35.28±0.005	37.72±0.005	36.00±0.00	36.84±0.005	37.80±0.00	37.39±0.004	38.24±0.005	37.74±0.00	37.69±0.00	37.99±0.005	38.18±0.00	38.015±0.00
1	36.08±0.005	41.99±0.005	47.45±0.00	48.52±0.005	50.28±0.00	49.77±0.00	46.82±0.005	88.12±0.00	98.41±0.005	183.06±0.00	202.30±0.005	253.38±0.005
2	36.20±0.00	49.01±0.005	48.70±0.005	53.50±0.005	55.07±0.005	54.57±0.00	51.56±0.005	90.82±0.00	101.60±0.005	167.98±0.00	199.86±0.005	252.40±0.005
3	36.09±0.004	53.92±0.00	41.18±0.005	55.78±0.005	59.15±0.00	54.87±0.00	55.85±0.005	93.51±0.008	104.79±0.005	152.90±0.00	192.07±0.00	245.75±0.005
4	36.63±0.005	51.13±0.00	59.85±0.005	67.69±0.005	61.33±0.004	59.67±0.00	58.79±0.005	96.22±0.00	107.98±0.005	137.82±0.00	179.37±0.00	235.59±0.005

5	36.74±0.00	57.16±0.00	62.37±0.00	75.06±0.00	61.99±0.00	63.21±0.00	59.57±0.005	98.92±0.00	111.17±0.005	122.74±0.00	180.75±0.005	225.30±0.00
6	37.11±0.00	61.75±0.00	65.24±0.005	79.59±0.00	66.42±0.005	68.05±0.005	64.22±0.005	101.62±0.00	114.36±0.005	107.66±0.00	179.03±0.005	224.32±0.00
7	36.54±0.00	61.84±0.00	68.48±0.00	87.04±0.005	73.69±0.005	69.75±0.00	68.56±0.00	104.32±0.00	117.55±0.005	94.58±0.00	170.07±0.00	209.21±0.00
8	35.57±0.005	66.74±0.005	71.17±0.005	94.23±0.00	78.31±0.00	73.69±0.005	71.59±0.00	107.02±0.00	120.74±0.005	88.50±0.00	158.81±0.00	198.60±0.00
9	35.65±0.00	77.36±0.005	79.04±0.005	99.12±0.00	75.68±0.00	78.27±0.00	78.44±0.003	109.72±0.00	123.93±0.005	81.12±0.00	156.37±0.00	190.46±0.005
10	35.70±0.00	77.23±0.008	81.96±0.005	103.06±0.005	79.98±0.00	83.52±0.00	78.73±0.00	112.42±0.00	127.12±0.005	76.87±0.006	148.98±0.00	179.67±0.005
11	35.74±0.00	79.97±0.005	84.39±0.00	108.90±0.00	87.74±0.005	76.26±0.00	81.62±0.005	115.12±0.00	130.31±0.005	65.86±0.006	145.01±0.00	167.08±0.005
12	36.83±0.005	83.03±0.006	89.07±0.007	113.47±0.005	87.94±0.009	85.83±0.00	87.89±0.005	117.82±0.00	133.50±0.005	58.09±0.007	141.13±0.00	158.45±0.005
13	36.35±0.00	92.89±0.00	99.5±0.00	123.27±0.00	101.92±0.005	96.21±0.00	92.09±0.005	120.52±0.00	136.69±0.005	41.89±0.006	136.98±0.00	146.85±0.005

Estriol

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	12.06±0.005	12.75±0.005	12.48±0.005	12.35±0.006	12.80±0.005	12.60±0.006	12.52±0.005	12.39±0.005	12.59±0.004	12.53±0.005	12.12±0.005	12.55±0.005
1	12.03±0.005	5.18±0.005	4.61±0.005	2.44±0.005	0.97±0.005	7.77±0.005	0.77±0.005	0.62±0.005	1.46±0.005	0.26±0.005	0.65±0.005	0.62±0.005
2	12.10±0.005	5.21±0.005	4.64±0.005	2.51±0.005	1.04±0.005	7.82±0.005	0.82±0.005	0.65±0.005	1.53±0.005	0.29±0.005	0.68±0.006	0.69±0.005
3	12.17±0.005	5.24±0.005	4.67±0.005	2.58±0.005	1.11±0.005	7.87±0.005	0.87±0.005	0.68±0.005	1.60±0.005	0.32±0.005	0.71±0.005	0.76±0.005
4	12.24±0.005	5.27±0.005	4.70±0.005	2.65±0.005	1.18±0.005	7.92±0.005	0.92±0.005	0.71±0.005	1.67±0.005	0.35±0.005	0.74±0.005	0.83±0.005
5	12.31±0.005	5.30±0.005	4.73±0.005	2.72±0.005	1.25±0.005	7.97±0.005	0.97±0.005	0.74±0.005	1.74±0.005	0.38±0.005	0.77±0.005	0.90±0.005
6	12.38±0.005	5.33±0.002	4.76±0.005	2.79±0.005	1.32±0.005	8.02±0.005	1.024±0.008	0.77±0.005	1.81±0.005	0.41±0.005	0.80±0.004	0.97±0.005
7	12.45±0.005	5.36±0.005	4.79±0.005	2.86±0.005	1.39±0.005	8.07±0.005	1.07±0.005	0.80±0.005	1.88±0.005	0.44±0.005	0.83±0.005	1.04±0.005
8	12.52±0.005	5.39±0.002	4.82±0.005	2.93±0.005	1.46±0.005	8.12±0.005	1.12±0.005	0.83±0.005	1.95±0.005	0.47±0.005	0.86±0.005	1.11±0.005
9	12.59±0.005	5.42±0.005	4.85±0.005	3.00±0.005	1.53±0.005	8.16±0.005	1.17±0.005	0.86±0.004	2.02±0.005	0.50±0.005	0.89±0.005	1.18±0.005
10	12.66±0.005	5.45±0.002	4.88±0.005	3.07±0.005	1.60±0.005	8.22±0.005	1.22±0.005	0.89±0.005	2.09±0.005	0.53±0.005	0.92±0.008	1.25±0.005
11	12.73±0.005	5.48±0.005	4.91±0.005	3.14±0.005	1.67±0.005	8.26±0.005	1.27±0.005	0.92±0.005	2.16±0.005	0.56±0.005	0.95±0.005	1.32±0.005
12	12.80±0.005	5.51±0.002	4.94±0.005	3.21±0.005	1.74±0.005	8.32±0.005	1.32±0.005	0.95±0.005	2.23±0.005	0.59±0.005	0.98±0.005	1.39±0.005
13	12.87±0.005	5.54±0.005	4.97±0.005	3.28±0.005	1.81±0.005	8.37±0.005	1.37±0.005	0.98±0.009	2.30±0.005	0.62±0.005	1.01±0.005	1.46±0.005

Prolactine

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	1.21±0.005	1.23±0.007	1.21±0.005	1.26±0.005	1.24±0.009	1.23±0.005	1.27±0.006	1.25±0.005	1.37±0.005	1.22±0.002	1.27±0.005	1.24±0.002
1	1.23±0.005	3.97±0.00	6.21±0.00	2.26±0.00	9.01±0.00	4.22±0.00	9.43±0.005	4.62±0.005	9.01±0.00	19.71±0.005	15.67±0.005	26.78±0.005
2	1.25±0.005	3.64±0.00	5.24±0.00	3.11±0.005	7.24±0.00	5.77±0.005	10.81±0.00	5.96±0.00	7.24±0.00	19.76±0.005	16.39±0.00	26.83±0.005
3	1.27±0.005	3.31±0.00	4.27±0.00	3.97±0.00	5.47±0.00	7.33±0.00	12.18±0.005	7.29±0.005	5.47±0.00	19.81±0.005	17.10±0.005	26.88±0.005
4	1.29±0.005	2.98±0.00	3.30±0.00	4.82±0.005	3.70±0.00	8.88±0.005	13.56±0.00	8.63±0.00	3.70±0.00	19.86±0.005	17.82±0.00	26.93±0.005
5	1.27±0.005	2.65±0.00	2.33±0.00	5.68±0.00	1.93±0.00	10.44±0.00	14.93±0.005	9.96±0.005	1.93±0.00	19.91±0.005	18.53±0.005	26.98±0.005
6	1.26±0.003	2.32±0.00	1.36±0.00	6.53±0.005	0.60±0.008	11.99±0.005	16.31±0.00	11.30±0.00	0.60±0.008	19.96±0.005	19.25±0.00	27.03±0.005
7	1.27±0.001	1.99±0.00	1.03±0.008	7.39±0.00	1.64±0.008	13.55±0.00	17.68±0.005	12.63±0.005	1.64±0.008	20.01±0.005	19.96±0.005	27.08±0.005
8	1.37±0.005	1.89±0.002	1.45±0.009	8.24±0.005	3.37±0.006	15.10±0.005	19.06±0.00	13.97±0.00	3.37±0.006	20.06±0.005	20.68±0.00	27.13±0.005
9	1.27±0.006	2.04±0.004	2.13±0.002	9.10±0.00	5.15±0.00	16.66±0.00	20.43±0.005	15.30±0.005	5.15±0.00	20.11±0.005	21.39±0.005	27.18±0.005
10	1.28±0.002	2.31±0.002	2.9±0.002	9.95±0.005	6.92±0.00	18.21±0.005	21.81±0.00	16.64±0.00	6.92±0.00	20.16±0.005	22.11±0.00	27.23±0.005
11	1.29±0.007	2.62±0.009	3.75±0.007	10.81±0.00	8.69±0.00	19.77±0.00	23.18±0.005	17.97±0.005	8.69±0.00	20.21±0.005	22.82±0.005	27.28±0.005
12	1.24±0.009	.00±0.003	4.64±0.008	11.66±0.005	10.46±0.00	21.32±0.005	24.56±0.00	19.31±0.00	10.46±0.00	20.26±0.005	23.54±0.00	27.33±0.005
13	1.23±0.007	3.40±0.00	5.56±0.008	12.52±0.00	12.23±0.00	22.88±0.00	25.93±0.005	20.64±0.005	12.23±0.00	20.31±0.005	24.25±0.005	27.38±0.005

Progesterone

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	0.16±0.003	0.15±0.005	0.16±0.00	0.15±0.002	0.16±0.005	0.14±0.005	0.14±0.005	0.14±0.005	0.15±0.003	0.15±0.002	0.15±0.00	0.16±0.003
1	0.17±0.003	1.43±0.00	1.84±0.005	2.97±0.005	4.10±0.005	3.15±0.00	6.21±0.00	5.29±0.005	7.04±0.005	1.72±0.005	9.01±0.00	9.43±0.005
2	0.14±0.005	1.46±0.00	1.86±0.005	2.98±0.005	4.15±0.005	3.35±0.00	5.24±0.00	5.34±0.005	7.09±0.005	2.36±0.00	7.24±0.00	10.81±0.00
3	0.15±0.001	1.49±0.00	1.88±0.005	2.99±0.005	4.20±0.005	3.55±0.00	4.27±0.00	5.39±0.005	7.14±0.005	2.99±0.005	5.47±0.00	12.18±0.005
4	0.14±0.003	1.52±0.00	1.90±0.005	2.99±0.005	4.25±0.005	3.75±0.00	3.30±0.00	5.44±0.005	7.19±0.005	3.63±0.00	3.70±0.00	13.56±0.00
5	0.15±0.00	1.55±0.00	1.92±0.005	3.01±0.005	4.30±0.005	3.95±0.00	2.33±0.00	5.49±0.005	7.24±0.005	4.26±0.005	1.93±0.00	14.93±0.005
6	0.16±0.005	1.58±0.00	1.94±0.005	3.02±0.005	4.35±0.005	2.80±0.00	1.36±0.00	5.54±0.005	7.29±0.001	4.90±0.00	0.60±0.008	16.31±0.00
7	0.14±0.005	1.61±0.00	1.96±0.005	3.02±0.005	4.40±0.005	2.05±0.00	1.03±0.008	5.59±0.005	7.34±0.005	5.53±0.005	1.64±0.008	17.68±0.005
8	0.17±0.00	1.64±0.00	1.98±0.005	3.04±0.005	4.45±0.005	4.55±0.00	1.45±0.009	5.64±0.005	7.39±0.001	6.17±0.00	3.37±0.006	19.06±0.00
9	0.14±0.005	1.67±0.00	2.00±0.005	3.05±0.005	4.50±0.005	3.43±0.00	2.13±0.002	5.69±0.005	7.44±0.001	6.80±0.005	5.15±0.00	20.43±0.005

10	0.15±0.005	1.7±0.00	2.02±0.005	3.05±0.005	4.55±0.005	4.95±0.00	2.92±0.00	5.74±0.005	7.49±0.005	7.44±0.00	6.92±0.00	21.81±0.00
11	0.16±0.005	1.73±0.00	2.04±0.005	3.07±0.005	4.60±0.005	2.22±0.00	3.75±0.009	5.79±0.005	7.54±0.001	8.07±0.005	8.69±0.00	23.18±0.005
12	0.17±0.005	1.76±0.00	2.06±0.005	3.08±0.005	4.65±0.005	2.95±0.00	4.64±0.008	5.84±0.005	7.59±0.005	8.71±0.00	10.46±0.00	24.56±0.00
13	0.18±0.005	1.79±0.00	2.08±0.005	3.08±0.005	4.70±0.005	2.59±0.00	5.56±0.008	5.89±0.005	7.64±0.001	9.34±0.005	12.23±0.00	25.93±0.005

Testosterone

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	0.03±0.003	0.03±0.006	0.037±0.009	0.03±0.009	0.031±0.005	0.03±0.003	0.034±0.003	0.03±0.003	0.03±0.001	0.03±0.007	0.03±0.006	0.03±0.002
1	0.03±0.003	0.18±0.005	0.89±0.005	1.02±0.005	1.51±0.005	3.43±0.005	0.80±0.005	2.07±0.005	5.70±0.005	1.84±0.00	6.76±0.005	8.30±0.005
2	0.03±0.004	0.19±0.005	0.90±0.005	1.07±0.005	1.53±0.005	3.44±0.005	0.82±0.005	2.08±0.005	5.71±0.005	2.54±0.005	6.81±0.005	8.35±0.005
3	0.03±0.003	0.20±0.005	0.91±0.005	1.12±0.005	1.55±0.005	3.45±0.005	0.84±0.005	2.09±0.005	5.72±0.005	3.25±0.00	6.86±0.005	8.40±0.005
4	0.03±0.001	0.21±0.005	0.92±0.003	1.17±0.005	1.57±0.005	3.45±0.005	0.86±0.005	2.10±0.005	5.72±0.005	3.95±0.005	6.91±0.005	8.45±0.005
5	0.031±0.005	0.22±0.005	0.93±0.005	1.22±0.005	1.59±0.005	3.47±0.005	0.88±0.005	2.11±0.005	5.74±0.005	4.66±0.00	6.96±0.005	8.50±0.005
6	0.03±0.005	0.23±0.005	0.94±0.005	1.27±0.005	1.61±0.005	3.48±0.005	0.90±0.005	2.12±0.005	5.75±0.005	5.36±0.005	7.01±0.004	8.54±0.006
7	0.03±0.003	0.24±0.005	0.95±0.003	1.32±0.005	1.63±0.005	3.48±0.005	0.92±0.005	2.13±0.005	5.75±0.005	6.07±0.00	7.06±0.005	8.60±0.005
8	0.03±0.006	0.25±0.005	0.96±0.005	1.37±0.005	1.65±0.005	3.50±0.005	0.94±0.003	2.14±0.005	5.77±0.005	6.77±0.005	7.11±0.004	8.64±0.006
9	0.03±0.009	0.26±0.005	0.97±0.005	1.42±0.005	1.67±0.005	3.51±0.005	0.96±0.005	2.15±0.005	5.78±0.005	7.48±0.00	7.16±0.004	8.69±0.006
10	0.03±0.007	0.27±0.005	0.98±0.004	1.47±0.005	1.69±0.005	3.51±0.005	0.98±0.005	2.16±0.005	5.78±0.005	8.18±0.005	7.21±0.005	8.75±0.005
11	0.03±0.007	0.28±0.005	0.99±0.005	1.52±0.005	1.71±0.005	3.53±0.005	1.00±0.004	2.17±0.005	5.80±0.005	8.89±0.00	7.26±0.004	8.79±0.006
12	0.03±0.001	0.29±0.005	1.00±0.005	1.57±0.005	1.73±0.005	3.54±0.005	1.02±0.005	2.18±0.005	5.81±0.005	9.59±0.005	7.31±0.005	8.85±0.005
13	0.03±0.006	0.30±0.005	1.01±0.006	1.62±0.005	1.75±0.005	3.54±0.005	1.04±0.005	2.19±0.005	5.81±0.005	10.30±0.00	7.36±0.004	8.89±0.006

Luteinizing Hormone

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	46.94±0.005	46.14±0.00	45.94±0.009	46.48±0.002	46.63±0.005	46.94±0.005	46.54±0.005	46.64±0.005	46.34±0.005	46.54±0.005	46.38±0.005	46.54±0.005
1	46.54±0.005	72.03±0.008	41.21±0.005	53.01±0.005	80.71±0.006	110.44±0.005	135.59±0.005	153.28±0.005	164.29±0.005	115.34±0.005	61.37±0.00	194.93±0.005
2	46.44±0.004	74.82±0.006	44.14±0.007	56.84±0.007	86.51±0.001	118.93±0.00	139.63±0.005	165.01±0.00	168.89±0.005	124.73±0.00	67.75±0.005	209.81±0.00
3	46.34±0.005	77.64±0.005	47.08±0.00	60.68±0.00	98.48±0.002	127.41±0.005	143.67±0.005	176.73±0.005	173.49±0.005	134.11±0.005	74.14±0.00	224.68±0.005

4	46.73±0.001	80.42±0.006	50.01±0.002	64.51±0.002	109.68±0.007	135.9±0.00	147.71±0.005	188.46±0.00	178.09±0.005	143.50±0.00	80.52±0.005	239.56±0.00
5	46.64±0.005	83.24±0.005	52.94±0.005	68.34±0.005	116.98±0.001	144.38±0.005	151.75±0.005	200.18±0.005	182.69±0.005	152.88±0.005	86.90±0.009	254.43±0.005
6	45.54±0.005	86.04±0.005	55.87±0.007	72.17±0.007	130.82±0.00	152.87±0.00	155.79±0.005	211.91±0.00	187.29±0.005	162.27±0.00	93.28±0.003	269.31±0.00
7	46.54±0.005	88.84±0.005	58.81±0.00	76.01±0.00	145.65±0.00	161.35±0.005	159.83±0.005	223.63±0.005	191.89±0.005	171.65±0.005	99.66±0.006	284.18±0.005
8	45.34±0.005	91.64±0.005	61.74±0.002	79.84±0.002	161.53±0.006	169.84±0.00	163.87±0.005	235.36±0.00	196.49±0.005	181.04±0.00	106.06±0.003	299.06±0.00
9	46.94±0.005	94.44±0.005	64.67±0.005	83.65±0.005	180.78±0.009	178.32±0.005	167.91±0.005	247.08±0.005	201.09±0.005	190.42±0.005	112.45±0.00	313.93±0.005
10	46.14±0.005	97.23±0.00	67.60±0.007	87.49±0.003	175.15±0.009	186.81±0.00	171.95±0.005	258.81±0.00	205.69±0.005	199.81±0.00	118.83±0.005	328.81±0.00
11	45.94±0.009	100.03±0.006	70.54±0.00	91.34±0.00	169.07±0.007	195.29±0.005	175.98±0.005	270.53±0.005	210.28±0.005	209.19±0.005	125.22±0.00	343.68±0.005
12	45.94±0.009	102.83±0.009	73.47±0.002	95.17±0.002	160.69±0.006	203.78±0.00	180.01±0.006	282.26±0.00	214.88±0.005	218.58±0.00	131.60±0.005	358.56±0.00
13	46.14±0.00	105.63±0.009	76.40±0.005	99.00±0.005	151.82±0.00	212.26±0.005	184.06±0.005	293.98±0.005	219.48±0.005	227.96±0.005	137.99±0.00	373.43±0.005

Follicle Stimulating Hormone

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	75.75±0.002	75.75±0.002	75.34±0.005	75.19±0.001	75.26±0.00	76.73±0.005	76.19±0.005	75.09±0.00	75.28±0.00	75.13±0.007	75.96±0.00	74.32±0.001
1	75.28±0.00	26.07±0.00	30.03±0.00	25.98±0.005	20.66±0.005	28.84±0.00	21.89±0.005	25.41±0.00	20.46±0.00	18.88±0.005	22.06±0.00	19.96±0.005
2	75.34±0.005	26.46±0.00	29.51±0.005	24.95±0.005	21.28±0.00	29.64±0.00	22.50±0.00	24.75±0.005	19.82±0.005	22.28±0.005	17.99±0.00	20.63±0.00
3	75.13±0.007	22.57±0.005	26.07±0.005	24.01±0.005	17.30±0.005	21.01±0.005	21.58±0.005	24.50±0.005	21.21±0.005	20.01±0.005	16.89±0.00	20.395±0.00
4	75.19±0.001	23.6±0.0085	29.92±0.005	17.72±0.00	19.13±0.005	19.53±0.00	15.49±0.005	25.51±0.005	19.63±0.005	19.18±0.005	12.01±0.00	17.28±0.00
5	75.96±0.00	21.69±0.00	24.86±0.005	20.56±0.00	20.24±0.005	22.62±0.00	17.73±0.00	28.10±0.00	18.68±0.005	17.09±0.005	13.25±0.00	16.68±0.005
6	75.26±0.00	22.53±0.00	24.08±0.00	13.18±0.005	16.18±0.00	18.65±0.00	19.19±0.00	22.49±0.005	16.16±0.00	17.52±0.005	16.29±0.00	18.79±0.00
7	75.40±0.005	18.42±0.00	22.80±0.00	14.72±0.00	19.54±0.00	21.58±0.00	14.40±0.00	22.96±0.005	14.67±0.00	12.55±0.005	8.53±0.00	11.53±0.004
8	75.09±0.00	16.87±0.005	25.48±0.00	17.87±0.005	12.91±0.00	14.49±0.005	12.88±0.004	23.97±0.005	13.90±0.00	11.36±0.005	5.81±0.00	11.65±0.009
9	76.39±0.00	19.51±0.005	20.15±0.00	12.92±0.009	13.21±0.00	17.32±0.005	14.21±0.00	22.01±0.005	11.06±0.00	10.98±0.005	4.8±0.00	12.50±0.003
10	76.73±0.005	12.79±0.005	16.80±0.00	9.51±0.003	10.79±0.001	15.76±0.005	12.70±0.005	20.73±0.00	13.08±0.00	6.22±0.005	6.76±0.00	10.29±0.00
11	75.91±0.005	18.09±0.00	24.16±0.00	10.14±0.006	8.82±0.00	15.84±0.00	7.59±0.00	21.38±0.00	18.07±0.00	6.89±0.003	4.62±0.005	10.95±0.005
12	76.19±0.005	14.15±0.00	16.76±0.00	8.93±0.007	10.72±0.001	8.68±0.009	9.70±0.00	20.00±0.004	9.74±0.00	4.45±0.009	2.31±0.00	7.84±0.00
13	74.32±0.001	9.32±0.009	12.19±0.004	6.51±0.005	8.62±0.008	8.09±0.00	10.11±0.00	20.42±0.009	12.25±0.005	3.65±0.002	10.48±0.00	7.60±0.005

Androstenedione

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	69.24±0.005	69.24±0.005	68.86±0.003	68.82±0.006	68.42±0.006	69.04±0.005	69.64±0.005	68.03±0.00	69.63±0.009	69.03±0.008	68.64±0.005	68.84±0.005
1	69.03±0.008	151.25±0.00	89.47±0.007	43.78±0.005	47.51±0.005	51.76±0.005	56.88±0.00	167.82±0.009	215.54±0.005	140.19±0.005	246.29±0.005	129.24±0.003
2	68.82±0.006	162.57±0.005	96.09±0.005	49.44±0.005	53.23±0.005	57.40±0.005	61.98±0.005	180.41±0.008	221.14±0.005	150.93±0.00	252.49±0.005	138.88±0.009
3	68.64±0.005	173.9±0.00	102.70±0.004	55.10±0.005	58.95±0.005	63.04±0.005	67.09±0.00	193.00±0.008	226.74±0.005	161.66±0.005	258.69±0.005	148.53±0.006
4	68.42±0.006	185.22±0.005	109.31±0.007	60.76±0.005	64.67±0.005	68.68±0.005	72.19±0.005	205.59±0.007	232.34±0.004	172.40±0.00	264.88±0.001	158.18±0.002
5	68.24±0.005	196.55±0.00	115.93±0.001	66.42±0.005	70.39±0.005	74.32±0.005	77.30±0.00	218.18±0.007	237.94±0.005	183.13±0.005	271.09±0.005	167.82±0.009
6	69.04±0.005	207.87±0.005	122.54±0.004	72.08±0.005	76.11±0.005	79.96±0.005	82.40±0.005	230.77±0.006	243.54±0.005	193.87±0.00	277.29±0.005	177.47±0.005
7	68.84±0.005	219.20±0.00	129.15±0.008	77.74±0.005	81.83±0.005	85.60±0.005	87.51±0.00	243.36±0.006	249.14±0.005	204.60±0.005	283.49±0.005	187.12±0.002
8	69.64±0.005	230.52±0.005	135.77±0.001	83.40±0.005	87.55±0.005	91.24±0.005	92.61±0.005	255.95±0.005	254.74±0.005	215.34±0.00	289.69±0.005	196.76±0.008
9	69.54±0.005	241.85±0.00	142.38±0.005	89.06±0.005	93.27±0.005	96.88±0.005	97.72±0.00	268.54±0.005	260.34±0.005	226.07±0.005	295.89±0.005	206.41±0.005
10	68.03±0.00	253.17±0.005	148.99±0.008	94.72±0.005	98.99±0.005	102.52±0.005	102.82±0.005	281.13±0.004	265.94±0.005	236.81±0.00	302.09±0.005	216.06±0.001
11	68.53±0.006	264.50±0.00	155.61±0.002	100.38±0.005	104.71±0.005	108.16±0.005	107.93±0.00	293.72±0.004	271.53±0.005	247.54±0.005	308.28±0.005	225.70±0.008
12	69.63±0.009	275.82±0.005	162.22±0.005	106.04±0.005	110.43±0.005	113.80±0.005	113.03±0.005	306.31±0.003	277.13±0.006	258.28±0.00	314.47±0.006	235.35±0.004
13	68.23±0.009	287.1±0.005	168.83±0.009	111.70±0.005	116.15±0.005	119.44±0.005	118.14±0.00	318.90±0.003	282.73±0.005	269.01±0.005	320.67±0.006	245.00±0.001

APPENDIX XIII

THYROID HORMONES

Thyroid Stimulating Hormone

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	0.88±0.005	1.68±0.005	0.84±0.005	0.97±0.00	0.93±0.005	0.99±0.00	1.09±0.005	0.93±0.003	0.96±0.002	0.90±0.008	0.86±0.005	0.87±0.006
1	0.89±0.005	1.69±0.005	2.24±0.00	6.93±0.005	14.96±0.005	18.72±0.005	20.06±0.005	23.02±0.005	26.73±0.005	30.90±0.005	35.13±0.005	40.55±0.005
2	0.90±0.005	1.70±0.005	1.24±0.005	6.94±0.005	14.99±0.005	18.77±0.005	20.09±0.005	24.25±0.00	26.80±0.005	30.91±0.005	35.20±0.005	40.56±0.005
3	0.91±0.005	1.71±0.005	0.25±0.00	6.95±0.005	15.02±0.005	18.82±0.005	20.12±0.005	25.47±0.005	26.87±0.005	30.92±0.005	35.27±0.005	40.57±0.005
4	0.92±0.003	1.72±0.005	0.74±0.005	6.96±0.005	15.05±0.005	18.87±0.005	20.15±0.005	26.70±0.00	26.94±0.005	30.91±0.006	35.34±0.005	40.56±0.006
5	0.93±0.005	1.73±0.005	1.74±0.00	6.97±0.005	15.08±0.005	18.92±0.005	20.18±0.005	27.92±0.005	27.01±0.005	30.94±0.005	35.41±0.005	40.59±0.005
6	0.94±0.005	1.74±0.005	2.73±0.005	6.98±0.005	15.11±0.005	18.97±0.005	20.21±0.005	29.15±0.00	27.08±0.005	30.95±0.005	35.48±0.005	40.60±0.005
7	0.95±0.003	1.75±0.005	3.73±0.00	6.99±0.005	15.14±0.005	19.02±0.005	20.24±0.005	30.37±0.005	27.15±0.005	30.94±0.006	35.55±0.005	40.59±0.006
8	0.96±0.005	1.76±0.005	4.72±0.005	7.00±0.005	15.17±0.005	19.07±0.005	20.27±0.005	31.60±0.00	27.22±0.005	30.97±0.005	35.62±0.005	40.62±0.005
9	0.97±0.005	1.77±0.005	5.72±0.00	7.002±0.001	15.20±0.005	19.12±0.005	20.30±0.005	32.82±0.005	27.29±0.005	30.98±0.005	35.69±0.005	40.63±0.005
10	0.98±0.004	1.78±0.005	6.70±0.008	7.02±0.005	15.23±0.005	19.17±0.005	20.33±0.005	34.05±0.00	27.36±0.005	30.97±0.006	35.76±0.005	40.62±0.006
11	0.99±0.005	1.79±0.005	7.71±0.00	7.03±0.005	15.26±0.005	19.22±0.005	20.36±0.005	35.27±0.005	27.43±0.005	31.00±0.005	35.83±0.005	40.65±0.005
12	1.00±0.005	1.80±0.005	8.70±0.005	7.04±0.005	15.29±0.005	19.27±0.005	20.39±0.005	36.50±0.00	27.50±0.005	31.01±0.005	35.90±0.005	40.66±0.005
13	1.01±0.004	1.81±0.004	9.70±0.00	7.05±0.005	15.32±0.005	19.32±0.005	20.42±0.005	37.72±0.005	27.57±0.005	31.02±0.005	35.97±0.005	51.65±0.006

Total Thyroxine

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	1.02±0.005	1.18±0.006	1.08±0.005	0.83±0.005	0.95±0.006	0.77±0.005	0.98±0.00	0.97±0.009	1.24±0.003	0.95±0.006	0.96±0.008	1.27±0.004
1	1.03±0.005	36.26±0.005	14.55±0.005	19.01±0.005	24.32±0.005	27.22±0.005	16.19±0.005	13.95±0.005	23.82±0.005	26.99±0.005	8.21±0.005	45.00±0.005
2	1.04±0.007	36.29±0.005	14.62±0.005	19.04±0.005	24.37±0.005	27.23±0.005	16.31±0.00	13.96±0.005	23.87±0.005	27.02±0.005	8.24±0.005	45.07±0.005
3	1.05±0.005	36.32±0.005	14.69±0.005	19.07±0.005	24.42±0.005	27.24±0.005	16.42±0.005	13.97±0.005	23.92±0.005	27.05±0.005	8.27±0.005	45.14±0.005
4	1.06±0.005	36.35±0.005	14.76±0.005	19.10±0.005	24.47±0.005	27.24±0.008	16.54±0.00	13.98±0.005	23.97±0.005	27.08±0.005	8.30±0.005	45.21±0.005
5	1.07±0.005	36.38±0.005	14.83±0.005	19.13±0.005	24.52±0.005	27.26±0.005	16.65±0.005	13.95±0.006	24.02±0.005	27.11±0.005	8.32±0.005	45.28±0.005

6	1.08±0.005	36.41±0.005	14.90±0.005	19.16±0.005	24.57±0.005	27.27±0.005	16.77±0.00	14.00±0.005	24.07±0.005	27.12±0.006	8.36±0.005	45.35±0.005
7	1.09±0.005	36.44±0.005	14.97±0.005	19.19±0.005	24.62±0.005	27.26±0.009	16.88±0.005	14.01±0.005	24.12±0.005	27.17±0.005	8.38±0.005	45.42±0.005
8	1.10±0.005	36.47±0.005	15.04±0.005	19.22±0.005	24.67±0.005	27.29±0.005	17.00±0.00	14.02±0.005	24.17±0.005	27.20±0.005	8.42±0.005	45.49±0.005
9	1.11±0.005	36.50±0.005	15.11±0.005	19.25±0.005	24.72±0.005	27.30±0.005	17.11±0.005	14.03±0.005	24.22±0.005	27.23±0.005	8.44±0.005	45.56±0.005
10	1.12±0.005	36.53±0.005	15.18±0.005	19.28±0.005	24.77±0.005	27.29±0.009	17.23±0.00	14.04±0.005	24.27±0.005	27.24±0.006	8.48±0.005	45.63±0.005
11	1.13±0.005	36.56±0.005	15.25±0.005	19.31±0.005	24.82±0.005	27.32±0.005	17.34±0.005	14.05±0.005	24.32±0.005	27.29±0.005	8.50±0.005	45.70±0.005
12	1.14±0.005	36.59±0.005	15.32±0.005	19.34±0.005	24.87±0.005	27.33±0.005	17.46±0.00	14.06±0.005	24.37±0.005	27.32±0.005	8.54±0.005	45.77±0.005
13	1.02±0.005	36.62±0.005	15.39±0.005	19.37±0.005	24.92±0.005	27.33±0.008	17.57±0.005	14.07±0.005	24.42±0.005	27.35±0.005	8.57±0.005	45.84±0.005

Free Thyroxine

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	1.45±0.005	0.90±0.002	1.30±0.007	0.94±0.005	1.27±0.008	0.83±0.001	1.24±0.00	1.08±0.005	1.50±0.002	1.36±0.008	1.47±0.006	1.18±0.00
1	1.32±0.005	6.62±0.005	13.88±0.005	16.51±0.005	23.27±0.005	26.36±0.005	14.90±0.005	11.62±0.005	21.86±0.005	25.61±0.005	33.93±0.005	44.79±0.005
2	1.33±0.005	6.67±0.005	13.91±0.005	16.58±0.005	23.32±0.005	26.39±0.005	14.97±0.005	11.67±0.005	21.89±0.005	25.68±0.005	33.94±0.005	45.11±0.00
3	1.34±0.005	6.72±0.005	13.94±0.005	16.65±0.005	23.37±0.005	26.42±0.005	15.04±0.005	11.72±0.005	21.92±0.005	25.75±0.005	33.95±0.005	45.42±0.005
4	1.34±0.005	6.77±0.005	13.97±0.005	16.72±0.005	23.42±0.005	26.45±0.005	15.11±0.005	11.77±0.005	21.95±0.005	25.82±0.005	33.94±0.006	45.74±0.00
5	1.36±0.005	6.82±0.005	14.00±0.005	16.79±0.005	23.47±0.005	26.48±0.005	15.18±0.005	11.82±0.005	21.98±0.005	25.89±0.005	33.97±0.005	46.05±0.005
6	1.37±0.005	6.87±0.005	14.03±0.005	16.86±0.005	23.52±0.005	26.51±0.005	15.25±0.005	11.87±0.005	22.01±0.005	25.96±0.005	33.98±0.005	46.37±0.00
7	1.38±0.005	6.92±0.005	14.06±0.005	16.93±0.005	23.57±0.005	26.54±0.005	15.32±0.005	11.92±0.005	22.04±0.005	26.03±0.005	33.97±0.006	46.68±0.005
8	1.39±0.005	6.97±0.005	14.09±0.005	17.00±0.005	23.62±0.005	26.57±0.005	15.39±0.005	11.97±0.005	22.07±0.005	26.10±0.005	34.00±0.005	47.00±0.00
9	1.40±0.005	7.01±0.005	14.12±0.005	17.07±0.005	23.67±0.005	26.60±0.005	15.46±0.005	12.02±0.005	22.10±0.005	26.17±0.005	34.01±0.005	47.31±0.005
10	1.41±0.005	7.07±0.005	14.15±0.005	17.14±0.005	23.72±0.005	26.63±0.005	15.53±0.005	12.07±0.005	22.13±0.005	26.24±0.005	34.00±0.006	47.63±0.00
11	1.42±0.005	7.11±0.005	14.18±0.005	17.21±0.005	23.77±0.005	26.66±0.005	15.60±0.005	12.12±0.005	22.16±0.005	26.31±0.005	34.03±0.005	47.94±0.005
12	1.43±0.005	7.17±0.005	14.21±0.005	17.28±0.005	23.82±0.005	26.69±0.005	15.67±0.005	12.17±0.005	22.19±0.005	26.38±0.005	34.04±0.005	48.26±0.00
13	1.44±0.005	7.22±0.005	14.24±0.005	17.35±0.005	23.87±0.005	26.72±0.005	15.74±0.005	12.22±0.005	22.22±0.005	26.45±0.005	34.03±0.006	48.57±0.005

Conjugated Thyroxine

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	6.24±0.00	7.93±0.005	8.19±0.00	8.89±0.00	7.11±0.00	8.83±0.00	6.11±0.00	7.03±0.00	5.37±0.00	7.16±0.00	6.33±0.00	8.22±0.00
1	8.18±0.00	1.59±0.00	0.67±0.00	2.50±0.00	1.05±0.00	0.85±0.00	1.29±0.00	2.33±0.00	1.95±0.00	1.38±0.00	2.33±0.00	0.24±0.00
2	5.18±0.00	1.57±0.00	0.70±0.009	2.46±0.00	1.05±0.00	0.83±0.00	1.33±0.005	2.29±0.00	1.98±0.00	1.34±0.00	2.34±0.00	0.03±0.004
3	7.20±0.00	1.55±0.00	0.74±0.00	2.42±0.00	1.05±0.00	0.81±0.00	1.38±0.00	2.25±0.00	1.99±0.00	1.30±0.00	2.36±0.00	0.28±0.005
4	6.25±0.00	1.53±0.00	0.78±0.009	2.38±0.00	1.05±0.00	0.79±0.00	1.42±0.005	2.21±0.00	2.01±0.00	1.26±0.00	2.40±0.009	0.52±0.00
5	6.22±0.00	1.50±0.00	0.82±0.008	2.34±0.00	1.05±0.00	0.77±0.00	1.47±0.00	2.13±0.001	2.03±0.00	1.22±0.00	2.41±0.00	0.76±0.00
6	8.23±0.00	1.49±0.00	0.86±0.007	2.30±0.00	1.05±0.00	0.75±0.00	1.51±0.005	2.13±0.00	2.05±0.00	1.16±0.001	2.42±0.00	1.01±0.00
7	7.24±0.00	1.46±0.00	0.91±0.00	2.26±0.00	1.05±0.00	0.72±0.00	1.56±0.00	2.09±0.00	2.07±0.00	1.14±0.00	2.46±0.009	1.25±0.00
8	7.25±0.00	1.45±0.00	0.94±0.009	2.22±0.00	1.05±0.00	0.71±0.002	1.60±0.005	2.05±0.00	2.09±0.00	1.10±0.00	2.47±0.00	1.50±0.005
9	7.26±0.00	1.43±0.00	0.98±0.008	2.18±0.00	1.05±0.00	0.68±0.009	1.65±0.00	2.01±0.00	2.11±0.00	1.06±0.00	2.48±0.00	1.75±0.00
10	5.27±0.00	1.41±0.00	1.03±0.00	2.14±0.00	1.05±0.00	0.66±0.00	1.69±0.005	1.97±0.00	2.13±0.00	1.00±0.001	2.52±0.009	1.99±0.00
11	8.28±0.00	1.39±0.00	1.07±0.00	2.10±0.00	1.05±0.00	0.65±0.00	1.74±0.00	1.93±0.00	2.15±0.00	0.98±0.00	2.52±0.00	2.24±0.00
12	5.29±0.00	1.37±0.00	1.11±0.00	2.06±0.00	1.05±0.00	0.63±0.00	1.78±0.005	1.89±0.00	2.18±0.00	0.94±0.00	2.55±0.00	2.48±0.005
13	7.23±0.005	1.35±0.00	1.15±0.00	2.02±0.00	1.05±0.00	0.61±0.00	1.83±0.00	1.85±0.00	2.19±0.00	0.90±0.00	2.58±0.009	2.72±0.00

Total Triiodothyronine

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	4.04±0.00	4.14±0.009	4.06±0.00	4.01±0.00	4.28±0.00	4.24±0.005	4.24±0.005	4.55±0.005	4.25±0.005	4.30±0.007	4.19±0.005	4.37±0.005
1	4.15±0.00	3.40±0.00	7.67±0.00	9.62±0.005	12.95±0.005	15.88±0.005	18.68±0.005	19.91±0.005	11.81±0.005	21.87±0.005	19.73±0.005	24.77±0.005
2	5.10±0.005	3.67±0.005	8.27±0.005	10.17±0.005	13.28±0.005	16.65±0.005	19.45±0.005	20.24±0.005	12.14±0.005	22.42±0.005	20.50±0.005	25.32±0.005
3	4.05±0.005	3.42±0.005	7.72±0.005	9.67±0.005	12.98±0.005	15.95±0.005	18.75±0.005	19.94±0.005	11.84±0.005	21.92±0.005	19.80±0.005	24.82±0.005
4	4.16±0.00	3.45±0.00	7.78±0.00	9.72±0.005	13.01±0.005	16.02±0.005	18.82±0.005	19.97±0.005	11.87±0.005	21.97±0.005	19.87±0.005	24.87±0.005
5	4.89±0.005	3.62±0.005	8.16±0.005	10.07±0.001	13.22±0.005	16.51±0.005	19.31±0.005	20.18±0.005	12.08±0.005	22.32±0.005	20.36±0.005	25.22±0.005
6	4.37±0.00	3.50±0.00	7.89±0.00	9.82±0.005	13.07±0.005	16.16±0.005	18.96±0.005	20.03±0.005	11.93±0.005	22.07±0.005	20.01±0.005	24.97±0.005
7	4.47±0.005	3.52±0.005	7.94±0.005	9.87±0.005	13.10±0.005	16.23±0.005	19.03±0.005	20.06±0.005	11.96±0.005	22.12±0.005	20.08±0.005	25.02±0.005
8	4.58±0.00	3.55±0.00	8.00±0.00	9.92±0.005	13.13±0.005	16.30±0.005	19.10±0.005	20.09±0.005	11.99±0.005	22.17±0.005	20.15±0.005	25.07±0.005
9	4.68±0.005	3.57±0.005	8.05±0.005	9.96±0.009	13.16±0.005	16.37±0.005	19.17±0.005	20.12±0.005	12.02±0.005	22.22±0.005	20.22±0.005	25.12±0.005

10	4.79±0.00	3.60±0.00	8.11±0.00	10.02±0.005	13.19±0.005	16.44±0.005	19.24±0.005	20.15±0.005	12.05±0.005	22.27±0.005	20.29±0.005	25.17±0.005
11	4.04±0.005	3.37±0.005	7.61±0.005	9.57±0.005	12.92±0.005	15.81±0.005	18.61±0.005	19.88±0.005	12.14±0.005	21.82±0.005	19.66±0.005	24.72±0.005
12	4.70±0.00	3.65±0.00	8.22±0.00	10.12±0.005	13.25±0.005	16.58±0.005	19.38±0.005	20.21±0.005	12.11±0.005	22.37±0.005	20.43±0.005	25.27±0.005
13	4.26±0.005	3.47±0.004	7.83±0.005	9.77±0.005	13.04±0.005	16.09±0.005	18.89±0.005	20.00±0.005	11.90±0.005	22.02±0.005	19.94±0.005	24.92±0.005

Free Triiodothyronine

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	2.33±0.005	2.45±0.005	2.70±0.007	2.34±0.00	2.37±0.004	2.38±0.006	2.57±0.002	2.85±0.005	2.12±0.007	2.39±0.00	2.38±0.007	2.77±0.005
1	2.33±0.00	1.88±0.005	6.68±0.00	7.41±0.005	5.78±0.005	8.62±0.005	13.13±0.005	16.02±0.005	10.22±0.005	15.57±0.005	10.79±0.005	18.54±0.005
2	2.12±0.005	1.91±0.005	6.73±0.005	7.48±0.005	5.81±0.005	8.67±0.005	12.77±0.005	16.09±0.005	9.85±0.005	15.62±0.005	10.82±0.005	18.61±0.005
3	2.22±0.00	1.94±0.005	6.79±0.00	7.55±0.005	5.84±0.005	8.72±0.005	12.80±0.005	16.16±0.005	9.89±0.005	15.67±0.005	10.85±0.005	18.68±0.005
4	2.31±0.005	1.97±0.005	6.84±0.005	7.62±0.005	5.87±0.005	8.77±0.005	12.83±0.005	16.23±0.005	9.92±0.005	15.72±0.005	10.88±0.005	18.75±0.005
5	2.41±0.00	2.00±0.005	6.90±0.00	7.69±0.005	5.90±0.005	8.82±0.005	12.86±0.005	16.30±0.005	9.95±0.002	15.77±0.005	10.91±0.005	18.82±0.005
6	2.37±0.005	2.03±0.005	6.95±0.005	7.75±0.009	5.92±0.005	8.87±0.005	12.89±0.005	16.37±0.005	9.98±0.005	15.82±0.005	10.94±0.005	18.89±0.005
7	2.45±0.00	2.06±0.005	7.00±0.009	7.83±0.005	5.96±0.005	8.92±0.005	12.92±0.005	16.44±0.005	10.01±0.005	15.87±0.005	10.97±0.005	18.96±0.005
8	2.46±0.005	2.09±0.005	7.06±0.005	7.89±0.009	5.98±0.005	8.97±0.005	12.95±0.005	16.51±0.005	10.04±0.005	15.92±0.005	11.00±0.005	19.03±0.005
9	2.63±0.00	2.12±0.005	7.12±0.00	7.97±0.005	6.02±0.005	9.01±0.005	12.98±0.005	16.58±0.005	10.07±0.001	15.97±0.005	11.03±0.005	19.10±0.005
10	2.58±0.005	2.15±0.005	7.17±0.005	8.03±0.009	6.04±0.005	9.07±0.005	13.01±0.005	16.65±0.005	10.10±0.005	16.02±0.005	11.06±0.005	19.17±0.005
11	2.58±0.00	2.18±0.005	7.23±0.00	8.10±0.009	6.08±0.005	9.11±0.005	13.04±0.005	16.72±0.005	10.13±0.005	16.07±0.005	11.09±0.005	19.24±0.005
12	2.76±0.00	2.21±0.005	7.28±0.004	8.18±0.005	6.10±0.005	9.17±0.005	13.07±0.005	16.79±0.005	10.16±0.005	16.12±0.005	11.12±0.005	19.31±0.005
13	3.17±0.00	2.24±0.005	7.34±0.00	8.25±0.005	6.14±0.005	9.22±0.005	13.10±0.005	16.86±0.005	10.19±0.005	16.17±0.005	11.15±0.005	19.38±0.005

Conjugated Triiodothyronine

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	21.63±0.005	19.00±0.005	18.55±0.005	22.10±0.005	21.15±0.005	18.20±0.005	21.25±0.005	19.30±0.005	20.35±0.005	18.40±0.005	19.45±0.005	22.50±0.005
1	21.69±0.005	1.49±0.00	0.93±0.005	2.16±0.00	7.14±0.00	7.19±0.00	5.48±0.00	3.86±0.00	1.92±0.00	6.25±0.00	8.87±0.00	6.18±0.00
2	21.75±0.005	1.48±0.005	0.93±0.005	2.14±0.00	7.14±0.00	7.21±0.00	5.91±0.00	3.82±0.00	1.96±0.00	6.25±0.00	8.91±0.00	6.16±0.00
3	21.81±0.005	1.48±0.00	0.93±0.005	2.12±0.00	7.14±0.00	7.23±0.00	5.95±0.00	3.78±0.00	1.95±0.00	6.25±0.00	8.95±0.00	6.14±0.00

4	21.87±0.005	1.47±0.005	0.93±0.005	2.10±0.00	7.14±0.00	7.25±0.00	5.99±0.00	3.74±0.00	1.95±0.00	6.25±0.00	8.99±0.00	6.12±0.00
5	21.93±0.005	1.46±0.009	0.93±0.005	2.08±0.00	7.14±0.00	7.27±0.00	6.03±0.00	3.70±0.00	1.95±0.004	6.25±0.00	9.03±0.00	6.10±0.00
6	21.99±0.005	1.46±0.005	0.93±0.005	2.06±0.006	7.15±0.00	7.29±0.00	6.07±0.00	3.66±0.00	1.95±0.00	6.25±0.00	9.07±0.00	6.08±0.00
7	22.05±0.005	1.46±0.00	0.93±0.006	2.04±0.00	7.14±0.00	7.31±0.00	6.11±0.00	3.62±0.00	1.95±0.00	6.25±0.00	9.11±0.00	6.06±0.00
8	22.11±0.005	1.45±0.005	0.93±0.005	2.02±0.006	7.15±0.00	7.33±0.00	6.15±0.00	3.58±0.00	1.95±0.00	6.25±0.00	9.15±0.00	6.04±0.00
9	22.17±0.005	1.45±0.00	0.93±0.005	1.99±0.004	7.14±0.00	7.36±0.00	6.19±0.00	3.54±0.00	1.95±0.003	6.25±0.00	9.19±0.00	6.02±0.00
10	22.23±0.005	1.44±0.005	0.93±0.005	1.98±0.006	7.15±0.00	7.37±0.00	6.23±0.00	3.50±0.00	1.95±0.00	6.25±0.00	9.23±0.00	6.00±0.00
11	22.29±0.005	1.44±0.00	0.93±0.005	1.96±0.002	7.14±0.00	7.40±0.00	6.27±0.00	3.46±0.00	1.95±0.00	6.25±0.00	9.27±0.00	5.98±0.00
12	22.35±0.005	1.43±0.005	0.93±0.006	1.94±0.00	7.15±0.00	7.41±0.00	6.31±0.00	3.42±0.00	1.95±0.00	6.25±0.00	9.31±0.00	5.96±0.00
13	21.93±0.005	1.43±0.00	0.93±0.005	1.92±0.00	7.14±0.00	7.43±0.00	6.35±0.00	3.38±0.00	1.95±0.00	6.25±0.00	9.35±0.00	5.94±0.00

APPENDIX XIV

INTERLEUKINS

IL-1

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	45.53±0.005	48.63±0.005	47.36±0.003	44.25±0.00	45.53±0.005	47.62±0.003	48.49±0.00	46.29±0.009	42.23±0.005	45.53±0.005	46.45±0.005	47.66±0.003
1	47.66±0.003	80.71±0.006	98.48±0.002	112.18±0.001	127.32±0.002	143.40±0.002	162.25±0.004	183.75±0.006	207.38±0.00	224.66±0.007	258.63±0.009	283.12±0.008
2	49.53±0.005	86.51±0.001	106.88±0.007	123.82±0.002	132.92±0.00	157.64±0.009	170.93±0.003	201.50±0.002	213.89±0.001	137.29±0.009	270.75±0.004	297.04±0.00
3	48.43±0.005	98.48±0.002	117.98±0.001	149.49±0.002	136.20±0.00	173.97±0.007	189.45±0.007	218.61±0.002	227.55±0.009	248.52±0.009	286.66±0.007	309.58±0.003
4	47.36±0.003	109.68±0.007	129.92±0.00	156.59±0.002	147.29±0.002	182.95±0.006	194.80±0.003	229.67±0.007	238.50±0.009	259.68±0.006	298.82±0.008	328.36±0.008
5	46.45±0.005	116.98±0.001	136.20±0.00	163.03±0.006	153.89±0.002	193.99±0.007	121.38±0.002	235.76±0.002	251.85±0.005	267.44±0.004	320.28±0.003	341.29±0.00
6	49.07±0.005	130.82±0.00	158.59±0.002	187.08±0.009	161.23±0.006	101.08±0.006	137.29±0.00	249.62±0.008	268.69±0.006	273.26±0.007	338.97±0.005	368.87±0.008
7	47.62±0.003	145.65±0.00	169.33±0.006	191.53±0.005	169.18±0.002	120.19±0.009	146.37±0.005	263.64±0.004	281.60±0.004	284.70±0.004	351.30±0.008	381.57±0.006
8	49.49±0.005	161.53±0.006	181.08±0.009	210.89±0.001	171.67±0.007	133.09±0.002	168.73±0.006	275.36±0.007	299.65±0.009	304.85±0.009	368.95±0.004	401.62±0.005
9	44.73±0.005	180.78±0.009	206.38±0.00	218.58±0.002	189.18±0.009	147.36±0.00	173.32±0.007	293.52±0.008	311.63±0.003	318.58±0.003	375.23±0.001	425.24±0.006
10	46.30±0.005	175.15±0.009	198.97±0.00	223.42±0.001	207.48±0.004	153.89±0.002	193.02±0.002	316.11±0.001	323.38±0.00	332.98±0.00	391.61±0.00	441.62±0.008
11	48.33±0.005	169.07±0.007	192.83±0.005	237.55±0.009	221.20±0.006	179.85±0.005	217.41±0.002	326.59±0.004	339.82±0.00	349.80±0.008	403.82±0.003	459.11±0.001
12	47.60±0.003	160.69±0.006	187.49±0.001	242.05±0.00	236.26±0.002	196.67±0.005	228.97±0.007	331.69±0.00	348.25±0.004	357.75±0.004	423.38±0.007	484.53±0.007
13	46.49±0.005	151.8±0.002	184.59±0.001	257.37±0.005	249.62±0.008	221.50±0.006	254.28±0.006	352.53±0.004	359.79±0.00	379.53±0.001	451.38±0.005	512.62±0.001

IL-2

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	50.62±0.001	55.91±0.009	47.36±0.003	43.11±0.005	56.30±0.003	51.77±0.005	54.09±0.008	50.10±0.005	59.30±0.005	51.48±0.005	59.71±0.005	56.87±0.005
1	52.59±0.006	95.43±0.009	98.48±0.002	45.39±0.005	108.944±0.009	166.34±0.005	209.39±0.005	248.34±0.005	289.34±0.005	326.24±0.005	373.39±0.003	406.18±0.006
2	54.99±0.006	106.19±0.009	106.88±0.007	47.67±0.005	112.46±0.005	170.98±0.005	214.87±0.005	254.58±0.005	296.38±0.005	333.99±0.005	382.06±0.005	415.50±0.005
3	53.29±0.008	129.27±0.007	148.90±0.004	49.95±0.005	115.98±0.005	175.62±0.005	220.35±0.005	260.82±0.005	303.42±0.005	341.76±0.005	390.75±0.005	424.83±0.005
4	57.28±0.003	142.43±0.007	177.23±0.009	52.23±0.003	119.50±0.005	180.26±0.005	225.83±0.004	267.05±0.005	310.45±0.005	349.51±0.005	399.42±0.005	434.14±0.005
5	54.30±0.002	153.64±0.001	193.00±0.00	54.51±0.005	123.02±0.005	184.90±0.005	231.31±0.005	273.30±0.005	317.50±0.005	357.28±0.005	408.11±0.005	443.47±0.005
6	50.27±0.005	173.52±0.001	234.44±0.001	56.79±0.005	126.54±0.005	189.54±0.005	236.79±0.005	279.54±0.005	324.54±0.005	365.04±0.005	416.79±0.005	452.79±0.005

7	57.29±0.006	206.46±0.006	257.52±0.005	59.07±0.005	130.06±0.005	194.18±0.005	242.27±0.005	285.78±0.005	331.58±0.005	372.80±0.005	425.47±0.005	462.11±0.005
8	53.27±0.007	245.17±0.005	279.86±0.002	61.35±0.005	133.58±0.005	198.82±0.005	247.75±0.005	292.02±0.005	338.62±0.005	380.56±0.005	434.15±0.005	471.43±0.005
9	54.47±0.004	271.72±0.008	295.58±0.001	63.63±0.005	137.10±0.005	203.46±0.005	253.23±0.005	298.26±0.005	345.66±0.006	388.32±0.00	442.83±0.00	480.75±0.00
10	56.35±0.005	300.15±0.009	341.19±0.002	65.91±0.005	140.62±0.005	208.10±0.005	258.71±0.005	304.50±0.005	352.70±0.005	396.08±0.002	451.50±0.005	490.06±0.005
11	53.94±0.005	324.75±0.005	374.48±0.009	68.19±0.003	144.15±0.00	212.79±0.002	264.18±0.005	310.73±0.005	359.73±0.005	403.83±0.005	460.18±0.005	499.38±0.005
12	57.39±0.001	369.12±0.006	350.39±0.008	70.47±0.003	147.65±0.008	217.37±0.002	269.65±0.007	316.97±0.005	366.77±0.003	411.59±0.002	468.86±0.00	508.68±0.009
13	56.60±0.003	397.81±0.006	326.52±0.006	72.76±0.002	151.21±0.001	222.01±0.002	275.13±0.007	323.21±0.005	373.81±0.003	419.35±0.001	477.53±0.009	518.01±0.007

IL-3

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	42.46±0.005	42.46±0.005	43.37±0.009	43.20±0.001	41.67±0.003	43.00±0.007	43.54±0.005	42.85±0.005	42.70±0.002	41.78±0.003	41.09±0.005	42.43±0.005
1	42.43±0.005	353.85±0.001	305.37±0.005	369.36±0.003	438.09±0.002	461.79±0.003	416.57±0.001	254.49±0.005	309.84±0.005	369.29±0.004	389.79±0.004	445.13±0.005
2	43.20±0.001	232.34±0.006	251.99±0.002	273.99±0.006	300.93±0.005	430.78±0.005	408.73±0.005	260.85±0.005	317.28±0.004	377.88±0.005	398.78±0.005	455.21±0.005
3	42.70±0.00	330.33±0.006	324.44±0.005	391.67±0.002	463.88±0.007	488.76±0.009	556.00±0.007	237.44±0.006	257.22±0.001	279.61±0.007	307.00±0.006	438.98±0.001
4	43.37±0.009	222.48±0.009	241.49±0.001	262.74±0.001	288.83±0.008	414.38±0.004	393.05±0.005	273.56±0.005	332.15±0.005	395.08±0.005	416.78±0.005	475.37±0.005
5	43.46±0.005	217.45±0.006	236.09±0.004	257.06±0.006	282.69±0.006	406.19±0.007	385.20±0.006	279.93±0.005	339.60±0.005	403.69±0.005	425.79±0.005	485.46±0.005
6	43.54±0.005	267.21±0.005	324.72±0.005	386.49±0.005	407.79±0.005	465.30±0.004	187.70±0.007	204.42±0.007	223.22±0.007	246.21±0.004	357.02±0.002	434.79±0.005
7	42.62±0.005	207.53±0.005	225.53±0.005	245.78±0.005	270.53±0.005	389.78±0.005	369.53±0.005	292.65±0.005	354.48±0.005	420.89±0.005	443.79±0.005	505.62±0.005
8	41.67±0.003	202.62±0.008	220.25±0.005	240.14±0.004	264.45±0.003	381.63±0.006	361.70±0.005	299.01±0.005	361.92±0.005	429.49±0.005	452.79±0.005	515.70±0.005
9	41.78±0.002	197.63±0.004	214.98±0.001	234.50±0.007	258.37±0.004	373.38±0.005	212.49±0.001	230.81±0.004	251.41±0.007	276.60±0.006	495.54±0.005	525.78±0.001
10	42.85±0.005	192.72±0.009	209.70±0.003	228.87±0.003	252.29±0.006	365.18±0.003	346.08±0.009	311.73±0.005	376.80±0.005	446.68±0.005	470.78±0.005	535.85±0.005
11	42.92±0.004	397.98±0.006	377.37±0.002	286.29±0.005	347.04±0.005	412.29±0.005	338.21±0.004	318.08±0.005	384.23±0.005	455.28±0.005	479.78±0.005	545.93±0.005
12	43.00±0.007	182.74±0.007	199.15±0.002	217.59±0.003	240.14±0.003	348.78±0.008	227.37±0.006	246.68±0.008	268.33±0.009	294.91±0.008	422.58±0.003	400.89±0.004
13	41.09±0.005	177.78±0.004	193.86±0.001	211.95±0.003	234.06±0.003	340.58±0.006	322.49±0.005	330.80±0.004	399.11±0.002	472.47±0.009	497.76±0.008	566.09±0.005

IL-4

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	47.18±0.005	46.96±0.002	49.73±0.005	49.54±0.005	48.88±0.006	47.95±0.004	49.09±0.001	48.21±0.005	47.18±0.005	46.14±0.008	47.97±0.004	47.29±0.005
1	49.73±0.005	400.03±0.007	416.08±0.005	264.67±0.007	379.54±0.002	302.02±0.006	469.73±0.005	446.45±0.001	292.06±0.006	260.64±0.005	315.99±0.005	244.84±0.009
2	48.45±0.004	510.42±0.007	320.55±0.008	199.89±0.002	485.11±0.008	230.04±0.003	596.45±0.004	346.59±0.008	222.00±0.003	338.39±0.004	406.70±0.001	183.70±0.009
3	47.29±0.005	409.23±0.005	408.12±0.002	259.34±0.002	388.33±0.005	296.04±0.006	480.29±0.005	438.13±0.005	286.23±0.005	267.12±0.005	323.54±0.007	239.68±0.005
4	46.14±0.008	482.83±0.005	344.44±0.001	216.09±0.002	458.73±0.005	248.03±0.008	564.77±0.005	371.62±0.002	239.52±0.002	318.96±0.005	384.03±0.004	199.00±0.004
5	47.97±0.004	436.84±0.005	384.24±0.001	243.08±0.004	414.74±0.005	278.03±0.002	511.98±0.005	413.18±0.007	268.71±0.001	286.56±0.005	346.23±0.005	224.44±0.005
6	49.54±0.005	446.04±0.005	376.28±0.005	237.67±0.007	423.54±0.005	272.02±0.006	522.54±0.005	404.90±0.003	262.86±0.006	293.04±0.005	353.79±0.005	219.28±0.004
7	48.88±0.006	501.21±0.009	328.52±0.00	205.34±0.008	476.33±0.003	236.04±0.003	585.88±0.006	354.86±0.003	227.84±0.003	331.91±0.005	399.14±0.002	188.82±0.003
8	47.95±0.004	464.44±0.005	360.36±0.003	226.83±0.003	441.14±0.005	260.03±0.004	543.66±0.005	388.15±0.004	251.19±0.004	306.00±0.005	368.91±0.005	209.13±0.009
9	49.41±0.002	473.64±0.004	352.38±0.002	221.49±0.002	449.94±0.002	254.03±0.004	554.22±0.004	379.94±0.004	245.35±0.005	312.48±0.002	376.47±0.007	204.06±0.004
10	49.09±0.001	455.24±0.005	368.32±0.005	232.28±0.005	432.34±0.005	266.03±0.003	533.10±0.005	396.53±0.005	257.08±0.005	299.52±0.005	361.35±0.005	214.30±0.002
11	47.31±0.009	492.03±0.005	336.48±0.005	210.68±0.004	467.53±0.005	242.03±0.004	575.33±0.005	363.19±0.002	233.66±0.009	325.43±0.005	391.58±0.005	194.02±0.002
12	46.9±0.006	418.44±0.005	400.15±0.008	253.91±0.008	397.14±0.005	290.10±0.004	490.86±0.001	429.81±0.003	280.40±0.004	273.60±0.005	331.11±0.005	234.64±0.004
13	48.21±0.005	427.63±0.005	392.19±0.007	248.52±0.007	405.93±0.005	284.03±0.002	501.41±0.005	421.49±0.003	274.55±0.005	280.07±0.005	338.66±0.005	229.52±0.007

IL-5

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	59.15±0.005	59.97±0.007	57.08±0.004	58.25±0.005	56.95±0.009	59.59±0.009	58.47±0.002	58.23±0.001	58.58±0.007	59.15±0.005	58.64±0.002	59.02±0.005
1	59.59±0.009	208.06±0.003	245.19±0.008	264.06±0.004	287.35±0.007	417.83±0.009	409.10±0.002	389.18±0.002	256.54±0.005	373.39±0.003	397.98±0.007	463.58±0.005
2	59.02±0.005	222.47±0.007	261.76±0.001	281.24±0.006	305.83±0.005	443.03±0.005	322.62±0.009	314.14±0.005	451.50±0.005	480.42±0.005	557.54±0.005	474.02±0.005
3	58.47±0.003	378.21±0.007	359.57±0.008	282.14±0.005	408.11±0.005	434.63±0.005	393.66±0.004	374.38±0.005	269.34±0.005	390.75±0.005	416.31±0.005	484.47±0.007
4	58.58±0.007	212.94±0.005	250.86±0.007	269.81±0.007	293.37±0.001	426.23±0.003	385.94±0.003	366.97±0.006	275.73±0.005	399.42±0.005	425.46±0.005	494.90±0.005
5	58.25±0.005	401.38±0.005	381.78±0.005	262.94±0.005	382.06±0.005	407.14±0.005	169.74±0.002	201.90±0.003	217.98±0.003	238.08±0.003	350.63±0.006	316.46±0.005
6	59.79±0.005	203.36±0.009	239.967±0.006	258.26±0.001	281.08±0.003	409.43±0.005	370.49±0.007	352.09±0.007	288.54±0.005	416.79±0.005	443.79±0.005	515.79±0.005
7	58.23±0.001	198.53±0.009	234.53±0.005	252.53±0.005	274.95±0.007	401.03±0.005	362.62±0.007	344.58±0.002	294.94±0.005	425.47±0.005	452.95±0.005	526.23±0.005
8	58.64±0.002	193.74±0.003	229.09±0.005	246.77±0.004	268.87±0.003	392.54±0.004	355.06±0.003	337.38±0.001	301.34±0.005	434.15±0.005	462.11±0.005	536.67±0.005
9	57.08±0.004	188.93±0.004	223.66±0.002	241.01±0.005	262.71±0.004	384.23±0.003	347.34±0.001	330.05±0.008	307.74±0.005	442.83±0.003	471.27±0.004	547.11±0.002

10	59.02±0.006	184.14±0.003	218.22±0.003	235.26±0.003	256.55±0.005	375.83±0.005	339.62±0.007	217.77±0.004	256.29±0.003	275.54±0.009	299.58±0.009	434.63±0.003
11	56.95±0.009	179.33±0.004	212.77±0.005	229.49±0.009	250.39±0.004	367.43±0.008	331.90±0.003	315.18±0.003	320.53±0.005	460.18±0.005	489.58±0.005	567.98±0.005
12	59.41±0.006	174.54±0.004	207.34±0.003	223.74±0.003	244.24±0.003	359.03±0.008	324.18±0.006	307.78±0.006	326.93±0.005	468.86±0.006	498.72±0.009	578.41±0.006
13	59.97±0.006	227.35±0.004	267.12±0.001	287.08±0.007	311.98±0.006	451.43±0.001	505.35±0.005	300.38±0.006	333.33±0.004	477.53±0.009	507.89±0.007	588.86±0.004

IL-6

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	44.08±0.005	44.68±0.005	44.73±0.001	43.14±0.001	43.52±0.002	44.74±0.005	44.32±0.006	43.96±0.003	44.08±0.005	44.88±0.007	43.21±0.004	41.40±0.001
1	43.24±0.009	313.94±0.005	443.96±0.001	259.46±0.004	282.10±0.007	299.53±0.006	377.49±0.003	449.23±0.005	244.77±0.008	266.79±0.005	393.88±0.009	421.55±0.001
2	41.40±0.001	321.45±0.008	435.68±0.005	254.38±0.005	276.43±0.007	293.58±0.003	386.24±0.005	459.39±0.005	239.59±0.002	273.39±0.005	402.96±0.005	413.63±0.005
3	43.56±0.001	328.98±0.005	427.40±0.003	249.06±0.006	270.74±0.009	287.62±0.004	395.01±0.005	469.56±0.003	234.46±0.001	279.99±0.005	412.05±0.005	405.71±0.004
4	44.73±0.001	336.49±0.005	419.12±0.003	243.61±0.005	265.04±0.004	281.65±0.007	403.76±0.005	479.71±0.005	229.45±0.006	286.58±0.005	421.12±0.005	397.78±0.008
5	44.68±0.005	344.02±0.005	410.84±0.004	238.33±0.006	259.24±0.002	275.70±0.007	412.53±0.005	489.88±0.005	224.43±0.001	293.19±0.005	430.21±0.005	389.86±0.006
6	44.74±0.005	351.54±0.005	402.56±0.001	233.09±0.002	253.73±0.001	269.73±0.006	421.29±0.005	500.04±0.005	219.36±0.001	299.79±0.005	439.29±0.005	381.94±0.006
7	43.21±0.004	359.06±0.005	394.28±0.005	227.70±0.002	248.03±0.005	263.78±0.005	430.05±0.005	510.20±0.005	214.28±0.001	306.39±0.005	448.37±0.005	374.03±0.005
8	44.32±0.006	366.58±0.005	385.99±0.008	222.38±0.002	242.35±0.004	257.82±0.004	438.81±0.005	520.36±0.005	209.21±0.001	312.99±0.005	457.45±0.005	366.01±0.002
9	43.52±0.002	374.10±0.002	377.72±0.001	217.15±0.002	236.67±0.006	251.86±0.004	447.57±0.002	530.52±0.003	204.12±0.006	319.59±0.009	466.53±0.003	358.19±0.004
10	41.96±0.003	381.62±0.004	369.44±0.006	211.78±0.004	231.00±0.003	245.90±0.009	456.32±0.005	540.67±0.005	199.05±0.001	326.19±0.005	475.60±0.005	350.21±0.005
11	44.44±0.004	389.13±0.005	361.16±0.008	206.50±0.005	225.31±0.003	239.93±0.009	465.08±0.005	550.83±0.005	194.01±0.003	332.78±0.005	484.68±0.005	342.35±0.006
12	44.88±0.007	396.65±0.002	352.88±0.008	201.19±0.002	219.64±0.003	233.99±0.003	473.84±0.003	560.98±0.007	188.90±0.002	339.38±0.004	493.74±0.009	334.43±0.006
13	43.14±0.001	404.17±0.002	344.58±0.008	195.87±0.002	213.96±0.003	228.03±0.003	482.58±0.008	571.15±0.005	183.81±0.001	345.98±0.004	502.82±0.008	326.51±0.005

IL-7

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	50.45±0.005	50.45±0.005	50.46±0.001	52.24±0.005	53.28±0.005	51.44±0.005	50.33±0.005	51.35±0.003	53.17±0.002	50.21±0.007	52.15±0.005	52.19±0.004
1	53.28±0.005	247.17±0.005	272.08±0.003	294.55±0.006	309.49±0.006	453.92±0.001	429.02±0.001	262.69±0.005	318.04±0.005	381.59±0.001	387.74±0.001	443.08±0.005
2	53.26±0.005	185.82±0.001	205.92±0.003	224.01±0.003	236.07±0.003	352.64±0.005	332.54±0.004	340.92±0.004	396.86±0.005	288.77±0.005	348.44±0.005	416.95±0.005
3	50.46±0.001	295.29±0.005	356.04±0.005	425.79±0.005	432.54±0.005	493.29±0.005	420.98±0.005	269.21±0.005	325.63±0.007	390.42±0.005	396.69±0.005	453.12±0.005

4	53.41±0.004	206.29±0.006	228.00±0.002	247.52±0.004	260.54±0.004	386.40±0.003	364.70±0.00	314.85±0.002	378.84±0.001	452.31±0.002	459.42±0.001	523.41±0.001
5	50.21±0.007	190.90±0.003	211.44±0.003	229.89±0.003	242.19±0.003	361.08±0.008	340.58±0.006	334.40±0.005	401.63±0.002	478.82±0.003	486.29±0.002	553.51±0.007
6	52.15±0.005	333.24±0.005	399.27±0.005	405.66±0.005	463.17±0.001	483.25±0.005	201.18±0.001	222.48±0.003	241.65±0.001	254.42±0.005	377.96±0.002	423.58±0.005
7	53.17±0.002	237.01±0.002	261.13±0.009	282.77±0.008	297.32±0.006	437.04±0.002	412.94±0.003	275.73±0.005	242.10±0.009	266.64±0.003	288.61±0.002	303.38±0.005
8	50.33±0.005	216.53±0.005	239.03±0.005	259.28±0.005	272.78±0.005	403.28±0.005	380.78±0.005	301.81±0.005	363.64±0.005	434.63±0.005	441.50±0.005	503.33±0.005
9	51.35±0.003	211.35±0.009	233.51±0.005	253.40±0.004	266.66±0.003	394.74±0.009	372.66±0.002	308.33±0.005	371.24±0.005	443.47±0.005	450.46±0.005	513.37±0.005
10	52.24±0.005	231.85±0.008	255.53±0.009	276.92±0.003	291.13±0.009	428.60±0.003	404.88±0.008	282.24±0.005	340.83±0.005	408.10±0.005	414.61±0.005	473.20±0.005
11	51.44±0.005	409.23±0.001	487.64±0.008	495.23±0.008	563.56±0.005	445.48±0.005	356.66±0.001	321.37±0.005	386.44±0.004	461.14±0.005	468.37±0.005	533.44±0.005
12	52.19±0.004	196.05±0.004	216.95±0.005	235.75±0.009	248.30±0.004	369.52±0.008	348.62±0.002	327.88±0.005	394.03±0.005	469.98±0.005	477.33±0.005	543.48±0.005
13	52.84±0.009	221.64±0.008	244.54±0.007	265.15±0.006	278.89±0.006	411.72±0.006	388.816±0.006	226.66±0.001	250.07±0.002	271.03±0.005	285.02±0.004	420.17±0.002

IL-8

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	53.81±0.005	52.06±0.004	53.25±0.006	52.32±0.003	53.36±0.009	52.66±0.001	53.81±0.005	52.94±0.001	52.75±0.001	51.79±0.005	53.10±0.009	51.61±0.005
1	53.10±0.009	346.53±0.001	316.55±0.005	453.91±0.005	478.01±0.005	373.70±0.002	232.33±0.007	418.53±0.005	258.59±0.005	375.44±0.003	395.93±0.008	448.94±0.001
2	52.66±0.001	319.36±0.009	259.23±0.007	465.66±0.005	283.78±0.006	298.48±0.005	227.43±0.002	356.77±0.005	232.28±0.005	517.07±0.005	254.78±0.005	268.28±0.008
3	52.75±0.005	326.85±0.005	253.88±0.003	203.03±0.005	370.53±0.005	297.23±0.005	427.76±0.005	450.66±0.005	271.47±0.005	392.88±0.005	414.18±0.005	432.22±0.003
4	52.94±0.001	410.53±0.002	265.03±0.005	384.15±0.005	405.05±0.005	440.58±0.005	217.64±0.004	394.52±0.007	277.90±0.005	401.59±0.005	423.29±0.005	423.86±0.003
5	51.61±0.005	341.81±0.005	243.08±0.005	496.51±0.005	266.38±0.004	280.36±0.001	212.71±0.005	386.53±0.001	284.35±0.005	410.32±0.005	432.42±0.005	415.49±0.004
6	51.79±0.005	349.29±0.005	237.67±0.007	506.79±0.005	260.58±0.002	274.32±0.006	207.90±0.006	378.53±0.005	290.79±0.005	419.04±0.005	441.54±0.005	407.14±0.002
7	52.06±0.004	322.52±0.008	335.86±0.004	480.06±0.009	505.35±0.008	348.62±0.008	311.89±0.005	264.67±0.007	455.38±0.005	289.57±0.006	304.51±0.006	398.78±0.005
8	53.36±0.009	364.25±0.005	226.88±0.005	527.35±0.005	248.98±0.004	262.24±0.003	198.15±0.007	362.53±0.003	303.67±0.005	436.48±0.005	459.78±0.005	390.41±0.008
9	53.64±0.004	371.73±0.002	221.49±0.002	537.63±0.001	243.18±0.005	256.20±0.004	193.16±0.009	354.51±0.002	310.11±0.005	445.20±0.004	468.90±0.003	382.06±0.002
10	52.32±0.003	379.21±0.004	216.09±0.002	547.90±0.005	237.39±0.003	250.16±0.007	171.86±0.007	334.32±0.005	248.46±0.002	486.22±0.005	272.18±0.002	286.40±0.000
11	53.37±0.004	386.68±0.005	210.68±0.004	558.18±0.005	231.579	244.12±0.004	183.55±0.003	338.53±0.005	322.98±0.005	462.63±0.005	487.13±0.005	365.34±0.009
12	53.25±0.006	394.16±0.002	205.29±0.002	568.45±0.006	225.79±0.003	238.09±0.003	163.04±0.002	330.53±0.007	329.42±0.005	471.35±0.003	496.23±0.009	356.99±0.001
13	53.74±0.005	401.64±0.002	199.89±0.002	578.74±0.005	219.99±0.003	232.05±0.003	173.75±0.007	475.95±0.004	278.00±0.009	292.43±0.009	222.58±0.006	402.52±0.008

IL-9

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	47.57±0.005	47.57±0.005	47.29±0.007	46.73±0.003	49.09±0.008	48.43±0.007	46.56±0.009	48.10±0.001	45.45±0.008	47.59±0.005	48.38±0.005	49.35±0.008
1	48.38±0.005	239.78±0.002	254.71±0.009	284.59±0.007	297.04±0.006	441.47±0.001	419.06±0.001	384.20±0.003	264.74±0.005	371.34±0.004	391.83±0.009	447.18±0.005
2	49.09±0.008	234.78±0.006	249.48±0.007	278.88±0.006	291.13±0.006	433.23±0.005	411.18±0.005	376.88±0.005	271.30±0.005	379.97±0.005	400.87±0.005	457.30±0.005
3	46.73±0.006	229.78±0.004	244.24±0.001	273.16±0.001	285.21±0.003	424.99±0.003	403.30±0.004	369.56±0.005	277.86±0.005	388.62±0.005	409.92±0.005	467.43±0.001
4	46.56±0.009	224.78±0.002	239.00±0.004	267.44±0.002	279.28±0.009	416.75±0.004	395.42±0.002	362.23±0.006	284.41±0.005	397.25±0.005	418.95±0.005	477.54±0.005
5	45.45±0.008	219.78±0.004	233.76±0.005	261.72±0.004	273.37±0.003	408.51±0.005	387.53±0.006	354.91±0.007	290.98±0.005	405.90±0.005	428.00±0.005	487.67±0.005
6	47.59±0.005	214.77±0.009	228.51±0.008	256.00±0.001	267.45±0.007	400.27±0.002	379.65±0.006	347.59±0.009	297.54±0.005	414.54±0.005	437.04±0.005	497.79±0.005
7	48.43±0.007	209.78±0.006	223.28±0.005	250.28±0.005	261.53±0.006	392.03±0.005	371.78±0.006	340.28±0.007	304.10±0.005	423.18±0.005	446.08±0.005	507.91±0.005
8	48.10±0.001	204.79±0.001	218.04±0.006	244.56±0.004	255.61±0.004	383.78±0.009	363.90±0.002	332.96±0.002	310.66±0.005	431.82±0.005	455.12±0.005	518.03±0.005
9	47.85±0.001	199.77±0.009	212.80±0.006	238.84±0.005	249.69±0.004	375.55±0.003	356.02±0.006	325.64±0.005	317.22±0.002	440.46±0.006	464.16±0.006	528.15±0.003
10	49.35±0.008	194.79±0.002	207.57±0.001	233.13±0.003	243.78±0.008	367.31±0.003	348.14±0.004	318.32±0.006	323.78±0.005	449.09±0.005	473.19±0.005	538.26±0.005
11	48.08±0.004	189.78±0.004	202.32±0.004	227.40±0.001	237.84±0.009	359.08±0.001	340.26±0.006	311.00±0.003	330.33±0.005	457.73±0.005	482.23±0.005	548.38±0.005
12	47.29±0.007	184.79±0.002	197.09±0.008	221.69±0.003	231.94±0.003	350.84±0.002	332.38±0.007	303.68±0.005	336.89±0.005	466.37±0.001	491.25±0.009	558.49±0.007
13	47.34±0.007	179.79±0.001	191.85±0.001	215.97±0.003	226.02±0.003	342.59±0.006	324.50±0.005	296.36±0.006	343.45±0.004	475.00±0.009	500.29±0.008	568.62±0.005

IL-10

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	65.44±0.005	65.44±0.005	66.44±0.005	65.67±0.008	65.17±0.005	67.74±0.003	66.04±0.005	67.33±0.005	65.45±0.002	65.04±0.005	65.28±0.002	64.46±0.001
1	65.04±0.005	113.04±0.005	106.89±0.004	102.79±0.004	96.64±0.004	143.79±0.005	214.87±0.009	129.44±0.005	174.54±0.005	215.54±0.005	246.29±0.005	318.04±0.005
2	65.17±0.005	116.64±0.005	110.37±0.005	106.19±0.004	99.92±0.004	147.99±0.005	210.28±0.008	133.36±0.005	179.34±0.005	221.14±0.005	252.49±0.005	325.63±0.007
3	66.44±0.005	120.24±0.005	113.85±0.005	109.59±0.005	103.20±0.005	152.19±0.005	205.68±0.005	137.28±0.005	184.14±0.005	226.74±0.005	258.69±0.005	333.24±0.005
4	67.33±0.005	123.84±0.005	117.33±0.005	112.99±0.005	106.48±0.004	156.39±0.005	201.08±0.003	141.20±0.005	188.94±0.005	232.34±0.003	264.88±0.005	340.83±0.005
5	64.46±0.001	127.44±0.005	120.81±0.005	116.39±0.005	109.76±0.005	160.59±0.005	196.48±0.004	145.12±0.005	193.74±0.005	237.94±0.005	271.09±0.005	348.44±0.005
6	66.04±0.005	131.04±0.005	124.29±0.005	119.79±0.005	113.04±0.005	164.79±0.005	191.87±0.009	149.04±0.005	198.54±0.005	243.54±0.005	277.29±0.005	356.04±0.005
7	65.67±0.008	134.64±0.005	127.77±0.005	123.19±0.005	116.32±0.005	168.99±0.005	187.29±0.003	152.96±0.005	203.34±0.005	249.14±0.005	283.49±0.005	363.64±0.005
8	65.28±0.002	138.24±0.005	131.25±0.005	126.59±0.005	119.60±0.005	173.19±0.005	182.69±0.007	156.88±0.005	208.14±0.005	254.74±0.005	289.69±0.005	371.24±0.005
9	67.84±0.002	141.84±0.005	134.73±0.005	129.99±0.005	122.88±0.005	177.39±0.005	159.69±0.005	160.80±0.005	212.94±0.005	260.34±0.005	295.89±0.005	378.84±0.003

10	65.45±0.002	145.44±0.005	138.21±0.005	133.39±0.005	126.16±0.005	181.59±0.005	164.29±0.005	164.72±0.005	217.74±0.005	265.94±0.005	302.09±0.005	386.44±0.004
11	66.04±0.009	149.04±0.002	141.68±0.009	136.78±0.008	129.43±0.006	185.78±0.007	168.89±0.005	168.64±0.004	222.53±0.007	271.53±0.005	308.28±0.005	394.03±0.005
12	67.74±0.003	152.63±0.006	145.16±0.004	140.18±0.003	132.71±0.003	189.98±0.003	173.49±0.005	172.55±0.005	227.33±0.001	277.12±0.007	314.48±0.005	401.63±0.002
13	66.25±0.003	156.23±0.006	148.64±0.006	143.58±0.005	135.99±0.003	194.18±0.001	178.09±0.005	176.47±0.005	232.13±0.004	282.72±0.006	320.68±0.005	409.23±0.001

IL-11

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	65.96±0.005	62.37±0.005	66.62±0.005	48.96±0.001	65.96±0.005	67.11±0.005	65.71±0.003	67.06±0.003	67.61±0.005	67.13±0.005	66.32±0.005	64.61±0.007
1	64.61±0.007	180.88±0.008	177.85±0.005	159.33±0.005	195.04±0.005	227.84±0.005	238.09±0.005	233.99±0.005	250.39±0.005	256.54±0.005	283.19±0.005	340.59±0.005
2	62.37±0.005	176.76±0.002	173.57±0.005	159.41±0.007	200.24±0.005	233.68±0.005	244.13±0.005	239.95±0.005	256.67±0.005	262.94±0.005	290.11±0.005	348.62±0.005
3	67.61±0.005	172.64±0.004	169.29±0.005	159.48±0.005	205.44±0.005	239.52±0.005	250.17±0.005	245.91±0.005	262.95±0.005	269.34±0.005	297.03±0.005	356.67±0.005
4	65.716±0.003	168.52±0.005	165.01±0.005	159.56±0.002	210.64±0.005	245.35±0.008	256.20±0.005	251.86±0.006	269.22±0.005	275.73±0.005	303.94±0.005	364.70±0.005
5	66.32±0.005	164.40±0.003	160.73±0.005	159.63±0.005	215.84±0.005	251.20±0.005	262.25±0.005	257.83±0.005	275.51±0.005	282.14±0.005	310.87±0.005	372.75±0.005
6	67.11±0.005	160.28±0.008	156.45±0.005	159.71±0.004	221.04±0.005	257.04±0.005	268.29±0.005	263.79±0.005	281.79±0.005	288.54±0.005	317.79±0.005	380.79±0.005
7	67.61±0.004	156.16±0.007	152.17±0.005	159.78±0.005	226.24±0.005	262.88±0.005	274.33±0.005	269.75±0.005	288.07±0.005	294.94±0.005	324.71±0.005	388.83±0.005
8	66.62±0.005	152.04±0.003	147.89±0.005	159.86±0.007	231.44±0.005	268.72±0.005	280.37±0.005	275.71±0.005	294.35±0.005	301.34±0.005	331.63±0.005	396.87±0.005
9	66.62±0.002	147.92±0.005	143.61±0.005	159.93±0.005	236.64±0.005	274.56±0.005	286.41±0.005	281.67±0.005	300.63±0.005	307.74±0.005	338.5±0.005	404.91±0.002
10	67.13±0.005	143.80±0.003	139.33±0.005	160.01±0.002	241.84±0.005	280.40±0.005	292.45±0.005	287.63±0.005	306.91±0.005	314.14±0.005	345.47±0.005	412.94±0.008
11	65.65±0.005	139.68±0.008	135.05±0.005	160.08±0.005	247.03±0.006	286.23±0.005	298.48±0.005	293.58±0.005	313.18±0.005	320.53±0.005	352.38±0.005	420.98±0.005
12	67.06±0.003	135.56±0.002	130.77±0.005	160.16±0.004	252.23±0.002	292.06±0.006	304.51±0.006	299.53±0.006	319.46±0.005	326.93±0.005	359.30±0.004	429.02±0.001
13	66.05±0.005	131.44±0.003	126.49±0.005	160.23±0.005	257.44±0.005	297.90±0.006	310.56±0.005	305.50±0.005	325.74±0.005	333.33±0.004	366.22±0.003	437.06±0.004

IL-12

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	53.60±0.005	52.06±0.005	53.00±0.006	54.55±0.006	53.25±0.007	54.59±0.007	53.60±0.005	53.33±0.009	53.43±0.005	55.29±0.005	52.69±0.004	52.18±0.005
1	54.59±0.007	424.04±0.001	416.57±0.001	401.63±0.002	391.67±0.002	381.71±0.003	366.77±0.003	356.81±0.004	429.02±0.001	438.98±0.001	463.88±0.008	481.3±0.001
2	53.00±0.006	416.08±0.005	408.73±0.005	394.03±0.005	384.23±0.005	374.43±0.005	359.73±0.005	349.93±0.005	420.98±0.005	430.78±0.005	455.28±0.005	472.43±0.005
3	53.43±0.005	408.12±0.004	400.89±0.004	386.43±0.005	376.79±0.005	367.15±0.005	352.68±0.006	343.04±0.006	412.94±0.003	422.58±0.003	446.68±0.002	463.54±0.001

4	53.25±0.007	400.16±0.004	393.05±0.005	378.83±0.005	369.34±0.006	359.86±0.006	345.64±0.007	336.16±0.007	404.90±0.004	414.38±0.004	438.08±0.003	454.67±0.002
5	52.18±0.005	392.20±0.005	385.20±0.006	371.22±0.006	361.90±0.007	352.58±0.007	338.60±0.009	329.29±0.009	396.86±0.005	406.18±0.005	429.47±0.004	445.79±0.003
6	55.29±0.00±0.005	384.23±0.006	377.36±0.006	363.62±0.007	354.46±0.008	345.31±0.001	331.57±0.002	322.41±0.001	388.81±0.006	397.98±0.002	420.88±0.008	436.91±0.003
7	54.67±0.00±0.006	376.28±0.005	369.53±0.006	356.03±0.007	347.03±0.007	338.03±0.007	324.53±0.005	315.53±0.006	380.78±0.005	389.78±0.005	412.28±0.006	428.03±0.007
8	52.06±0.00±0.005	368.32±0.001	361.69±0.002	348.43±0.002	339.59±0.001	330.75±0.001	317.49±0.004	308.65±0.005	372.74±0.007	381.57±0.002	403.67±0.007	419.14±0.006
9	52.69±0.00±0.004	360.36±0.001	353.85±0.001	340.83±0.002	332.15±0.005	323.47±0.005	310.45±0.007	301.77±0.006	364.70±0.001	373.38±0.003	395.08±0.002	410.27±0.002
10	54.55±0.00±0.006	352.40±0.003	346.01±0.005	333.23±0.006	324.71±0.007	316.19±0.006	303.41±0.004	294.89±0.003	356.66±0.002	365.18±0.003	386.483±0.008	401.39±0.003
11	53.30±0.00±0.005	344.44±0.006	338.17±0.006	325.63±0.005	317.27±0.003	308.91±0.003	296.37±0.003	288.01±0.004	348.62±0.006	356.99±0.001	377.88±0.008	392.52±0.002
12	53.33±0.00±0.009	336.48±0.009	330.33±0.007	318.03±0.006	309.83±0.006	301.63±0.005	289.33±0.005	281.13±0.007	340.59±0.002	348.79±0.002	369.29±0.001	383.63±0.009
13	53.25±0.00±0.008	328.52±0.005	322.49±0.005	310.43±0.006	302.39±0.006	294.35±0.007	282.30±0.002	274.26±0.004	332.54±0.004	340.58±0.006	360.68±0.008	374.75±0.008

IL-13

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	72.80±0.002	72.83±0.005	73.99±0.007	71.18±0.005	72.80±0.002	73.36±0.005	71.92±0.009	72.99±0.005	72.01±0.005	73.78±0.005	72.87±0.005	71.75±0.006
1	73.99±0.003	414.24±0.005	426.44±0.005	390.40±0.001	371.92±0.007	369.46±0.005	344.83±0.005	359.47±0.008	377.27±0.002	398.97±0.002	410.90±0.007	419.91±0.003
2	72.99±0.005	392.93±0.005	423.07±0.005	374.32±0.004	361.16±0.005	354.25±0.002	341.33±0.005	344.68±0.004	361.74±0.007	382.54±0.003	393.98±0.002	402.61±0.002
3	71.92±0.002	371.62±0.005	419.70±0.005	358.24±0.007	350.41±0.001	339.043±0.005	337.83±0.005	329.88±0.009	346.20±0.007	366.10±0.007	377.05±0.002	385.31±0.001
4	73.36±0.005	350.31±0.005	416.33±0.005	342.17±0.007	339.65±0.005	323.83±0.005	334.33±0.005	315.09±0.005	330.67±0.005	349.67±0.005	360.12±0.005	368.01±0.001
5	73.78±0.005	329.00±0.005	412.96±0.005	326.09±0.003	328.90±0.002	308.626±0.005	330.83±0.005	300.30±0.005	315.14±0.002	333.24±0.002	343.19±0.007	350.70±0.009
6	72.83±0.005	307.69±0.005	409.59±0.005	310.01±0.006	318.14±0.005	293.41±0.008	327.33±0.005	285.50±0.006	299.61±0.002	316.81±0.002	326.27±0.004	333.40±0.008
7	71.75±0.006	286.38±0.005	406.22±0.005	293.93±0.009	307.39±0.007	278.20±0.009	323.83±0.005	270.71±0.001	284.07±0.007	300.37±0.007	309.34±0.002	316.10±0.007
8	71.18±0.005	265.07±0.005	402.85±0.005	277.86±0.002	296.63±0.005	263.00±0.001	320.33±0.005	255.91±0.007	268.54±0.005	283.94±0.005	292.41±0.005	298.80±0.006
9	72.04±0.005	243.76±0.005	399.48±0.005	261.78±0.005	285.88±0.002	247.79±0.002	316.83±0.005	241.12±0.002	253.01±0.002	267.51±0.002	275.48±0.007	281.50±0.005
10	72.87±0.005	222.45±0.005	396.11±0.005	245.70±0.008	275.12±0.005	232.58±0.004	313.33±0.005	226.32±0.008	237.48±0.003	251.08±0.001	258.56±0.001	264.20±0.004
11	71.74±0.007	201.14±0.005	392.74±0.005	229.63±0.001	264.37±0.002	217.37±0.005	309.83±0.005	211.53±0.003	221.94±0.007	234.64±0.007	241.63±0.002	246.90±0.003
12	72.01±0.005	190.912±0.009	389.37±0.005	213.55±0.004	253.61±0.005	202.16±0.002	306.33±0.005	196.73±0.009	206.41±0.002	218.21±0.005	224.70±0.005	229.60±0.002
13	72.51±0.001	181.05±0.002	386.00±0.005	197.47±0.007	242.85±0.009	186.95±0.007	302.83±0.005	181.94±0.004	190.88±0.001	201.78±0.002	207.77±0.007	212.30±0.003

IL-14

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	44.26±0.002	44.26±0.008	43.75±0.001	44.72±0.005	45.58±0.005	45.29±0.005	44.30±0.009	43.86±0.005	43.78±0.005	42.16±0.001	45.23±0.005	44.37±0.007
1	45.23±0.005	155.75±0.005	167.08±0.005	178.41±0.005	193.86±0.005	210.34±0.005	228.88±0.005	245.36±0.005	256.69±0.005	283.47±0.005	309.22±0.005	372.05±0.005
2	44.30±0.009	146.95±0.002	157.51±0.003	168.07±0.002	182.47±0.003	197.83±0.007	215.11±0.002	230.47±0.002	241.03±0.003	265.99±0.003	289.99±0.002	348.5±0.005
3	43.78±0.005	138.14±0.005	147.93±0.005	157.72±0.005	171.07±0.005	185.31±0.005	201.33±0.005	215.57±0.005	225.36±0.005	248.50±0.005	270.75±0.005	325.04±0.005
4	43.75±0.001	129.34±0.006	138.36±0.001	147.38±0.002	159.68±0.002	172.80±0.004	187.56±0.007	200.68±0.006	209.70±0.002	231.02±0.002	251.52±0.004	301.53±0.002
5	44.37±0.007	120.53±0.005	128.78±0.005	137.03±0.005	148.28±0.005	160.28±0.005	173.78±0.005	185.78±0.005	194.03±0.005	213.53±0.005	232.28±0.005	278.03±0.005
6	45.58±0.005	111.73±0.002	119.21±0.004	126.69±0.007	136.89±0.006	147.77±0.007	160.01±0.002	170.89±0.002	178.37±0.002	196.05±0.006	213.05±0.006	254.53±0.003
7	43.76±0.006	102.92±0.005	109.63±0.005	116.34±0.005	125.49±0.005	135.25±0.005	146.23±0.005	155.99±0.005	162.70±0.005	178.56±0.005	193.81±0.005	231.02±0.005
8	44.72±0.005	94.11±0.009	100.05±0.009	106.00±0.004	114.10±0.001	122.74±0.004	132.46±0.002	141.10±0.003	147.04±0.002	161.08±0.007	174.58±0.002	207.52±0.001
9	42.16±0.001	85.31±0.003	90.48±0.003	95.65±0.003	102.70±0.005	110.22±0.005	118.68±0.005	126.20±0.005	131.37±0.005	143.59±0.005	155.34±0.005	184.01±0.005
10	45.29±0.005	76.51±0.007	80.91±0.004	85.31±0.007	91.31±0.004	97.71±0.001	104.91±0.001	111.31±0.007	115.71±0.002	126.11±0.003	136.11±0.006	160.51±0.001
11	44.02±0.009	67.70±0.005	71.33±0.005	74.96±0.005	79.91±0.005	85.19±0.005	91.13±0.005	96.41±0.005	100.04±0.005	108.62±0.002	116.87±0.003	137.00±0.004
12	43.86±0.005	58.89±0.007	61.75±0.007	64.61±0.007	68.51±0.006	72.67±0.006	77.35±0.006	81.51±0.004	84.37±0.005	91.13±0.005	97.65±0.006	113.49±0.003
13	43.98±0.005	50.09±0.005	52.18±0.005	54.27±0.005	57.12±0.005	60.16±0.005	63.58±0.005	66.62±0.005	68.71±0.005	73.65±0.005	78.40±0.005	89.99±0.005

IL-15

	CONT.	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	36.28±0.005	35.72±0.005	35.09±0.004	36.04±0.005	35.37±0.005	35.39±0.004	35.94±0.005	34.24±0.003	36.69±0.003	36.39±0.005	36.18±0.002	34.51±0.005
1	35.08±0.005	41.99±0.005	47.45±0.006	48.52±0.005	50.28±0.003	49.77±0.003	46.82±0.005	88.12±0.005	98.41±0.005	183.06±0.004	202.30±0.005	253.38±0.005
2	36.20±0.002	49.01±0.005	48.70±0.005	53.50±0.005	55.07±0.005	54.57±0.008	51.56±0.005	90.82±0.002	101.60±0.005	167.98±0.001	199.86±0.005	252.40±0.005
3	35.09±0.004	53.92±0.002	41.18±0.005	55.78±0.005	59.15±0.002	54.87±0.006	55.85±0.005	93.51±0.008	104.79±0.005	152.90±0.002	192.07±0.004	245.75±0.005
4	36.83±0.005	51.13±0.007	59.85±0.005	67.69±0.005	61.33±0.004	59.67±0.007	58.79±0.005	96.22±0.004	107.98±0.005	137.82±0.008	179.37±0.008	235.59±0.005
5	35.94±0.004	57.16±0.001	62.37±0.003	75.06±0.001	61.99±0.003	63.21±0.00	59.57±0.005	98.92±0.001	111.17±0.005	122.74±0.002	180.755±0.006	225.30±0.003
6	36.71±0.003	61.75±0.006	65.24±0.005	79.59±0.008	66.42±0.005	68.05±0.005	64.22±0.005	101.62±0.001	114.36±0.005	107.66±0.007	179.035±0.009	224.32±0.001
7	36.54±0.007	61.84±0.007	68.48±0.001	87.04±0.005	73.69±0.005	69.75±0.002	68.56±0.004	104.32±0.001	117.55±0.005	94.58±0.003	170.07±0.007	209.21±0.002
8	35.37±0.005	66.74±0.005	71.17±0.005	94.23±0.002	78.31±0.001	73.69±0.005	71.59±0.002	107.02±0.001	120.74±0.005	88.50±0.006	158.81±0.009	198.60±0.003
9	35.65±0.008	77.36±0.005	79.04±0.005	99.12±0.001	75.68±0.002	78.27±0.001	78.44±0.003	109.72±0.001	123.93±0.005	81.12±0.002	156.37±0.006	190.46±0.005

10	35.70±0.007	77.23±0.008	81.96±0.005	103.06±0.005	79.98±0.002	83.52±0.006	78.73±0.004	112.42±0.001	127.12±0.005	76.87±0.006	148.98±0.003	179.67±0.005
11	35.74±0.00	79.97±0.005	84.39±0.007	108.90±0.004	87.74±0.005	76.26±0.001	81.62±0.005	115.12±0.001	130.31±0.005	65.86±0.006	145.01±0.004	167.08±0.005
12	34.83±0.005	83.03±0.006	89.07±0.007	113.47±0.005	87.94±0.009	85.83±0.002	87.89±0.005	117.82±0.001	133.50±0.005	58.009±0.007	141.13±0.002	158.45±0.005
13	34.75±0.004	92.89±0.003	99.50±0.002	123.27±0.002	101.92±0.005	96.21±0.004	92.09±0.005	120.52±0.001	136.69±0.005	41.89±0.006	136.98±0.008	146.85±0.005

IL-16

	CONT.	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	43.66±0.002	43.87±0.005	42.27±0.001	42.99±0.005	42.90±0.003	42.84±0.001	42.67±0.001	43.00±0.00	42.91±0.003	42.16±0.001	43.37±0.001	43.81±0.002
1	42.05±0.001	46.65±0.001	51.70±0.003	52.77±0.004	54.43±0.007	48.17±0.003	52.76±0.005	93.97±0.002	101.21±0.005	182.59±0.002	204.29±0.002	261.03±0.005
2	44.31±0.002	53.95±0.005	52.60±0.005	57.30±0.005	57.42±0.005	50.27±0.006	55.91±0.002	99.88±0.005	103.40±0.005	180.39±0.005	200.18±0.005	257.56±0.004
3	43.07±0.001	53.29±0.005	54.63±0.005	63.63±0.005	58.35±0.008	54.51±0.008	59.46±0.008	105.84±0.005	109.59±0.005	178.20±0.004	196.08±0.006	254.08±0.005
4	42.83±0.004	57.09±0.002	63.95±0.005	69.69±0.005	69.53±0.005	61.02±0.001	66.11±0.005	109.64±0.005	112.28±0.005	171.50±0.005	191.97±0.005	250.61±0.003
5	42.09±0.008	58.00±0.005	64.77±0.002	79.08±0.005	69.29±0.001	64.91±0.002	67.95±0.005	112.81±0.005	115.96±0.002	164.81±0.007	187.87±0.008	247.13±0.005
6	43.85±0.002	61.53±0.004	69.54±0.005	82.94±0.002	71.88±0.002	67.60±0.005	72.36±0.002	117.92±0.002	121.28±0.005	158.11±0.005	183.76±0.005	243.66±0.007
7	42.20±0.007	61.45±0.004	76.48±0.004	92.64±0.005	73.74±0.006	71.75±0.001	75.59±0.005	124.10±0.005	127.90±0.005	155.92±0.001	179.66±0.003	240.18±0.005
8	43.87±0.002	67.54±0.005	78.42±0.001	99.83±0.002	79.76±0.001	75.995±0.00	78.38±0.007	129.07±0.005	131.86±0.004	153.72±0.005	175.55±0.005	236.71±0.002
9	43.13±0.001	76.20±0.003	81.84±0.005	97.51±0.009	79.83±0.002	78.77±0.007	84.31±0.005	131.43±0.005	137.95±0.003	142.53±0.002	171.45±0.002	233.23±0.005
10	43.39±0.001	82.65±0.001	92.06±0.005	106.41±0.004	84.58±0.007	82.52±0.003	88.31±0.001	135.41±0.005	141.17±0.005	140.33±0.005	167.34±0.005	229.76±0.003
11	42.15±0.002	86.12±0.007	95.04±0.002	110.04±0.004	86.49±0.005	85.25±0.008	89.61±0.005	140.83±0.005	143.27±0.005	129.14±0.001	163.24±0.001	226.28±0.005
12	43.91±0.001	88.57±0.005	94.91±0.007	118.62±0.005	91.65±0.007	88.55±0.005	91.54±0.005	143.55±0.005	148.39±0.005	131.44±0.005	159.13±0.005	222.81±0.001
13	43.17±0.001	105.55±0.005	110.25±0.008	129.77±0.003	98.29±0.007	99.56±0.003	102.20±0.005	149.65±0.001	153.80±0.005	133.75±0.002	155.03±0.007	219.33±0.005

IL-17

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	93.41±0.007	93.67±0.001	92.92±0.005	93.74±0.005	94.46±0.008	93.27±0.005	93.63±0.002	92.71±0.008	94.46±0.001	94.43±0.005	94.87±0.002	92.16±0.002
1	94.75±0.002	158.55±0.006	163.71±0.002	171.24±0.005	177.62±0.005	145.07±0.004	129.69±0.002	128.77±0.005	159.33±0.005	186.74±0.005	207.19±0.004	269.07±0.002
2	93.09±0.004	156.82±0.005	162.50±0.005	170.95±0.002	172.90±0.003	146.97±0.002	128.06±0.005	129.03±0.005	156.91±0.003	184.72±0.005	206.10±0.005	263.74±0.005
3	94.43±0.007	155.10±0.002	161.30±0.002	170.65±0.005	168.17±0.005	144.37±0.009	126.44±0.001	129.29±0.005	154.48±0.005	182.70±0.005	205.02±0.007	258.42±0.001

4	94.77±0.002	153.37±0.005	160.09±0.005	170.36±0.006	163.45±0.008	141.77±0.001	124.81±0.005	129.55±0.005	152.06±0.006	180.68±0.005	203.93±0.005	253.09±0.005
5	94.11±0.009	151.65±0.002	158.89±0.002	170.06±0.005	158.72±0.005	143.67±0.003	123.19±0.008	129.81±0.005	149.63±0.005	178.66±0.005	202.85±0.006	247.77±0.002
6	94.44±0.009	149.92±0.005	157.68±0.005	169.77±0.001	154.00±0.006	141.07±0.002	121.56±0.005	130.07±0.005	147.21±0.003	176.64±0.005	201.76±0.005	242.44±0.005
7	93.78±0.008	148.20±0.002	156.48±0.001	169.47±0.005	149.27±0.005	138.47±0.006	119.94±0.005	130.33±0.005	144.78±0.005	174.62±0.005	200.68±0.002	237.12±0.001
8	93.12±0.008	146.47±0.005	155.27±0.005	169.18±0.002	144.55±0.006	135.87±0.008	118.31±0.005	130.59±0.005	142.36±0.006	172.60±0.005	199.59±0.005	231.79±0.005
9	93.46±0.008	144.75±0.008	154.07±0.007	168.88±0.005	139.82±0.005	137.77±0.002	116.69±0.007	130.85±0.005	139.93±0.005	170.58±0.005	198.51±0.003	226.47±0.007
10	93.80±0.007	143.02±0.005	152.86±0.005	168.59±0.004	135.10±0.002	135.17±0.004	115.06±0.005	131.11±0.005	137.51±0.002	168.56±0.005	197.42±0.005	221.14±0.005
11	93.14±0.007	141.30±0.001	151.66±0.001	168.29±0.005	130.37±0.005	128.07±0.007	113.44±0.003	131.37±0.005	135.08±0.005	166.54±0.005	196.34±0.004	215.82±0.009
12	92.48±0.007	139.57±0.005	150.45±0.005	168.00±0.002	125.65±0.007	129.97±0.008	111.81±0.005	131.63±0.005	132.66±0.003	164.52±0.005	195.25±0.005	210.49±0.005
13	94.82±0.007	137.85±0.002	149.25±0.002	167.70±0.005	120.92±0.005	127.37±0.001	110.19±0.002	131.89±0.005	130.23±0.005	162.50±0.005	194.17±0.003	205.17±0.001

IL-18

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	79.41±0.002	79.41±0.004	78.65±0.001	97.46±0.001	78.21±0.001	78.95±0.005	77.59±0.005	79.31±0.001	77.46±0.005	79.29±0.001	78.53±0.005	77.03±0.005
1	78.53±0.005	121.76±0.005	133.09±0.005	144.42±0.005	358.66±0.005	339.09±0.005	289.65±0.005	260.81±0.005	225.79±0.005	275.23±0.005	323.64±0.005	376.17±0.005
2	78.65±0.006	115.27±0.001	125.83±0.006	136.39±0.008	336.07±0.003	317.83±0.002	271.75±0.002	244.87±0.002	212.23±0.002	258.31±0.003	303.43±0.002	352.39±0.003
3	79.40±0.005	108.77±0.005	118.56±0.005	128.35±0.005	313.47±0.005	296.56±0.005	253.84±0.005	228.92±0.005	198.66±0.005	241.38±0.005	283.21±0.005	328.60±0.005
4	78.21±0.003	102.27±0.008	111.30±0.004	120.32±0.001	290.87±0.004	275.30±0.001	235.94±0.001	212.98±0.001	185.10±0.002	224.46±0.002	263.00±0.008	304.81±0.004
5	77.03±0.005	95.78±0.005	104.03±0.005	112.28±0.005	268.28±0.005	254.03±0.005	218.03±0.005	197.03±0.005	171.53±0.005	207.53±0.005	242.78±0.005	281.03±0.005
6	77.59±0.005	89.29±0.001	96.77±0.003	104.25±0.002	245.69±0.002	232.77±0.008	200.13±0.007	181.09±0.004	157.97±0.001	190.61±0.008	222.57±0.002	257.25±0.007
7	77.82±0.003	82.78±0.005	89.49±0.005	96.20±0.005	223.09±0.005	211.50±0.005	182.22±0.005	165.14±0.005	144.40±0.005	173.68±0.005	202.35±0.005	233.46±0.005
8	77.46±0.005	76.29±0.002	82.23±0.002	88.17±0.001	200.50±0.002	190.24±0.002	164.32±0.001	149.20±0.007	130.84±0.004	156.76±0.002	182.14±0.007	209.68±0.003
9	79.29±0.007	69.79±0.005	74.96±0.005	80.13±0.005	177.90±0.005	168.97±0.005	146.41±0.005	133.25±0.005	117.27±0.005	139.83±0.005	161.92±0.005	185.89±0.005
10	78.95±0.005	63.31±0.001	67.71±0.007	72.11±0.002	155.31±0.001	147.71±0.001	128.51±0.001	117.31±0.001	103.71±0.002	122.91±0.001	141.71±0.002	162.11±0.006
11	77.67±0.002	56.81±0.005	60.44±0.005	64.07±0.005	132.71±0.005	126.44±0.005	110.60±0.005	101.36±0.005	90.14±0.0015	105.98±0.005	121.49±0.005	138.32±0.005
12	79.31±0.003	50.31±0.002	53.17±0.002	56.03±0.003	110.11±0.003	105.17±0.006	92.69±0.004	85.41±0.006	76.57±0.006	89.05±0.001	101.27±0.001	114.53±0.003
13	78.78±0.005	43.82±0.005	45.91±0.005	48.00±0.005	87.52±0.005	83.91±0.005	74.79±0.005	69.47±0.005	63.01±0.005	72.13±0.005	81.06±0.005	90.75±0.005

IL-21

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	17.54±0.001	18.84±0.002	18.92±0.005	17.51±0.001	17.44±0.005	17.09±0.005	17.04±0.005	17.34±0.005	18.29±0.005	18.88±0.001	16.39±0.002	17.42±0.002
1	17.72±0.005	40.94±0.006	51.76±0.005	57.91±0.003	72.03±0.008	102.79±0.001	154.04±0.005	113.04±0.005	82.28±0.005	61.79±0.003	43.97±0.001	23.59±0.005
2	16.71±0.005	43.64±0.002	57.40±0.005	62.31±0.007	74.83±0.005	106.19±0.003	158.44±0.005	116.64±0.005	85.28±0.005	64.38±0.009	48.78±0.005	29.77±0.001
3	17.79±0.005	46.34±0.009	63.04±0.005	66.71±0.007	77.64±0.005	109.59±0.005	162.84±0.005	120.24±0.005	88.29±0.005	66.99±0.005	53.60±0.006	35.94±0.005
4	18.88±0.007	49.04±0.007	68.68±0.005	71.11±0.008	80.43±0.005	112.99±0.005	167.24±0.005	123.84±0.005	91.28±0.006	69.58±0.005	58.41±0.005	42.12±0.002
5	18.46±0.005	51.74±0.008	74.32±0.005	75.51±0.008	83.24±0.005	116.39±0.005	171.64±0.005	127.44±0.005	94.29±0.005	72.19±0.005	63.23±0.002	48.29±0.005
6	18.75±0.001	54.44±0.004	79.96±0.005	79.91±0.002	86.04±0.005	119.79±0.005	176.04±0.005	131.04±0.005	97.29±0.005	74.79±0.005	68.04±0.005	54.47±0.008
7	18.53±0.005	57.14±0.003	85.60±0.005	84.31±0.001	88.84±0.005	123.19±0.005	180.44±0.005	134.64±0.005	100.29±0.005	77.39±0.005	72.86±0.001	60.64±0.005
8	17.82±0.001	59.84±0.004	91.24±0.005	88.71±0.004	91.64±0.005	126.59±0.005	184.84±0.005	138.24±0.005	103.29±0.005	79.99±0.005	77.67±0.005	66.82±0.002
9	17.10±0.005	62.54±0.002	96.88±0.005	93.11±0.002	94.44±0.005	129.99±0.005	189.24±0.005	141.84±0.005	106.29±0.005	82.59±0.005	82.49±0.003	72.99±0.005
10	16.39±0.001	65.24±0.002	102.52±0.005	97.51±0.004	97.23±0.009	133.39±0.005	193.64±0.005	145.44±0.005	109.29±0.004	85.19±0.004	87.30±0.004	79.17±0.001
11	16.17±0.005	67.94±0.001	108.16±0.005	101.91±0.006	100.04±0.009	136.78±0.005	198.03±0.005	149.03±0.005	112.28±0.005	87.78±0.008	92.11±0.009	85.34±0.005
12	16.56±0.007	70.64±0.001	113.80±0.005	106.30±0.009	102.83±0.002	140.18±0.002	202.43±0.004	152.63±0.002	115.28±0.003	90.38±0.004	96.93±0.004	91.52±0.002
13	18.45±0.005	73.34±0.001	119.44±0.005	110.70±0.009	105.66±0.003	143.58±0.002	206.82±0.009	156.22±0.006	118.27±0.006	92.98±0.008	101.74±0.009	97.69±0.005

IL-22

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	22.84±0.001	22.84±0.001	28.71±0.007	21.45±0.001	22.29±0.006	21.02±0.007	20.68±0.003	22.11±0.002	22.82±0.005	21.33±0.005	22.76±0.004	21.39±0.005
1	22.82±0.005	38.56±0.005	33.26±0.005	44.99±0.005	106.89±0.003	67.94±0.003	98.68±0.009	55.64±0.005	160.19±0.005	88.43±0.005	65.89±0.001	52.88±0.001
2	22.11±0.003	41.34±0.001	37.82±0.003	51.14±0.004	110.37±0.005	70.65±0.006	102.01±0.001	58.12±0.002	164.71±0.005	91.55±0.006	68.56±0.007	57.78±0.005
3	21.39±0.005	44.11±0.005	42.37±0.005	57.28±0.005	113.85±0.005	73.38±0.005	105.33±0.005	60.60±0.005	169.23±0.005	94.68±0.005	71.25±0.005	62.69±0.003
4	22.95±0.005	71.86±0.005	87.92±0.005	118.73±0.005	148.64±0.002	100.60±0.001	138.52±0.002	85.40±0.002	214.41±0.009	125.86±0.006	98.04±0.001	111.74±0.002
5	20.68±0.002	46.89±0.004	46.93±0.002	63.43±0.003	117.33±0.005	76.09±0.005	108.65±0.004	63.07±0.008	173.75±0.005	97.79±0.009	73.92±0.005	67.59±0.005
6	21.96±0.005	49.66±0.005	51.48±0.005	69.57±0.005	120.81±0.005	78.82±0.005	111.97±0.005	65.56±0.005	178.27±0.005	100.92±0.005	76.61±0.005	72.50±0.002
7	21.45±0.003	52.44±0.007	56.04±0.008	75.72±0.006	124.29±0.005	81.54±0.005	115.29±0.005	68.04±0.005	182.79±0.005	104.04±0.005	79.29±0.005	77.40±0.005
8	21.33±0.005	55.21±0.005	60.59±0.005	81.86±0.005	127.77±0.005	84.26±0.005	118.61±0.005	70.52±0.005	187.31±0.005	107.16±0.005	81.975	82.30±0.009
9	22.29±0.006	63.54±0.002	74.26±0.001	100.30±0.009	138.21±0.005	92.42±0.001	128.57±0.005	77.96±0.003	200.87±0.005	116.52±0.005	90.00±0.009	97.02±0.005

10	21.02±0.008	57.99±0.002	65.15±0.00	88.01±0.006	131.25±0.005	86.98±0.005	121.93±0.005	73.00±0.005	191.83±0.005	110.28±0.005	84.65±0.005	87.21±0.005
11	22.20±0.005	60.76±0.005	69.70±0.005	94.15±0.005	134.73±0.005	89.70±0.005	125.25±0.005	75.48±0.005	196.35±0.005	113.40±0.005	87.33±0.005	92.12±0.004
12	22.27±0.005	66.31±0.005	78.81±0.005	106.44±0.005	141.68±0.005	95.06±0.009	131.88±0.005	80.45±0.001	205.38±0.005	119.63±0.005	92.68±0.008	101.93±0.002
13	22.76±0.002	69.09±0.001	83.37±0.002	112.59±0.009	145.16±0.002	97.79±0.008	135.20±0.003	82.92±0.002	209.90±0.002	122.75±0.003	95.35±0.009	106.83±0.005

IL-23

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	24.64±0.006	23.82±0.005	25.77±0.005	24.90±0.005	25.25±0.005	25.34±0.005	24.64±0.002	24.52±0.002	24.28±0.003	24.89±0.003	23.25±0.002	24.12±0.003
1	24.97±0.006	27.47±0.007	56.88±0.009	63.84±0.002	74.08±0.007	92.53±0.006	178.64±0.005	110.99±0.005	78.18±0.005	59.74±0.004	54.10±0.005	36.25±0.005
2	25.61±0.003	203.04±0.005	128.79±0.005	92.79±0.005	72.54±0.005	62.87±0.009	54.43±0.007	143.03±0.005	104.47±0.002	82.78±0.001	69.89±0.008	68.97±0.001
3	25.79±0.005	34.76±0.007	67.09±0.003	69.12±0.005	79.77±0.005	98.94±0.005	188.40±0.005	118.11±0.005	84.03±0.005	64.86±0.005	57.61±0.005	43.52±0.005
4	24.28±0.004	38.40±0.005	72.19±0.005	71.75±0.005	82.60±0.005	102.14±0.001	193.28±0.005	121.67±0.005	86.94±0.005	67.41±0.006	59.37±0.002	47.16±0.003
5	24.96±0.005	67.56±0.005	113.03±0.005	92.87±0.004	105.32±0.002	127.73±0.003	198.16±0.005	125.23±0.005	89.87±0.005	69.98±0.005	61.12±0.004	50.79±0.005
6	23.25±0.001	45.69±0.005	82.40±0.005	77.04±0.005	88.29±0.005	108.54±0.005	52.98±0.005	92.61±0.005	82.32±0.005	93.97±0.005	114.94±0.005	227.43±0.005
7	24.73±0.005	49.34±0.002	87.51±0.003	79.68±0.005	91.13±0.005	111.74±0.005	207.92±0.005	132.35±0.005	95.71±0.005	75.10±0.005	64.63±0.004	58.06±0.005
8	24.52±0.001	42.05±0.004	77.30±0.006	74.40±0.005	85.45±0.005	105.34±0.005	212.80±0.005	135.91±0.005	98.63±0.005	77.66±0.005	66.38±0.009	61.70±0.008
9	25.50±0.005	56.63±0.002	97.72±0.008	84.96±0.005	96.81±0.005	118.14±0.005	217.68±0.005	139.47±0.005	101.55±0.005	80.22±0.005	68.14±0.003	65.33±0.005
10	24.89±0.002	60.27±0.005	102.82±0.005	87.59±0.009	99.65±0.003	121.34±0.005	222.56±0.005	63.92±0.002	107.93±0.004	90.24±0.008	102.40±0.006	124.53±0.005
11	23.57±0.005	95.73±0.008	183.52±0.005	114.55±0.005	81.10±0.005	62.30±0.006	55.86±0.004	39.89±0.007	31.11±0.005	61.98±0.005	66.47±0.008	76.92±0.005
12	23.06±0.002	146.58±0.005	107.38±0.001	85.33±0.009	71.65±0.003	72.60±0.005	232.30±0.009	150.14±0.002	110.29±0.006	87.90±0.004	73.40±0.008	76.24±0.004
13	24.25±0.005	71.21±0.006	118.14±0.002	95.51±0.002	108.16±0.001	130.93±0.002	237.11±0.006	153.69±0.006	113.21±0.006	90.45±0.006	75.16±0.003	79.87±0.005

IL-25

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	36.84±0.004	38.15±0.005	38.42±0.005	37.67±0.005	36.96±0.001	36.37±0.005	37.10±0.005	38.36±0.005	37.83±0.007	37.33±0.005	36.84±0.002	38.40±0.009
1	37.83±0.007	36.34±0.005	42.61±0.005	57.69±0.005	76.13±0.006	98.68±0.009	190.94±0.005	119.19±0.005	84.33±0.005	61.79±0.003	49.23±0.005	42.07±0.005
2	38.51±0.004	41.15±0.004	45.80±0.005	60.21±0.001	79.01±0.005	102.01±0.001	196.06±0.005	122.91±0.005	87.37±0.005	64.38±0.009	53.51±0.007	45.75±0.007
3	38.39±0.005	45.95±0.005	48.99±0.005	62.73±0.005	81.90±0.005	105.33±0.005	201.18±0.005	126.63±0.005	90.42±0.005	66.99±0.005	57.78±0.005	49.42±0.005

4	38.18±0.007	50.76±0.003	52.18±0.005	65.24±0.007	84.77±0.005	108.65±0.004	206.30±0.005	130.35±0.005	93.45±0.007	69.58±0.005	62.06±0.004	53.10±0.006
5	38.36±0.005	55.56±0.005	55.37±0.005	67.77±0.005	87.66±0.005	111.97±0.005	211.42±0.005	134.07±0.005	96.50±0.005	72.19±0.005	66.33±0.005	56.77±0.005
6	37.15±0.004	60.37±0.007	58.56±0.005	70.29±0.005	90.54±0.005	115.29±0.005	216.54±0.005	137.79±0.005	99.54±0.005	74.79±0.005	70.61±0.007	60.45±0.007
7	37.33±0.005	65.17±0.005	61.75±0.005	72.81±0.005	93.42±0.005	118.61±0.005	221.66±0.005	141.51±0.005	102.58±0.005	77.39±0.005	74.88±0.005	64.12±0.005
8	38.52±0.008	69.98±0.001	64.94±0.005	75.33±0.005	96.30±0.005	121.93±0.005	226.78±0.005	145.23±0.005	105.62±0.005	79.99±0.005	79.16±0.001	67.80±0.008
9	37.10±0.005	74.78±0.005	68.13±0.005	77.85±0.005	99.18±0.005	125.25±0.005	231.90±0.005	148.95±0.005	108.66±0.005	82.59±0.005	83.43±0.005	71.47±0.005
10	37.19±0.004	79.59±0.001	71.32±0.005	80.37±0.002	102.06±0.001	128.57±0.005	237.02±0.005	152.67±0.005	111.70±0.005	85.19±0.003	87.70±0.009	75.15±0.002
11	37.67±0.005	84.39±0.005	74.51±0.005	82.90±0.002	104.85±0.006	131.88±0.005	242.13±0.005	156.38±0.005	114.73±0.005	87.78±0.008	91.98±0.004	78.82±0.005
12	38.26±0.001	89.20±0.003	77.70±0.005	85.41±0.004	107.80±0.008	135.20±0.003	247.17±0.006	160.10±0.002	117.77±0.003	90.38±0.004	96.26±0.002	82.5
13	38.15±0.005	94.00±0.005	80.89±0.005	87.93±0.002	110.66±0.007	138.52±0.002	252.29±0.006	163.81±0.006	120.80±0.006	92.98±0.008	100.53±0.005	86.175

IL-26

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	31.64±0.004	31.55±0.003	31.14±0.006	32.48±0.005	32.23±0.005	31.64±0.008	31.86±0.005	31.19±0.004	32.28±0.005	32.15±0.005	31.71±0.002	31.12±0.005
1	32.23±0.006	34.31±0.005	46.75±0.005	59.74±0.004	92.08±0.005	96.63±0.008	199.14±0.005	108.94±0.004	80.23±0.005	230.82±0.005	57.47±0.004	43.78±0.005
2	32.91±0.007	37.08±0.007	49.27±0.009	62.30±0.006	95.04±0.005	99.92±0.009	204.42±0.005	112.46±0.005	83.19±0.005	236.10±0.005	61.43±0.006	49.44±0.005
3	30.99±0.005	39.84±0.005	51.78±0.005	64.86±0.005	98.00±0.005	103.20±0.005	209.70±0.005	115.98±0.005	86.16±0.005	241.38±0.005	65.39±0.007	55.10±0.005
4	32.18±0.000	42.61±0.001	70.67±0.005	67.41±0.006	100.96±0.005	106.48±0.003	214.98±0.005	119.50±0.005	89.11±0.005	246.66±0.005	69.35±0.007	60.76±0.005
5	31.86±0.005	45.37±0.005	73.11±0.005	69.98±0.005	103.92±0.005	109.76±0.005	220.26±0.005	123.02±0.005	54.30±0.003	251.93±0.005	73.30±0.009	66.42±0.005
6	32.85±0.001	48.14±0.001	75.55±0.004	72.54±0.005	106.88±0.003	113.04±0.005	225.54±0.005	126.54±0.005	56.81±0.005	257.13±0.006	77.26±0.009	72.08±0.005
7	32.43±0.005	50.90±0.005	77.99±0.003	75.10±0.005	86.55±0.005	116.32±0.005	69.98±0.009	130.06±0.005	59.33±0.006	262.48±0.007	81.22±0.009	77.74±0.005
8	32.42±0.003	53.67±0.003	80.43±0.005	77.66±0.005	89.31±0.005	119.60±0.005	72.74±0.005	133.58±0.005	61.84±0.005	58.47±0.005	85.18±0.008	83.40±0.005
9	31.70±0.005	56.43±0.005	82.86±0.009	80.22±0.005	92.07±0.005	122.88±0.005	75.51±0.005	137.10±0.005	64.36±0.006	60.90±0.009	89.14±0.008	89.06±0.005
10	31.19±0.002	59.20±0.004	69.39±0.003	82.78±0.001	94.82±0.009	126.16±0.005	78.26±0.005	140.62±0.005	66.87±0.005	63.35±0.005	93.10±0.008	94.72±0.005
11	31.27±0.005	61.96±0.005	71.90±0.005	85.34±0.006	97.61±0.009	129.43±0.005	81.03±0.005	144.13±0.005	109.83±0.005	65.79±0.005	97.07±0.003	100.38±0.005
12	32.96±0.002	64.73±0.003	74.42±0.003	87.90±0.008	100.26±0.001	132.71±0.003	83.79±0.005	147.65±0.002	112.78±0.006	68.23±0.005	101.03±0.006	106.04±0.005
13	32.95±0.005	67.49±0.005	76.93±0.005	90.44±0.002	103.04±0.006	135.99±0.002	56.03±0.003	151.17±0.002	115.74±0.006	53.59±0.005	104.99±0.001	111.70±0.005

IL-27

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	28.04±0.008	27.96±0.005	29.61±0.002	28.04±0.007	27.25±0.006	28.52±0.003	27.28±0.007	28.06±0.003	29.39±0.005	29.97±0.008	27.73±0.005	28.89±0.002
1	29.97±0.004	57.69±0.005	56.47±0.005	22.85±0.005	74.08±0.007	104.84±0.002	61.79±0.003	115.09±0.005	47.51±0.005	86.38±0.005	55.50±0.005	156.095
2	29.61±0.001	60.21±0.001	59.53±0.007	26.31±0.005	76.92±0.005	108.28±0.004	72.81±0.005	74.80±0.005	43.61±0.005	91.13±0.005	125.48±0.005	57.80±0.002
3	29.39±0.005	62.73±0.005	62.58±0.005	29.77±0.005	88.29±0.005	122.04±0.005	74.79±0.005	133.29±0.005	76.11±0.005	92.55±0.005	60.09±0.005	164.97±0.005
4	27.28±0.003	65.24±0.007	65.64±0.008	33.23±0.005	82.60±0.005	115.16±0.005	69.58±0.005	126.01±0.005	64.67±0.005	95.62±0.008	62.39±0.003	169.41±0.005
5	27.96±0.005	67.77±0.005	68.69±0.005	36.69±0.005	85.45±0.005	118.60±0.005	72.19±0.005	129.65±0.005	70.39±0.005	98.71±0.005	64.68±0.005	173.85±0.005
6	27.25±0.001	64.38±0.009	118.73±0.005	53.23±0.005	89.46±0.005	87.78±0.005	82.89±0.008	87.02±0.005	57.45±0.005	102.50±0.002	139.23±0.005	160.53±0.005
7	27.73±0.005	151.48±0.005	104.71±0.005	117.18±0.005	78.45±0.005	200.48±0.005	77.39±0.005	136.93±0.005	81.83±0.005	104.87±0.005	69.27±0.005	182.73±0.005
8	28.52±0.009	75.33±0.005	77.86±0.001	47.07±0.005	93.97±0.005	128.92±0.005	79.99±0.005	140.57±0.005	87.55±0.005	107.95±0.005	71.57±0.002	187.17±0.005
9	27.50±0.005	77.85±0.005	80.91±0.005	50.53±0.005	96.81±0.005	132.36±0.005	82.59±0.005	144.21±0.005	93.27±0.005	111.03±0.005	73.86±0.005	191.61±0.005
10	28.89±0.007	80.37±0.002	83.97±0.003	53.99±0.005	99.65±0.006	135.80±0.005	85.19±0.002	147.85±0.005	98.99±0.005	114.11±0.005	76.16±0.008	196.05±0.005
11	27.57±0.005	79.77±0.005	111.72±0.005	66.99±0.005	122.37±0.005	58.95±0.005	101.79±0.005	66.98±0.004	178.29±0.005	70.29±0.005	71.75±0.001	40.15±0.005
12	28.06±0.008	85.41±0.002	90.08±0.004	60.91±0.005	105.33±0.002	142.67±0.002	90.38±0.006	155.12±0.002	110.43±0.005	120.26±0.003	80.75±0.007	204.92±0.003
13	27.25±0.005	87.92±0.008	93.13±0.005	64.37±0.005	108.16±0.001	146.11±0.002	92.93±0.003	158.75±0.006	116.15±0.005	123.33±0.006	83.04±0.004	209.35±0.009

IL-30

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	46.10±0.001	46.17±0.002	47.21±0.004	47.85±0.003	46.89±0.003	48.53±0.006	48.32±0.001	46.57±0.003	48.25±0.007	47.01±0.004	46.73±0.001	48.69±0.003
1	48.25±0.006	39.23±0.005	22.14±0.005	21.45±0.007	28.33±0.005	27.93±0.005	50.83±0.005	34.28±0.005	31.90±0.005	39.04±0.005	55.70±0.005	73.42±0.009
2	48.41±0.004	40.38±0.005	23.99±0.005	22.87±0.005	30.61±0.007	31.48±0.005	53.35±0.007	37.01±0.007	34.45±0.002	42.13±0.006	60.05±0.008	78.81±0.008
3	46.57±0.002	41.53±0.005	25.84±0.005	24.30±0.002	32.88±0.005	35.03±0.005	55.86±0.005	39.73±0.005	36.99±0.005	45.21±0.005	64.39±0.005	84.20±0.008
4	46.73±0.008	42.68±0.005	27.69±0.005	25.72±0.005	35.16±0.002	38.58±0.005	58.38±0.009	42.46±0.004	39.54±0.004	48.30±0.007	68.74±0.009	89.59±0.007
5	46.89±0.003	43.83±0.005	29.54±0.005	27.15±0.003	37.43±0.005	42.13±0.005	60.89±0.005	45.18±0.005	42.08±0.005	51.38±0.005	73.08±0.005	94.98±0.007
6	47.05±0.004	44.98±0.005	31.39±0.005	28.57±0.005	39.71±0.002	45.68±0.005	63.40±0.009	47.91±0.002	44.63±0.008	54.47±0.007	77.43±0.004	100.376±0.005
7	47.21±0.003	46.13±0.005	33.24±0.005	30.00±0.008	41.98±0.005	49.23±0.005	65.92±0.004	50.63±0.005	47.17±0.005	57.55±0.005	81.77±0.005	105.76±0.005
8	47.37±0.005	47.28±0.005	35.09±0.005	31.42±0.005	44.26±0.001	52.78±0.005	68.43±0.009	53.36±0.007	49.72±0.002	60.64±0.003	86.12±0.001	111.155±0.005
9	48.53±0.005	48.43±0.005	36.94±0.005	32.85±0.001	46.53±0.005	56.33±0.005	70.95±0.004	56.08±0.005	52.26±0.005	63.72±0.005	90.46±0.005	116.54±0.005

10	48.69±0.007	49.58±0.005	38.79±0.005	34.27±0.005	48.81±0.008	59.88±0.005	73.46±0.008	58.81±0.003	54.81±0.009	66.81±0.002	94.81±0.003	121.93±0.004
11	47.85±0.003	50.73±0.005	40.64±0.005	35.70±0.004	51.08±0.005	63.43±0.005	75.98±0.003	61.53±0.005	57.35±0.005	69.89±0.005	99.15±0.005	127.32±0.004
12	47.01±0.009	51.88±0.005	42.49±0.005	37.12±0.005	53.36±0.009	66.98±0.005	78.49±0.009	64.26±0.008	59.90±0.002	72.98±0.009	103.50±0.004	132.71±0.003
13	46.17±0.006	53.03±0.005	44.34±0.005	38.55±0.007	55.63±0.005	70.53±0.005	81.01±0.004	66.98±0.005	62.44±0.005	76.06±0.005	107.84±0.005	138.10±0.003

IL-31

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	47.43±0.005	47.43±0.005	48.07±0.005	48.00±0.001	47.15±0.005	48.39±0.005	49.63±0.004	48.17±0.004	47.66±0.003	49.16±0.001	47.76±0.003	48.45±0.005
1	48.45±0.005	26.39±0.004	26.22±0.009	25.60±0.005	29.52±0.005	31.64±0.005	55.92±0.003	90.48±0.005	47.37±0.005	53.32±0.005	60.46±0.005	79.50±0.005
2	48.07±0.005	28.85±0.001	28.01±0.008	26.55±0.003	31.89±0.004	34.44±0.002	58.57±0.004	93.64±0.007	51.09±0.007	57.49±0.003	65.17±0.004	85.65±0.007
3	48.29±0.005	31.31±0.007	29.80±0.008	27.49±0.005	34.25±0.005	37.23±0.005	61.22±0.001	96.81±0.005	54.80±0.005	61.65±0.005	69.87±0.005	91.79±0.005
4	48.00±0.001	33.77±0.008	31.59±0.007	28.44±0.007	36.62±0.003	40.03±0.006	63.87±0.007	99.97±0.002	58.52±0.008	65.82±0.007	74.58±0.001	97.93±0.006
5	47.13±0.005	36.23±0.003	33.38±0.007	29.38±0.005	38.98±0.005	42.82±0.005	66.52±0.002	103.13±0.005	62.23±0.005	69.98±0.005	79.28±0.005	104.08±0.005
6	47.15±0.005	38.69±0.004	35.176±0.005	30.33±0.008	41.35±0.001	45.62±0.002	69.17±0.003	106.29±0.005	65.95±0.002	74.15±0.008	83.99±0.003	110.23±0.003
7	47.77±0.005	41.15±0.009	36.96±0.006	31.27±0.005	43.71±0.005	48.41±0.005	71.82±0.001	109.45±0.005	69.66±0.005	78.31±0.005	88.69±0.005	116.37±0.005
8	48.39±0.005	43.61±0.007	38.75±0.005	32.22±0.007	46.08±0.007	51.21±0.001	74.47±0.008	112.61±0.005	73.38±0.005	82.48±0.007	93.40±0.001	122.52±0.002
9	49.01±0.005	46.07±0.008	40.54±0.005	33.16±0.005	48.44±0.005	54.00±0.005	77.12±0.006	115.77±0.005	77.09±0.005	86.64±0.005	98.10±0.005	128.66±0.005
10	49.63±0.004	23.93±0.001	42.33±0.004	34.11±0.006	50.81±0.009	56.80±0.004	79.77±0.001	118.93±0.005	80.81±0.001	90.81±0.008	102.81±0.002	134.81±0.004
11	49.25±0.004	50.99±0.003	44.12±0.004	35.05±0.005	53.17±0.005	59.59±0.004	82.42±0.002	122.08±0.005	84.52±0.005	94.97±0.005	107.51±0.005	140.95±0.005
12	48.17±0.004	53.45±0.004	45.91±0.003	36.00±0.003	55.54±0.002	62.38±0.009	85.07±0.007	125.24±0.003	88.24±0.006	99.14±0.004	112.22±0.006	147.10±0.006
13	47.29±0.003	55.91±0.008	47.70±0.003	36.94±0.005	57.90±0.005	65.18±0.004	87.72±0.004	128.40±0.002	91.95±0.005	103.30±0.005	116.92±0.005	153.24±0.005

IL-32

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	45.08±0.003	45.60±0.001	45.20±0.005	44.89±0.003	44.13±0.005	46.26±0.001	45.65±0.001	46.11±0.003	46.62±0.001	45.23±0.001	45.12±0.009	45.63±0.009
1	45.33±0.005	36.08±0.006	29.41±0.005	22.12±0.005	35.88±0.005	40.39±0.005	51.21±0.003	65.89±0.001	82.28±0.005	55.13±0.009	69.18±0.001	74.49±0.001
2	44.89±0.004	37.96±0.002	31.44±0.007	23.34±0.005	37.63±0.005	42.53±0.002	53.77±0.002	68.56±0.007	85.28±0.005	59.13±0.003	74.24±0.006	79.96±0.001
3	44.34±0.005	39.84±0.004	33.48±0.003	24.56±0.005	39.38±0.005	44.66±0.005	56.33±0.001	71.25±0.005	88.29±0.005	63.12±0.008	79.31±0.002	85.43±0.002

4	45.60±0.004	41.72±0.006	35.51±0.002	25.78±0.005	41.13±0.005	46.80±0.004	58.89±0.004	73.92±0.005	91.28±0.006	67.12±0.002	84.37±0.007	90.90±0.002
5	44.98±0.009	34.20±0.001	37.54±0.005	27.00±0.005	42.88±0.005	48.93±0.005	61.45±0.002	76.61±0.005	94.29±0.005	71.11±0.007	89.44±0.003	96.37±0.003
6	46.11±0.002	45.48±0.007	39.57±0.007	28.22±0.005	44.63±0.005	51.07±0.002	64.01±0.003	79.29±0.005	97.29±0.005	75.11±0.001	94.50±0.008	101.84±0.003
7	44.86±0.005	47.36±0.009	41.61±0.007	29.44±0.005	46.38±0.005	53.20±0.005	66.57±0.001	81.97±0.005	100.29±0.005	79.10±0.006	99.57±0.002	107.31±0.004
8	46.62±0.007	49.24±0.002	43.64±0.002	30.66±0.005	48.13±0.005	55.34±0.007	69.13±0.007	84.65±0.005	103.29±0.005	83.09±0.009	104.63±0.009	112.78±0.004
9	46.37±0.005	51.12±0.006	45.67±0.005	31.88±0.005	49.88±0.005	57.47±0.005	71.69±0.003	87.33±0.005	106.29±0.005	87.09±0.002	109.70±0.005	118.25±0.005
10	45.12±0.009	52.99±0.009	47.70±0.007	33.10±0.005	51.63±0.005	59.61±0.001	74.25±0.008	90.00±0.009	109.29±0.004	91.08±0.003	114.77±0.005	123.72±0.005
11	45.78±0.004	54.87±0.009	49.74±0.003	34.32±0.005	53.38±0.005	61.74±0.005	76.81±0.007	92.68±0.008	112.28±0.005	95.08±0.004	119.83±0.006	129.19±0.006
12	45.63±0.009	56.75±0.009	51.77±0.002	35.54±0.005	55.13±0.005	63.88±0.004	79.37±0.006	95.35±0.009	115.28±0.003	99.07±0.008	124.90±0.001	134.66±0.006
13	45.39±0.004	58.63±0.009	53.80±0.005	36.76±0.005	56.88±0.005	66.01±0.005	81.92±0.009	98.04±0.004	118.27±0.006	103.07±0.003	129.96±0.007	140.13±0.007

IL-33

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	20.16±0.003	20.16±0.004	20.37±0.002	18.99±0.003	19.41±0.005	20.56±0.001	19.16±0.007	20.36±0.001	18.80±0.002	19.96±0.001	18.15±0.009	20.16±0.002
1	19.96±0.007	27.02±0.005	22.37±0.005	29.93±0.005	30.75±0.002	41.29±0.005	47.70±0.005	50.89±0.001	57.38±0.001	64.81±0.005	71.30±0.005	77.67±0.007
2	20.56±0.004	25.28±0.002	24.37±0.008	30.25±0.005	33.08±0.005	43.49±0.005	51.13±0.002	54.56±0.001	61.54±0.006	69.54±0.007	76.53±0.002	83.39±0.005
3	20.86±0.009	30.51±0.005	26.37±0.006	30.57±0.005	35.42±0.001	45.69±0.005	54.56±0.003	58.23±0.002	65.71±0.002	74.28±0.008	81.76±0.003	89.10±0.004
4	19.36±0.002	32.26±0.001	28.37±0.009	30.89±0.005	37.75±0.005	47.89±0.005	57.98±0.007	61.90±0.002	69.87±0.007	79.01±0.002	86.98±0.007	94.81±0.007
5	19.66±0.003	34.00±0.005	30.37±0.002	31.21±0.005	40.09±0.009	50.09±0.005	61.41±0.005	65.57±0.003	74.04±0.003	83.74±0.005	92.21±0.005	100.53±0.001
6	20.36±0.006	35.75±0.006	32.37±0.008	31.53±0.005	42.42±0.005	52.29±0.005	64.84±0.002	69.24±0.003	78.20±0.008	88.47±0.007	97.44±0.002	106.24±0.004
7	19.76±0.002	37.49±0.005	34.37±0.003	31.85±0.005	44.76±0.002	54.49±0.005	68.27±0.008	72.91±0.004	82.37±0.004	93.20±0.008	102.66±0.008	111.95±0.008
8	19.16±0.007	39.24±0.002	36.37±0.009	32.17±0.005	47.09±0.005	56.69±0.005	71.69±0.007	76.58±0.004	86.53±0.007	97.93±0.009	107.89±0.007	117.67±0.001
9	19.15±0.009	40.98±0.005	38.37±0.008	32.49±0.005	49.43±0.007	58.89±0.005	75.12±0.005	80.25±0.005	90.70±0.001	102.67±0.003	113.12±0.005	123.38±0.005
10	18.15±0.009	42.73±0.004	40.37±0.004	32.81±0.005	51.76±0.005	61.09±0.005	78.55±0.002	83.92±0.002	94.86±0.004	107.40±0.007	118.35±0.002	129.0985
11	20.95±0.009	44.47±0.005	42.37±0.007	33.13±0.005	54.10±0.003	63.29±0.004	81.98±0.002	87.59±0.006	99.03±0.006	112.14±0.003	123.58±0.007	134.812
12	18.80±0.002	46.22±0.002	44.37±0.002	33.45±0.005	56.43±0.005	65.49±0.004	85.40±0.007	91.26±0.006	103.20±0.001	116.87±0.002	128.80±0.007	140.5255
13	20.25±0.009	47.96±0.005	46.37±0.009	33.77±0.005	58.77±0.008	67.69±0.004	88.83±0.005	94.93±0.007	107.36±0.007	121.60±0.005	134.03±0.005	146.239

APPENDIX XV

INFLAMMATORY BIOMAKERS

Hydrogen Peroxidase

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	12.06±0.005	12.06±0.005	12.24±0.005	12.10±0.005	12.52±0.005	12.38±0.005	12.66±0.005	12.87±0.005	12.03±0.005	12.17±0.005	12.80±0.005	12.73±0.005
1	12.03±0.005	5.18±0.005	0.97±0.005	0.74±0.005	1.74±0.005	0.38±0.005	0.77±0.005	0.90±0.005	4.97±0.005	3.28±0.005	1.81±0.005	8.37±0.005
2	12.10±0.005	5.21±0.005	0.92±0.004	0.77±0.005	1.81±0.005	0.41±0.005	0.804±0.006	0.97±0.005	0.98±0.004	2.30±0.005	0.62±0.005	1.01±0.005
3	12.17±0.005	5.24±0.005	0.89±0.005	2.09±0.005	0.53±0.005	0.82±0.004	1.25±0.005	0.92±0.005	2.16±0.005	0.56±0.005	0.95±0.005	1.32±0.005
4	12.24±0.005	5.27±0.005	4.70±0.005	2.65±0.005	1.18±0.005	7.92±0.005	0.92±0.005	0.95±0.005	2.23±0.005	0.59±0.005	0.98±0.005	1.39±0.005
5	12.31±0.005	5.30±0.005	4.73±0.005	2.72±0.005	1.25±0.005	7.97±0.005	5.45±0.002	4.88±0.005	3.07±0.005	1.60±0.005	8.22±0.005	1.22±0.005
6	12.38±0.005	5.33±0.002	4.76±0.005	2.79±0.005	1.32±0.005	8.02±0.005	5.48±0.005	4.91±0.005	3.14±0.005	1.67±0.005	8.26±0.005	1.27±0.005
7	12.45±0.005	0.62±0.005	1.46±0.005	0.26±0.005	0.65±0.005	0.62±0.005	5.51±0.002	4.94±0.005	3.21±0.005	1.74±0.005	8.32±0.005	1.32±0.005
8	12.52±0.005	0.65±0.005	1.53±0.005	0.29±0.005	0.68±0.001	0.69±0.005	5.36±0.005	4.79±0.005	2.86±0.005	1.39±0.005	8.07±0.005	1.46±0.005
9	12.59±0.005	0.68±0.005	1.60±0.005	0.32±0.005	0.71±0.005	0.76±0.005	5.39±0.002	4.82±0.005	2.93±0.005	1.46±0.005	8.12±0.005	5.54±0.005
10	12.66±0.005	0.71±0.005	1.67±0.005	0.35±0.005	0.74±0.005	0.83±0.005	5.42±0.005	4.85±0.005	3.00±0.005	1.53±0.005	8.16±0.005	1.37±0.005
11	12.73±0.005	4.61±0.005	2.44±0.005	0.97±0.005	7.77±0.005	1.07±0.005	0.80±0.005	1.88±0.005	0.44±0.005	0.83±0.005	1.04±0.005	0.77±0.005
12	12.80±0.005	4.64±0.005	2.51±0.005	1.04±0.005	7.82±0.005	1.12±0.005	0.83±0.005	1.95±0.005	0.47±0.005	0.86±0.005	1.11±0.005	0.82±0.005
13	12.87±0.005	4.67±0.005	2.58±0.005	1.11±0.005	7.87±0.005	1.17±0.005	0.77±0.007	2.02±0.005	0.50±0.005	0.89±0.005	1.18±0.005	0.87±0.005

Hydrogen peroxide

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	12.38±0.005	12.49±0.005	12.65±0.005	12.73±0.005	12.61±0.005	12.59±0.005	12.87±0.005	12.62±0.005	12.45±0.005	12.66±0.005	12.78±0.005	12.94±0.005
1	12.45±0.005	59.84±0.005	69.78±0.005	85.22±0.005	91.83±0.005	109.66±0.004	121.93±0.005	136.70±0.005	147.38±0.005	163.01±0.006	184.29±0.009	227.84±0.005
2	12.52±0.005	59.91±0.005	69.85±0.005	85.27±0.005	91.90±0.005	109.71±0.003	122.00±0.005	136.73±0.005	147.41±0.005	163.09±0.005	184.37±0.005	233.68±0.005
3	12.59±0.005	59.98±0.005	69.92±0.005	85.32±0.005	91.97±0.005	109.76±0.006	122.07±0.005	136.76±0.005	147.44±0.005	163.16±0.001	184.43±0.009	239.52±0.005
4	12.66±0.005	60.05±0.005	69.99±0.005	85.37±0.005	92.04±0.005	109.97±0.005	122.14±0.005	136.79±0.005	147.47±0.005	163.23±0.005	184.51±0.005	245.36±0.004
5	12.73±0.005	60.12±0.005	70.06±0.005	85.42±0.005	92.11±0.005	110.01±0.002	122.21±0.005	136.82±0.005	147.50±0.005	163.30±0.001	184.57±0.009	251.20±0.005

6	12.80±0.005	60.19±0.005	70.13±0.004	85.47±0.005	92.16±0.006	109.91±0.004	122.28±0.005	136.82±0.006	147.53±0.005	163.37±0.005	184.65±0.005	257.04±0.005
7	12.87±0.005	60.26±0.005	70.20±0.005	85.52±0.005	92.25±0.005	109.96±0.002	122.35±0.005	136.87±0.004	147.56±0.005	163.44±0.004	184.71±0.009	262.88±0.005
8	12.94±0.005	60.33±0.005	70.27±0.006	85.57±0.005	92.30±0.006	110.11±0.005	122.42±0.005	136.91±0.005	147.59±0.005	163.51±0.005	184.79±0.005	268.72±0.005
9	12.61±0.005	60.40±0.005	70.34±0.005	85.57±0.007	92.39±0.005	110.22±0.005	122.49±0.005	136.94±0.005	147.62±0.005	163.57±0.005	184.85±0.009	274.56±0.005
10	12.78±0.005	60.47±0.005	70.41±0.003	85.67±0.005	92.44±0.006	110.26±0.001	122.56±0.005	136.97±0.005	147.65±0.005	163.65±0.005	184.93±0.005	280.40±0.005
11	12.65±0.005	60.54±0.005	70.47±0.001	85.67±0.003	92.51±0.006	110.25±0.004	122.63±0.005	137.00±0.005	147.68±0.005	163.71±0.005	184.99±0.009	286.23±0.005
12	12.62±0.005	60.61±0.005	70.55±0.005	85.77±0.005	92.60±0.005	110.33±0.002	122.70±0.005	136.95±0.007	147.71±0.005	163.79±0.005	185.07±0.005	292.07±0.005
13	12.49±0.005	60.68±0.005	70.62±0.005	85.82±0.005	92.57±0.006	110.39±0.001	122.77±0.005	137.05±0.004	147.74±0.005	163.86±0.001	185.13±0.009	297.91±0.005

Myeloperoxidase

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	100.16±0.004	99.30±0.003	100.33±0.005	99.67±0.005	100.44±0.002	100.16±0.004	100.16±0.004	97.01±0.008	86.21±0.006	100.07±0.005	99.77±0.006	99.95±0.004
1	100.44±0.002	96.74±0.009	85.60±0.005	71.82±0.005	17.73±0.005	28.03±0.005	45.54±0.005	57.90±0.005	56.87±0.005	39.40±0.003	36.46±0.002	14.27±0.003
2	100.09±0.001	96.77±0.009	85.67±0.005	71.87±0.005	18.31±0.003	27.91±0.007	44.23±0.003	55.75±0.003	54.79±0.002	36.57±0.004	34.17±0.008	16.02±0.007
3	99.77±0.006	96.80±0.009	85.74±0.005	96.99±0.007	86.44±0.005	72.42±0.005	24.63±0.005	26.53±0.005	52.70±0.005	33.74±0.007	31.88±0.004	30.02±0.003
4	99.82±0.005	96.83±0.009	85.81±0.005	71.97±0.005	19.46±0.001	27.66±0.002	41.59±0.005	72.37±0.005	24.06±0.007	26.66±0.002	31.07±0.003	34.19±0.004
5	99.87±0.005	22.91±0.004	27.03±0.005	40.64±0.009	72.27±0.005	99.87±0.005	40.28±0.005	96.87±0.002	85.88±0.005	72.02±0.005	20.03±0.005	27.53±0.005
6	99.90±0.004	96.87±0.006	85.93±0.006	72.07±0.005	20.61±0.003	27.41±0.004	38.97±0.002	47.13±0.001	46.45±0.002	25.25±0.001	25.01±0.002	24.77±0.004
7	99.95±0.004	49.28±0.005	48.53±0.005	28.08±0.007	27.30±0.006	26.52±0.006	37.65±0.005	44.97±0.005	44.36±0.005	22.42±0.003	22.72±0.006	23.02±0.001
8	100.02±0.005	96.95±0.008	86.07±0.006	72.17±0.005	21.76±0.001	27.16±0.001	36.34±0.006	42.82±0.003	42.28±0.009	19.59±0.002	20.43±0.001	21.27±0.004
9	100.07±0.005	96.99±0.002	86.16±0.005	72.17±0.003	22.33±0.005	51.43±0.003	50.61±0.004	30.91±0.009	29.59±0.001	28.27±0.008	18.14±0.006	19.52±0.002
10	100.12±0.005	71.92±0.005	18.88±0.005	27.78±0.005	42.91±0.005	53.59±0.005	33.71±0.008	38.51±0.003	38.11±0.002	13.93±0.006	15.85±0.005	17.77±0.008
11	100.16±0.004	97.05±0.001	86.28±0.006	72.27±0.004	23.48±0.005	26.78±0.005	32.39±0.005	96.74±0.006	86.02±0.005	72.12±0.005	21.18±0.005	27.28±0.005
12	100.22±0.005	97.05±0.006	86.37±0.005	40.18±0.008	16.76±0.007	99.90±0.004	35.02±0.005	26.91±0.004	33.93±0.004	8.27±0.008	11.27±0.002	14.27±0.003
13	100.27±0.005	36.35±0.005	36.02±0.005	11.10±0.001	13.56±0.002	16.02±0.001	29.76±0.005	32.04±0.005	31.85±0.0015	5.43±0.009	8.98±0.004	12.52±0.007

APPENDIX XVI

ENDOGENOUS ANTIOXIDANT MARKERS

SUPEROXIDE DISMUTASE (SOD)

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	60.92±0.00	61.93±0.005	61.36±0.002	61.36±0.005	60.96±0.001	62.23±0.003	60.54±0.005	61.48±0.005	60.64±0.002	61.00±0.001	61.00±0.002	62.5±0.001
1	60.28±0.002	33.19±0.005	35.46±0.005	37.08±0.003	26.84±0.005	35.40±0.005	33.97±0.005	30.64±0.002	28.62±0.005	27.15±0.005	24.75±0.008	21.80±0.005
2	61.40±0.005	30.88±0.005	38.73±0.007	33.71±0.004	25.07±0.005	30.48±0.005	32.08±0.005	25.48±0.005	27.45±0.004	26.68±0.005	23.47±0.004	24.81±0.003
3	61.09±0.003	35.91±0.004	35.11±0.006	33.53±0.005	26.09±0.005	33.38±0.002	27.52±0.003	26.99±0.004	27.58±0.008	23.33±0.005	24.35±0.009	24.03±0.005
4	60.75±0.002	30.58±0.005	35.00±0.006	30.57±0.006	23.02±0.001	31.87±0.005	26.75±0.005	27.59±0.005	20.78±0.006	26.28±0.005	22.35±0.007	22.54±0.002
5	60.18±0.004	26.07±0.006	30.03±0.009	25.98±0.005	20.66±0.005	28.84±0.001	21.89±0.005	25.41±0.001	20.46±0.009	18.88±0.005	22.06±0.004	19.96±0.005
6	60.54±0.005	26.46±0.004	29.51±0.005	24.95±0.005	21.28±0.001	29.64±0.004	22.50±0.006	24.75±0.005	19.82±0.005	22.28±0.005	17.99±0.006	20.63±0.004
7	60.52±0.005	22.57±0.005	26.07±0.005	24.01±0.005	17.30±0.005	21.01±0.005	21.58±0.005	24.50±0.005	21.21±0.005	20.01±0.005	16.89±0.002	20.39±0.005
8	60.19±0.001	23.68±0.005	29.92±0.005	17.72±0.002	19.13±0.005	19.53±0.007	15.49±0.005	25.51±0.005	19.63±0.005	19.18±0.005	12.01±0.009	17.28±0.001
9	62.36±0.001	21.69±0.001	24.86±0.005	20.56±0.002	20.24±0.005	22.62±0.002	17.73±0.001	28.10±0.008	18.68±0.005	17.09±0.005	13.25±0.007	16.68±0.005
10	60.26±0.003	22.53±0.001	24.08±0.002	13.18±0.005	16.18±0.003	18.65±0.003	19.19±0.007	22.49±0.005	16.16±0.001	17.52±0.005	16.29±0.001	18.79±0.001
11	61.40±0.005	18.42±0.007	22.80±0.003	14.72±0.003	19.54±0.006	21.58±0.005	14.40±0.002	22.96±0.005	14.67±0.001	12.55±0.005	8.53±0.001	11.53±0.004
12	61.09±0.002	16.87±0.005	25.48±0.004	17.87±0.005	12.91±0.002	14.49±0.005	12.88±0.004	23.97±0.005	13.90±0.007	11.36±0.005	5.82±0.006	11.66±0.006
13	60.39±0.001	19.51±0.005	20.15±0.007	12.92±0.009	13.21±0.006	17.32±0.005	14.21±0.006	22.01±0.005	11.06±0.001	10.98±0.005	4.80±0.004	12.50±0.003

Catalase

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	74.09±0.001	74.36±0.004	73.19±0.005	73.78±0.002	74.77±0.001	75.37±0.002	73.66±0.003	72.46±0.004	74.08±0.005	73.39±0.005	74.195	74.32±0.005
1	74.19±0.005	50.69±0.005	48.34±0.001	30.03±0.004	34.28±0.005	34.37±0.008	48.36±0.004	37.67±0.006	21.22±0.005	38.33±0.005	27.005	22.82±0.005
2	75.30±0.003	49.03±0.009	43.29±0.005	27.56±0.002	28.80±0.004	32.37±0.006	44.91±0.003	35.23±0.002	21.35±0.005	37.51±0.007	26.95	22.11±0.002
3	74.68±0.005	47.36±0.005	38.25±0.001	25.09±0.001	23.31±0.005	30.37±0.009	41.63±0.006	32.80±0.006	21.48±0.005	36.68±0.005	26.895	21.39±0.005
4	74.32±0.005	45.73±0.007	33.20±0.005	22.62±0.008	17.83±0.004	28.37±0.007	38.38±0.003	30.42±0.002	21.61±0.005	35.86±0.009	26.84	20.68±0.004
5	74.86±0.005	44.03±0.005	28.16±0.004	20.15±0.003	12.34±0.005	26.37±0.001	35.13±0.002	28.07±0.002	21.74±0.005	35.03±0.005	26.785	19.96±0.005

6	72.39±0.005	42.37±0.008	23.11±0.005	17.68±0.008	6.86±0.008	24.37±0.004	31.81±0.007	25.49±0.002	21.87±0.005	34.21±0.001	26.73	19.25±0.002
7	73.11±0.005	40.70±0.005	29.29±0.007	15.21±0.001	27.69±0.005	22.37±0.002	28.53±0.005	23.06±0.004	22.00±0.005	33.38±0.005	26.675	18.53±0.005
8	72.08±0.005	39.04±0.006	25.36±0.004	12.74±0.009	25.84±0.005	20.37±0.009	25.26±0.007	20.78±0.006	22.13±0.005	32.56±0.007	26.62	17.82±0.003
9	74.74±0.005	37.37±0.005	20.80±0.008	10.27±0.009	23.99±0.005	18.37±0.007	22.04±0.003	18.19±0.006	22.26±0.005	31.73±0.005	26.565	17.10±0.005
10	73.78±0.003	35.71±0.009	16.78±0.006	7.80±0.004	22.14±0.005	16.37±0.004	18.71±0.009	15.90±0.008	22.39±0.005	30.91±0.004	26.51	16.39±0.001
11	73.02±0.002	34.04±0.006	12.56±0.004	19.68±0.006	20.29±0.005	14.37±0.008	15.53±0.008	13.39±0.006	22.52±0.005	30.08±0.005	26.455	15.67±0.005
12	73.19±0.005	32.47±0.008	8.39±0.001	13.96±0.005	18.44±0.005	12.36±0.009	12.18±0.009	10.89±0.005	22.65±0.005	29.27±0.003	26.4	14.96±0.007
13	74.68±0.003	30.71±0.005	7.68±0.003	7.67±0.005	39.83±0.006	10.69±0.001	35.08±0.006	8.45±0.002	22.78±0.005	28.43±0.005	26.345	24.25±0.005

TOTAL ANTIOXIDANT CAPACITY

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	253.62±0.005	253.07±0.006	252.72±0.005	253.62±0.005	253.60±0.005	252.96±0.005	253.56±0.005	252.48±0.005	252.00±0.004	253.08±0.005	254.52±0.005	253.24±0.005
1	253.24±0.005	151.46±0.004	143.58±0.008	116.81±0.005	104.67±0.004	86.94±0.008	73.97±0.005	60.50±0.007	54.85±0.007	41.94±0.005	37.44±0.003	22.78±0.005
2	252.96±0.005	144.93±0.007	139.12±0.006	107.36±0.005	103.99±0.007	85.03±0.006	73.20±0.003	57.49±0.005	54.13±0.005	41.51±0.003	36.64±0.005	21.07±0.005
3	252.48±0.005	138.40±0.002	134.66±0.009	97.91±0.005	103.31±0.008	83.15±0.004	72.43±0.003	54.49±0.003	53.42±0.003	41.07±0.005	35.85±0.007	19.36±0.005
4	252.00±0.004	131.87±0.004	130.20±0.009	88.46±0.009	102.63±0.006	81.22±0.005	71.67±0.002	51.48±0.005	52.70±0.005	40.64±0.006	35.05±0.005	17.65±0.005
5	254.52±0.005	125.34±0.006	125.74±0.003	79.01±0.005	101.95±0.009	79.32±0.007	70.94±0.003	48.48±0.006	51.99±0.001	40.20±0.005	34.26±0.001	15.94±0.006
6	253.04±0.005	118.81±0.002	121.28±0.007	69.56±0.005	101.27±0.003	77.41±0.005	70.12±0.002	45.47±0.004	51.27±0.005	39.77±0.007	33.46±0.005	14.23±0.005
7	253.56±0.005	112.28±0.007	116.82±0.008	60.12±0.004	100.59±0.007	75.51±0.007	69.35±0.002	42.55±0.009	50.56±0.006	39.33±0.005	32.67±0.001	12.52±0.005
8	253.08±0.005	105.75±0.002	112.36±0.009	51.88±0.004	99.91±0.009	73.60±0.004	68.61±0.002	39.46±0.004	49.84±0.005	38.90±0.003	31.87±0.005	10.81±0.005
9	253.60±0.005	99.22±0.008	107.90±0.003	46.91±0.008	99.23±0.009	71.78±0.008	67.90±0.005	36.46±0.008	49.13±0.001	38.46±0.005	31.07±0.009	9.10±0.005
10	252.12±0.005	92.69±0.002	103.52±0.009	44.56±0.006	98.63±0.009	69.80±0.002	67.04±0.003	33.45±0.005	48.42±0.005	38.03±0.004	30.28±0.004	7.39±0.005
11	252.72±0.005	86.15±0.009	98.97±0.008	43.80±0.006	97.98±0.009	67.97±0.008	66.27±0.002	30.44±0.009	47.70±0.001	37.59±0.005	29.48±0.008	5.81±0.007
12	252.15±0.009	83.42±0.004	94.51±0.006	44.94±0.002	97.18±0.008	65.98±0.004	65.59±0.002	27.44±0.004	46.98±0.005	37.16±0.007	28.69±0.003	4.64±0.004
13	253.07±0.006	79.47±0.009	90.08±0.009	47.27±0.004	96.50±0.009	64.07±0.008	64.73±0.001	24.45±0.008	46.27±0.003	36.72±0.005	27.93±0.003	3.71±0.007

NITRIC OXIDE (NO)

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	35.76±0.005	36.67±0.005	36.53±0.005	36.39±0.005	36.25±0.005	36.11±0.005	35.97±0.005	36.60±0.005	35.83±0.005	36.32±0.005	36.18±0.005	36.46±0.005
1	35.83±0.005	62.02±0.005	44.51±0.005	54.81±0.005	85.71±0.005	92.92±0.005	100.13±0.005	84.68±0.005	93.95±0.005	104.25±0.005	99.10±0.005	105.86±0.007
2	35.90±0.005	59.49±0.002	43.27±0.001	52.87±0.002	81.66±0.002	88.3±0.008	95.10±0.002	80.70±0.007	89.34±0.004	89.04±0.004	94.14±0.004	104.87±0.006
3	35.97±0.005	57.15±0.005	42.02±0.005	50.92±0.005	77.62±0.005	83.85±0.005	90.08±0.005	76.73±0.005	84.74±0.005	93.64±0.005	89.19±0.005	103.88±0.009
4	36.04±0.005	54.71±0.004	40.77±0.006	48.97±0.006	73.57±0.007	79.31±0.001	85.05±0.007	72.75±0.000	80.13±0.003	88.33±0.004	84.23±0.003	102.89±0.004
5	36.11±0.005	52.28±0.005	39.53±0.005	47.03±0.005	69.53±0.005	74.78±0.005	80.03±0.005	68.78±0.005	75.53±0.005	83.03±0.005	79.28±0.005	101.90±0.007
6	36.18±0.005	49.85±0.003	38.29±0.003	45.09±0.004	65.49±0.009	70.25±0.007	75.01±0.009	64.81±0.001	70.93±0.006	77.73±0.007	74.33±0.007	100.91±0.009
7	36.25±0.005	47.41±0.005	37.04±0.005	43.14±0.005	61.44±0.005	65.71±0.005	69.97±0.002	60.83±0.005	66.32±0.005	72.40±0.008	69.36±0.003	99.92±0.008
8	36.32±0.005	44.98±0.007	35.80±0.007	41.20±0.001	57.39±0.006	61.17±0.008	64.95±0.001	56.85±0.007	61.71±0.001	67.11±0.001	64.41±0.001	98.92±0.009
9	36.39±0.005	42.52±0.006	34.55±0.005	39.24±0.002	53.33±0.006	56.62±0.006	59.91±0.006	52.86±0.006	57.09±0.006	61.79±0.006	59.44±0.006	97.93±0.009
10	36.46±0.005	40.11±0.008	33.31±0.004	37.31±0.006	49.31±0.009	52.11±0.004	54.91±0.008	48.91±0.009	52.51±0.009	56.51±0.008	54.51±0.004	96.94±0.008
11	36.53±0.005	37.67±0.005	32.06±0.005	35.36±0.005	45.26±0.005	47.57±0.005	49.88±0.005	44.93±0.005	47.90±0.005	51.20±0.005	49.55±0.005	95.95±0.008
12	36.60±0.005	35.23±0.002	30.71±0.002	33.41±0.001	41.21±0.003	43.03±0.003	44.75±0.002	40.95±0.007	43.29±0.007	45.79±0.002	44.49±0.002	94.96±0.008
13	36.67±0.005	32.80±0.005	29.57±0.005	31.47±0.005	37.17±0.005	38.50±0.005	39.83±0.005	36.98±0.005	38.69±0.005	40.59±0.005	39.64±0.005	93.96±0.009

Nitrotyrosine

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	9.27±0.005	9.92±0.005	9.81±0.006	9.71±0.005	9.62±0.005	9.52±0.005	9.42±0.005	9.32±0.005	9.87±0.005	9.77±0.005	9.67±0.005	9.42±0.005
1	9.32±0.005	116.05±0.004	309.42±0.009	111.31±0.004	214.70±0.005	113.04±0.005	185.28±0.001	200.42±0.005	412.44±0.003	594.79±0.004	487.49±0.001	666.54±0.007
2	9.37±0.005	140.30±0.009	332.81±0.006	134.21±0.006	214.73±0.005	116.64±0.005	185.35±0.005	200.45±0.005	443.68±0.009	607.79±0.008	524.46±0.001	681.03±0.002
3	9.42±0.005	164.50±0.007	356.20±0.008	157.30±0.004	214.76±0.005	120.24±0.005	185.41±0.006	200.48±0.005	474.93±0.006	620.79±0.005	561.43±0.002	695.34±0.005
4	9.47±0.005	188.78±0.008	379.60±0.004	180.22±0.006	214.79±0.005	123.84±0.005	185.49±0.005	200.49±0.006	506.18±0.002	633.85±0.004	598.40±0.002	709.64±0.004
5	9.52±0.005	212.93±0.002	402.98±0.007	203.19±0.006	214.82±0.005	127.44±0.005	185.55±0.006	200.54±0.005	537.42±0.009	646.79±0.005	635.37±0.003	724.14±0.005
6	9.57±0.005	237.11±0.005	426.37±0.006	226.13±0.005	214.85±0.005	131.04±0.005	185.63±0.005	200.57±0.005	568.67±0.005	659.79±0.005	672.34±0.003	738.54±0.005
7	9.62±0.005	261.3±0.002	449.76±0.006	249.08±0.002	214.88±0.005	134.64±0.005	185.69±0.009	200.60±0.005	599.92±0.002	672.79±0.005	709.32±0.001	752.94±0.005
8	9.67±0.005	285.59±0.005	473.15±0.005	272.02±0.005	214.91±0.005	138.24±0.005	185.77±0.005	200.61±0.006	631.16±0.008	685.79±0.003	746.29±0.007	767.34±0.007
9	9.71±0.005	309.73±0.006	496.54±0.005	294.97±0.007	214.94±0.005	141.84±0.005	185.83±0.001	200.66±0.005	662.28±0.005	698.79±0.005	783.07±0.009	781.74±0.005

10	9.77±0.005	333.93±0.007	519.934±0.005	317.91±0.008	214.97±0.005	145.44±0.005	185.91±0.005	200.69±0.005	693.55±0.006	711.70±0.002	820.23±0.006	796.15±0.005
11	9.81±0.006	358.14±0.008	543.32±0.004	340.86±0.004	215.00±0.005	149.09±0.005	185.97±0.001	200.72±0.005	724.90±0.008	724.79±0.006	857.19±0.006	810.53±0.005
12	9.87±0.005	382.33±0.009	566.713±0.005	363.80±0.004	215.03±0.005	152.64±0.004	186.05±0.005	200.73±0.006	756.15±0.004	737.92±0.006	894.16±0.006	824.93±0.003
13	9.92±0.005	406.55±0.005	590.10±0.003	386.75±0.008	215.06±0.005	156.18±0.003	186.09±0.008	200.78±0.005	787.40±0.001	750.83±0.007	931.13±0.007	839.33±0.007

Protein carbonyl

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	2.95±0.001	2.85±0.002	2.95±0.007	3.51±0.003	2.80±0.001	3.05±0.001	3.38±0.003	2.47±0.005	3.18±0.005	2.85±0.001	2.44±0.003	2.95±0.00
1	3.15±0.001	5.28±0.003	4.77±0.005	5.22±0.004	8.39±0.005	13.08±0.002	3.37±0.005	1.78±0.005	3.38±0.005	8.29±0.004	1.84±0.006	8.96±0.003
2	3.35±0.004	5.40±0.002	3.90±0.003	5.20±0.006	7.79±0.009	12.88±0.005	3.18±0.002	2.38±0.006	3.58±0.005	8.19±0.002	2.54±0.008	9.97±0.005
3	3.55±0.003	7.20±0.001	4.16±0.006	8.21±0.009	7.21±0.005	12.69±0.007	2.98±0.005	8.97±0.005	3.78±0.005	8.10±0.006	3.25±0.002	8.98±0.005
4	3.75±0.002	5.33±0.002	4.10±0.006	5.23±0.003	6.62±0.005	12.49±0.005	2.79±0.007	3.57±0.002	3.98±0.005	8.00±0.005	3.95±0.005	8.98±0.004
5	3.95±0.002	7.30±0.001	3.77±0.004	5.24±0.006	6.03±0.005	13.30±0.002	2.59±0.005	4.16±0.005	4.18±0.005	7.90±0.005	4.66±0.001	11.00±0.005
6	2.80±0.003	5.60±0.004	4.10±0.007	5.26±0.003	6.10±0.005	12.10±0.005	2.40±0.003	4.76±0.002	4.38±0.005	7.81±0.003	5.36±0.005	9.01±0.005
7	2.05±0.001	6.25±0.001	4.06±0.002	2.27±0.008	6.13±0.009	11.91±0.004	2.20±0.005	7.35±0.005	4.58±0.005	7.72±0.002	6.07±0.001	9.01±0.004
8	3.05±0.002	5.40±0.004	2.35±0.002	5.28±0.006	5.80±0.005	11.71±0.005	2.01±0.001	5.95±0.004	4.78±0.005	7.62±0.005	6.77±0.005	9.03±0.005
9	3.43±0.004	5.95±0.002	4.10±0.004	5.30±0.005	6.11±0.007	11.52±0.001	2.59±0.005	6.54±0.005	4.98±0.005	7.53±0.001	7.48±0.003	12.04±0.005
10	2.85±0.001	6.50±0.004	4.89±0.002	4.31±0.004	6.05±0.005	11.32±0.005	3.57±0.003	8.14±0.004	5.18±0.005	7.43±0.003	8.18±0.005	9.04±0.004
11	2.22±0.001	6.05±0.009	4.10±0.006	5.32±0.007	5.79±0.005	10.13±0.006	3.37±0.005	7.73±0.005	5.38±0.005	7.33±0.008	8.89±0.002	10.06±0.005
12	2.95±0.003	5.76±0.005	3.45±0.002	5.34±0.001	6.02±0.009	10.93±0.002	3.18±0.007	2.33±0.001	5.58±0.005	7.24±0.005	9.59±0.005	9.07±0.005
13	2.59±0.003	6.97±0.006	4.98±0.003	5.35±0.002	6.26±0.003	16.73±0.004	2.98±0.005	8.92±0.005	5.78±0.005	7.15±0.004	10.30±0.004	9.07±0.004

APPENDIX XVII

LIPID PEROXIDATION

MDA

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	28.47±0.005	28.92±0.005	28.92±0.005	28.82±0.005	28.62±0.005	28.87±0.005	28.82±0.005	28.52±0.005	28.72±0.005	28.57±0.005	28.67±0.005	28.47±0.005
1	28.52±0.005	108.78±0.005	131.12±0.005	158.84±0.005	206.87±0.005	283.84±0.005	319.34±0.005	392.44±0.005	286.99±0.005	309.72±0.005	418.35±0.004	483.14±0.005
2	28.57±0.005	108.83±0.009	131.15±0.005	158.87±0.005	206.90±0.005	280.26±0.006	317.20±0.005	398.56±0.005	288.14±0.005	310.94±0.005	421.94±0.007	480.94±0.007
3	28.62±0.005	108.89±0.004	131.18±0.005	158.90±0.005	206.93±0.005	276.67±0.005	315.06±0.005	404.68±0.005	289.29±0.005	312.16±0.005	425.53±0.003	478.73±0.005
4	28.67±0.005	109.07±0.005	131.21±0.005	158.93±0.005	206.96±0.005	273.09±0.002	334.32±0.005	410.80±0.005	290.44±0.005	313.38±0.005	429.12±0.002	476.53±0.004
5	28.72±0.005	109.11±0.004	131.24±0.005	158.96±0.005	206.99±0.005	269.50±0.005	332.18±0.005	416.92±0.005	291.59±0.005	314.60±0.005	432.71±0.001	474.32±0.005
6	28.77±0.005	109.04±0.009	131.26±0.004	158.99±0.005	207.02±0.005	265.92±0.004	330.04±0.005	386.32±0.005	292.74±0.005	315.82±0.005	436.30±0.008	472.12±0.006
7	28.82±0.005	109.08±0.008	131.29±0.004	159.02±0.005	207.05±0.005	262.33±0.005	327.90±0.005	392.44±0.005	293.89±0.005	317.04±0.005	439.89±0.004	469.91±0.005
8	28.87±0.005	109.23±0.007	131.33±0.005	159.05±0.005	207.08±0.005	287.43±0.002	325.76±0.005	398.56±0.005	295.04±0.005	318.26±0.005	443.48±0.002	467.71±0.003
9	28.92±0.005	109.32±0.005	131.36±0.005	159.08±0.005	207.11±0.005	283.84±0.005	323.62±0.005	441.40±0.005	296.19±0.005	319.48±0.005	447.07±0.007	465.50±0.005
10	28.97±0.005	109.36±0.001	131.39±0.005	159.11±0.005	207.14±0.005	280.26±0.004	321.48±0.005	447.52±0.005	297.34±0.005	320.70±0.005	450.66±0.001	463.30±0.002
11	28.82±0.005	109.38±0.002	131.42±0.005	159.14±0.005	207.17±0.005	276.67±0.005	319.34±0.005	386.32±0.005	298.49±0.005	321.92±0.005	454.25±0.003	461.09±0.005
12	28.77±0.005	109.44±0.007	131.43±0.008	159.17±0.005	207.20±0.005	273.09±0.003	317.20±0.005	392.44±0.005	299.64±0.005	323.14±0.005	457.83±0.009	458.89±0.009
13	28.92±0.005	109.49±0.001	131.41±0.007	159.20±0.005	207.23±0.005	269.50±0.005	315.06±0.005	398.56±0.005	300.79±0.005	324.36±0.005	461.43±0.008	456.68±0.005

Lipid Hydroperoxide

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	2.70±0.002	2.50±0.001	2.49±0.004	2.62±0.009	2.65±0.003	2.44±0.004	2.81±0.001	2.39±0.002	2.31±0.002	2.98±0.001	2.32±0.003	2.70±0.002
1	3.17±0.004	17.73±0.005	18.76±0.005	27.02±0.005	29.26±0.006	11.27±0.001	8.27±0.003	22.12±0.005	28.48±0.007	26.66±0.007	19.79±0.005	27.96±0.008
2	3.04±0.006	18.31±0.003	19.27±0.001	28.77±0.001	30.08±0.005	13.56±0.001	11.10±0.002	23.34±0.005	29.09±0.004	26.78±0.005	20.23±0.001	28.43±0.007
3	2.81±0.001	18.88±0.005	19.77±0.005	30.51±0.005	30.91±0.007	15.85±0.001	13.93±0.006	24.56±0.005	29.72±0.002	26.91±0.004	20.66±0.005	28.91±0.006
4	2.98±0.001	19.46±0.002	20.28±0.004	32.26±0.007	31.73±0.005	18.14±0.006	16.76±0.004	25.78±0.005	30.32±0.004	27.03±0.005	21.10±0.003	29.42±0.005
5	2.65±0.003	20.03±0.005	20.78±0.005	34.00±0.005	32.45±0.005	20.43±0.002	19.59±0.007	27.00±0.005	30.94±0.002	27.16±0.001	21.53±0.005	29.85±0.009

6	2.32±0.007	20.61±0.008	21.29±0.002	35.75±0.009	33.36±0.009	22.72±0.009	22.42±0.003	28.22±0.005	31.54±0.007	27.28±0.005	21.97±0.002	30.33±0.003
7	2.49±0.009	21.18±0.005	21.79±0.005	37.49±0.005	34.20±0.003	25.01±0.003	25.25±0.001	29.44±0.005	32.17±0.002	27.41±0.007	22.40±0.005	30.76±0.002
8	2.39±0.002	21.76±0.006	22.30±0.004	39.24±0.006	35.02±0.008	27.30±0.001	28.08±0.008	30.66±0.005	32.78±0.003	27.53±0.005	22.84±0.006	31.28±0.004
9	2.44±0.004	22.33±0.005	22.80±0.005	40.98±0.005	35.85±0.003	29.59±0.002	30.91±0.002	31.88±0.005	33.32±0.007	27.66±0.003	23.27±0.005	31.68±0.008
10	2.31±0.002	22.91±0.003	23.31±0.001	42.73±0.007	36.67±0.003	31.88±0.002	33.74±0.004	33.10±0.005	34.01±0.003	27.78±0.005	23.71±0.001	32.23±0.008
11	2.62±0.009	23.48±0.005	23.81±0.005	44.47±0.005	37.31±0.001	34.17±0.008	36.57±0.007	34.32±0.005	34.59±0.009	27.91±0.009	24.14±0.005	32.71±0.008
12	2.70±0.003	24.06±0.002	24.32±0.002	46.22±0.009	38.31±0.009	36.46±0.002	39.40±0.002	35.54±0.005	35.25±0.006	28.03±0.005	24.58±0.004	33.18±0.007
13	2.50±0.003	24.63±0.005	24.82±0.005	47.96±0.005	39.15±0.003	38.75±0.004	42.23±0.003	36.76±0.005	35.87±0.002	28.16±0.007	25.01±0.005	33.66±0.006

OXIDIZED LIPIDS

OXPL-LDL

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	39.52±0.005	39.13±0.004	39.39±0.001	39.27±0.003	39.03±0.002	39.27±0.005	39.93±0.001	39.55±0.005	39.45±0.005	39.62±0.005	39.93±0.001	39.82±0.005
1	39.57±0.005	35.60±0.002	40.28±0.007	41.52±0.002	44.00±0.009	54.47±0.005	32.65±0.004	41.64±0.001	44.23±0.002	92.74±0.005	49.53±0.007	94.6±0.0012
2	39.62±0.005	38.97±0.004	42.17±0.003	43.77±0.008	46.97±0.002	55.02±0.005	36.41±0.005	45.91±0.004	49.21±0.005	79.05±0.003	55.66±0.008	97.72±0.002
3	39.67±0.005	42.34±0.006	44.06±0.004	46.02±0.009	49.94±0.003	55.57±0.005	40.17±0.007	50.03±0.005	54.10±0.006	65.35±0.005	62.10±0.003	100.82±0.005
4	39.72±0.005	45.71±0.003	45.95±0.006	48.27±0.001	52.91±0.007	56.12±0.005	43.92±0.006	54.08±0.008	58.98±0.005	54.32±0.004	68.37±0.006	103.93±0.003
5	39.77±0.005	49.08±0.002	47.84±0.009	50.52±0.004	55.88±0.001	56.67±0.005	47.70±0.004	58.47±0.004	63.87±0.002	51.49±0.004	74.58±0.004	107.03±0.005
6	39.82±0.005	52.45±0.007	49.73±0.002	52.77±0.007	58.85±0.004	57.22±0.005	51.46±0.005	62.65±0.005	68.66±0.006	53.18±0.008	80.65±0.005	110.14±0.006
7	39.87±0.005	45.82±0.006	51.62±0.009	55.02±0.004	41.82±0.008	57.77±0.005	55.19±0.004	66.85±0.008	73.62±0.004	57.47±0.002	87.24±0.008	113.24±0.005
8	39.92±0.005	49.19±0.001	53.51±0.001	57.27±0.007	44.79±0.006	58.32±0.005	58.99±0.005	71.02±0.005	78.52±0.005	64.59±0.007	93.53±0.002	116.35±0.001
9	39.93±0.001	52.56±0.004	55.40±0.007	59.52±0.003	47.76±0.001	58.87±0.004	62.76±0.003	75.21±0.002	83.34±0.006	72.21±0.006	99.81±0.003	119.45±0.005
10	39.22±0.005	55.93±0.002	57.28±0.002	61.77±0.001	50.73±0.009	59.42±0.005	66.52±0.005	71.29±0.006	88.22±0.001	81.79±0.005	105.77±0.002	122.56±0.003
11	39.93±0.001	59.30±0.007	59.07±0.006	60.77±0.009	53.70±0.004	59.98±0.003	70.29±0.007	83.58±0.006	93.12±0.002	91.37±0.004	112.27±0.007	125.66±0.005
12	39.12±0.005	62.67±0.006	60.96±0.006	59.77±0.003	56.67±0.003	60.52±0.004	73.97±0.002	87.76±0.005	98.072±0.003	101.35±0.002	118.73±0.002	128.77±0.003
13	39.27±0.005	66.04±0.002	62.95±0.002	58.77±0.006	59.64±0.001	61.07±0.005	77.82±0.008	91.95±0.003	92.45±0.004	112.82±0.004	124.95±0.002	131.87±0.005

MDA-LDL

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	39.07±0.005	39.11±0.003	39.25±0.001	39.19±0.005	39.07±0.005	39.11±0.005	39.47±0.005	38.17±0.005	38.50±0.005	38.93±0.005	39.64±0.005	37.95±0.003
1	39.12±0.005	34.84±0.006	33.41±0.004	41.08±0.005	31.39±0.009	40.17±0.007	82.28±0.005	41.21±0.003	43.03±0.003	42.25±0.004	44.49±0.002	226.07±0.005
2	39.17±0.005	36.57±0.008	35.57±0.008	43.99±0.005	32.86±0.001	43.92±0.006	85.27±0.006	45.24±0.006	47.64±0.004	46.56±0.006	49.54±0.005	241.90±0.002
3	39.22±0.005	38.30±0.002	37.73±0.006	46.89±0.005	34.50±0.002	47.70±0.009	88.29±0.005	49.31±0.008	52.10±0.005	50.90±0.006	54.50±0.006	257.72±0.005
4	39.27±0.005	40.03±0.001	39.89±0.002	49.79±0.005	35.95±0.003	51.46±0.005	91.27±0.007	53.24±0.006	56.57±0.007	55.03±0.003	59.45±0.005	273.55±0.007
5	39.32±0.005	41.76±0.004	42.05±0.007	52.69±0.005	37.30±0.009	55.23±0.002	94.29±0.005	57.39±0.009	61.17±0.005	59.55±0.006	64.41±0.002	289.37±0.005
6	39.37±0.005	43.49±0.002	44.21±0.009	55.59±0.005	38.76±0.006	58.99±0.005	97.29±0.005	61.43±0.005	65.70±0.005	63.87±0.005	69.36±0.005	203.74±0.004
7	39.42±0.005	45.22±0.006	46.37±0.001	58.49±0.005	40.12±0.003	62.76±0.004	100.29±0.005	65.48±0.007	70.21±0.007	68.19±0.009	74.30±0.004	220.33±0.009
8	39.47±0.005	46.95±0.008	48.53±0.006	61.39±0.005	41.79±0.005	66.52±0.005	103.29±0.005	69.53±0.002	74.78±0.009	72.52±0.005	79.27±0.005	236.93±0.003
9	39.49±0.001	48.68±0.006	50.69±0.009	64.28±0.007	43.21±0.004	70.29±0.003	106.29±0.005	73.57±0.004	79.31±0.001	76.85±0.003	84.23±0.007	253.52±0.008
10	39.57±0.005	50.41±0.009	52.85±0.007	67.19±0.007	44.67±0.007	74.08±0.007	109.29±0.004	77.61±0.007	83.81±0.006	81.176±0.004	89.18±0.005	270.12±0.002
11	39.59±0.001	52.14±0.003	55.01±0.003	42.98±0.008	46.14±0.002	77.72±0.008	112.21±0.002	81.69±0.002	88.38±0.003	85.50±0.003	94.14±0.002	286.71±0.007
12	39.67±0.005	53.87±0.004	57.17±0.004	45.89±0.006	47.48±0.007	81.58±0.005	115.25±0.002	85.71±0.003	92.91±0.005	89.82±0.005	99.10±0.002	214.64±0.004
13	39.72±0.005	55.60±0.008	59.33±0.001	48.87±0.008	49.05±0.004	85.35±0.007	118.30±0.002	89.75±0.007	97.45±0.001	94.15±0.002	93.55±0.002	232.13±0.009

CML-LDL

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	38.78±0.005	38.89±0.003	39.13±0.001	39.15±0.004	38.38±0.005	39.20±0.002	38.80±0.005	38.57±0.005	38.47±0.005	39.05±0.005	39.21±0.005	44.58±0.005
1	38.81±0.005	36.08±0.007	37.80±0.009	37.06±0.003	33.05±0.002	36.08±0.008	35.24±0.004	30.53±0.008	33.33±0.003	100.05±0.008	45.28±0.008	51.36±0.006
2	38.84±0.005	38.17±0.002	43.47±0.003	37.97±0.006	35.01±0.006	37.96±0.001	37.57±0.009	31.86±0.008	35.18±0.002	95.11±0.008	50.54±0.002	58.03±0.005
3	38.87±0.005	40.26±0.006	49.14±0.008	38.88±0.001	36.90±0.007	39.84±0.006	40.00±0.003	33.17±0.006	37.09±0.003	90.07±0.006	55.70±0.006	64.90±0.004
4	38.90±0.005	42.35±0.001	54.81±0.009	39.79±0.001	38.67±0.004	41.72±0.003	42.28±0.004	34.45±0.003	39.06±0.007	85.06±0.008	60.76±0.007	71.67±0.005
5	38.93±0.005	44.44±0.006	60.48±0.002	40.70±0.006	40.65±0.006	43.60±0.008	44.95±0.009	35.59±0.008	41.18±0.007	72.33±0.005	66.03±0.008	63.05±0.007
6	38.96±0.005	46.53±0.008	66.15±0.004	41.61±0.004	42.43±0.008	45.48±0.002	47.31±0.002	36.84±0.006	42.96±0.003	67.31±0.004	71.10±0.003	85.22±0.008
7	38.99±0.005	48.62±0.003	58.32±0.007	42.52±0.007	44.39±0.005	47.36±0.004	49.84±0.007	37.99±0.006	45.07±0.007	69.98±0.001	61.33±0.004	92.09±0.006
8	39.02±0.005	50.68±0.007	63.99±0.003	43.43±0.008	46.30±0.002	49.24±0.008	52.27±0.005	39.52±0.008	47.02±0.009	64.96±0.002	81.39±0.005	98.78±0.003
9	39.05±0.005	52.70±0.004	69.66±0.009	44.34±0.002	48.15±0.001	51.12±0.001	54.71±0.002	40.75±0.004	48.96±0.002	59.92±0.005	86.61±0.006	84.25±0.003

10	39.08±0.005	54.87±0.005	75.33±0.006	45.24±0.009	49.94±0.007	52.99±0.009	57.06±0.004	42.01±0.006	50.89±0.001	54.91±0.007	91.77±0.005	101.42±0.008
11	39.11±0.005	56.97±0.006	81.00±0.007	46.16±0.007	51.90±0.002	54.87±0.009	59.52±0.009	43.16±0.008	52.86±0.001	49.87±0.009	97.03±0.006	119.07±0.002
12	39.14±0.005	58.85±0.007	86.67±0.003	47.07±0.002	53.77±0.004	56.75±0.009	62.00±0.007	44.21±0.007	54.80±0.005	44.76±0.008	91.68±0.005	125.86±0.002
13	39.17±0.005	61.03±0.009	92.34±0.002	47.98±0.003	55.65±0.002	58.63±0.009	64.45±0.004	45.75±0.002	56.75±0.003	39.83±0.005	86.05±0.003	132.65±0.004

HNE-LDL

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	39.59±0.005	39.87±0.002	38.06±0.004	39.56±0.001	39.41±0.005	39.13±0.005	39.09±0.005	39.69±0.005	39.98±0.005	39.86±0.005	39.42±0.005	39.06±0.005
1	39.62±0.005	36.32±0.008	39.49±0.001	43.05±0.005	39.55±0.005	34.45±0.009	41.29±0.005	61.79±0.003	107.67±0.008	92.53±0.006	50.31±0.004	51.87±0.009
2	39.65±0.005	37.77±0.006	41.92±0.006	46.55±0.007	42.69±0.005	36.49±0.008	43.49±0.005	64.28±0.001	108.20±0.005	95.72±0.009	56.70±0.007	58.69±0.007
3	39.68±0.005	39.22±0.003	44.35±0.004	50.04±0.005	45.83±0.005	38.90±0.003	45.69±0.005	66.99±0.005	108.74±0.006	98.94±0.005	63.30±0.008	65.70±0.003
4	39.71±0.005	40.67±0.008	46.78±0.003	53.54±0.003	48.97±0.005	40.78±0.001	47.89±0.005	69.57±0.006	109.27±0.005	102.14±0.001	69.78±0.006	72.61±0.005
5	39.74±0.005	42.12±0.006	49.21±0.007	57.03±0.005	52.11±0.005	43.35±0.002	50.09±0.005	72.19±0.005	109.81±0.009	105.34±0.005	76.20±0.004	79.53±0.008
6	39.77±0.005	43.57±0.009	51.64±0.004	42.53±0.006	55.25±0.005	45.49±0.008	52.29±0.005	74.79±0.005	110.34±0.005	108.54±0.005	82.76±0.006	86.34±0.004
7	39.80±0.005	45.02±0.002	54.07±0.002	46.02±0.005	58.39±0.005	47.80±0.004	54.49±0.005	77.39±0.005	110.88±0.007	111.74±0.005	89.29±0.001	93.34±0.004
8	39.83±0.005	46.47±0.007	56.52±0.008	49.52±0.001	61.53±0.005	50.02±0.005	56.69±0.005	79.99±0.005	111.41±0.005	114.94±0.005	95.77±0.005	100.27±0.005
9	39.86±0.005	47.92±0.004	58.92±0.009	53.01±0.005	64.67±0.005	52.25±0.006	58.89±0.005	82.59±0.005	111.95±0.004	118.14±0.005	102.32±0.004	107.20±0.004
10	39.89±0.005	49.37±0.001	61.35±0.005	56.51±0.007	67.81±0.005	54.38±0.007	61.09±0.005	85.18±0.001	112.48±0.005	121.34±0.005	108.67±0.003	114.01±0.003
11	39.92±0.005	50.82±0.009	63.78±0.009	60.00±0.005	70.95±0.005	56.70±0.001	63.29±0.004	87.78±0.006	113.02±0.003	124.51±0.009	115.27±0.004	121.03±0.004
12	39.95±0.005	52.27±0.003	66.21±0.009	63.50±0.002	74.09±0.005	58.92±0.004	65.49±0.004	90.38±0.005	113.55±0.005	127.75±0.004	121.75±0.006	127.94±0.001
13	39.98±0.005	53.72±0.008	68.65±0.008	66.99±0.005	77.23±0.005	61.15±0.001	67.69±0.004	92.98±0.009	114.09±0.009	130.90±0.002	128.19±0.003	134.85±0.005

Isoprostane

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	45.09±0.005	45.09±0.005	45.49±0.005	45.89±0.005	45.49±0.005	45.69±0.005	45.09±0.005	43.49±0.005	45.49±0.005	45.49±0.005	45.29±0.005	45.69±0.005
1	45.29±0.005	187.18±0.001	219.64±0.005	306.13±0.005	787.48±0.008	459.35±0.005	622.56±0.009	721.87±0.005	1101.14±0.005	911.87±0.005	1139.18±0.005	1156.49±0.005
2	45.49±0.005	201.24±0.006	225.32±0.005	287.11±0.007	804.24±0.005	459.87±0.003	623.56±0.002	734.79±0.009	1124.02±0.005	914.79±0.004	1143.38±0.005	1180.45±0.005
3	45.69±0.005	215.31±0.002	231.00±0.005	268.08±0.005	821.01±0.005	460.38±0.005	624.48±0.007	747.70±0.005	1146.90±0.005	917.70±0.005	1147.58±0.005	1204.41±0.005

4	45.89±0.005	229.37±0.007	236.68±0.001	249.06±0.009	837.78±0.005	460.90±0.001	625.57±0.002	760.62±0.002	1169.78±0.005	920.62±0.009	1151.78±0.005	1228.36±0.006
5	45.49±0.005	243.44±0.003	242.36±0.005	363.21±0.003	854.53±0.005	461.41±0.005	626.54±0.003	773.53±0.005	1192.66±0.005	923.53±0.005	1155.98±0.005	1252.33±0.005
6	45.49±0.005	257.50±0.008	248.04±0.005	344.18±0.005	871.29±0.005	461.93±0.008	627.56±0.007	786.45±0.006	1215.54±0.005	926.45±0.003	1160.18±0.005	1276.29±0.005
7	45.49±0.005	271.57±0.004	253.72±0.005	325.16±0.007	888.05±0.005	462.44±0.005	628.58±0.003	799.36±0.004	1238.42±0.005	929.36±0.009	1164.38±0.005	1300.25±0.005
8	45.69±0.005	116.85±0.003	259.40±0.005	306.13±0.005	904.81±0.004	462.96±0.003	629.59±0.001	812.28±0.006	1261.30±0.005	932.27±0.009	1168.58±0.005	1324.21±0.005
9	45.89±0.005	130.91±0.009	265.08±0.005	287.11±0.002	921.57±0.005	463.47±0.005	630.59±0.006	825.21±0.002	1284.18±0.005	935.21±0.002	1172.78±0.005	1348.17±0.005
10	45.09±0.005	144.98±0.004	270.76±0.005	363.21±0.007	938.32±0.005	463.99±0.001	631.60±0.001	838.05±0.006	1307.06±0.005	938.12±0.006	1176.98±0.005	1372.12±0.008
11	45.79±0.005	159.05±0.007	276.43±0.005	344.18±0.005	955.08±0.005	464.50±0.004	632.61±0.005	851.02±0.009	1329.93±0.005	941.02±0.003	1181.18±0.005	1396.08±0.005
12	43.49±0.005	173.11±0.005	282.11±0.005	325.16±0.009	971.90±0.008	465.01±0.009	633.61±0.001	863.93±0.009	1352.81±0.001	943.93±0.008	1185.38±0.005	1420.04±0.001
13	45.69±0.005	187.18±0.001	287.79±0.005	306.135	978.67±0.003	465.543	634.58±0.007	876.85±0.004	1375.69±0.001	946.85±0.003	1189.58±0.005	1444.00±0.001

Hexanyllysine

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	9.07±0.005	9.62±0.005	9.42±0.004	9.12±0.005	9.32±0.005	9.57±0.005	9.22±0.005	9.37±0.005	9.02±0.005	9.47±0.005	9.27±0.005	9.48±0.007
1	9.02±0.005	110.12±0.003	177.38±0.005	214.16±0.005	107.63±0.001	97.22±0.005	87.07±0.005	214.70±0.005	311.09±0.005	388.28±0.005	457.47±0.005	540.66±0.005
2	9.07±0.005	110.17±0.007	177.41±0.005	214.19±0.005	107.68±0.006	97.29±0.005	87.14±0.005	214.73±0.005	313.69±0.003	390.38±0.001	460.07±0.009	544.17±0.008
3	9.12±0.005	110.22±0.002	177.44±0.005	214.22±0.005	107.72±0.001	97.36±0.005	87.21±0.005	214.76±0.005	316.28±0.005	392.47±0.005	462.66±0.005	547.84±0.007
4	9.17±0.005	110.32±0.005	177.47±0.005	214.25±0.005	107.82±0.005	97.43±0.005	87.28±0.005	214.79±0.005	318.88±0.007	394.57±0.004	465.26±0.007	551.46±0.008
5	9.22±0.005	110.33±0.002	177.50±0.005	214.26±0.006	107.87±0.005	97.50±0.005	87.35±0.005	214.82±0.005	321.47±0.005	396.66±0.005	467.85±0.005	555.03±0.005
6	9.27±0.005	110.37±0.006	177.53±0.005	214.31±0.005	107.88±0.001	97.56±0.005	87.41±0.005	214.85±0.005	324.07±0.009	398.76±0.009	470.45±0.003	558.63±0.006
7	9.32±0.005	110.42±0.003	177.56±0.005	214.34±0.005	107.92±0.001	97.64±0.005	87.49±0.005	214.88±0.005	326.66±0.005	400.85±0.005	473.04±0.005	562.22±0.006
8	9.37±0.005	110.47±0.007	177.59±0.005	214.37±0.005	108.02±0.005	97.70±0.005	87.55±0.005	214.91±0.005	329.26±0.004	402.95±0.003	475.64±0.008	565.88±0.004
9	9.41±0.005	110.57±0.005	177.62±0.005	214.38±0.006	108.07±0.005	97.78±0.005	87.63±0.005	214.94±0.005	331.85±0.005	405.04±0.005	478.23±0.005	569.44±0.002
10	9.47±0.005	110.59±0.001	177.65±0.005	214.43±0.005	108.12±0.005	97.84±0.005	87.69±0.005	214.97±0.005	334.45±0.006	407.14±0.001	480.83±0.009	573.01±0.001
11	9.59±0.005	110.62±0.004	177.68±0.005	214.46±0.005	108.12±0.002	97.91±0.005	87.76±0.005	215.00±0.005	337.04±0.005	409.23±0.005	483.42±0.005	576.59±0.007
12	9.57±0.005	110.68±0.006	177.71±0.005	214.49±0.005	108.22±0.005	97.99±0.005	87.84±0.005	215.03±0.005	339.64±0.008	411.33±0.002	486.02±0.007	580.22±0.009
13	9.62±0.005	110.72±0.001	177.74±0.005	214.50±0.006	108.27±0.005	98.06±0.005	87.91±0.005	215.06±0.005	342.23±0.005	413.42±0.005	488.63±0.002	583.74±0.007

APPENDIX XVIII

PEROXIREDOXINE SYSTEM

PRX-1

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	197.25±0.002	192.59±0.002	191.82±0.005	196.82±0.005	193.85±0.003	195.87±0.007	193.92±0.005	192.48±0.005	193.36±0.003	197.36±0.002	192.06±0.001	197.52±0.002
1	196.07±0.005	89.70±0.008	44.37±0.005	63.53±0.001	105.86±0.002	128.77±0.002	54.47±0.005	36.80±0.004	60.99±0.006	30.09±0.005	47.60±0.005	14.27±0.004
2	194.90±0.004	82.80±0.003	35.92±0.005	61.23±0.005	104.87±0.002	125.67±0.005	55.02±0.005	31.11±0.005	58.63±0.009	29.83±0.007	46.15±0.002	16.02±0.004
3	197.72±0.005	75.90±0.007	28.90±0.001	58.94±0.009	103.88±0.004	122.56±0.001	55.57±0.005	25.67±0.002	56.26±0.005	29.56±0.005	44.69±0.005	30.02±0.001
4	193.55±0.003	69.01±0.007	26.82±0.005	56.64±0.005	102.89±0.001	119.45±0.005	56.12±0.005	22.71±0.001	53.99±0.008	29.30±0.002	43.34±0.001	28.27±0.007
5	189.37±0.005	62.11±0.005	27.57±0.009	54.35±0.008	101.9±0.006	116.40±0.003	56.67±0.005	21.71±0.006	51.53±0.005	29.03±0.005	41.78±0.005	26.52±0.009
6	197.744±0.005	55.22±0.009	30.17±0.005	52.05±0.004	100.91±0.003	113.29±0.007	57.22±0.005	22.14±0.006	49.17±0.009	28.77±0.006	40.33±0.003	24.77±0.002
7	194.33±0.009	48.32±0.005	33.82±0.004	49.75±0.009	99.93±0.007	110.16±0.001	57.77±0.005	23.50±0.001	46.80±0.005	28.50±0.005	38.87±0.005	23.02±0.006
8	195.93±0.003	41.43±0.001	38.93±0.001	47.46±0.004	98.93±0.007	107.06±0.005	58.32±0.005	25.67±0.005	44.44±0.003	28.24±0.001	37.42±0.006	21.27±0.003
9	192.52±0.008	34.53±0.005	44.12±0.005	45.17±0.007	97.93±0.009	103.94±0.004	58.87±0.004	28.47±0.002	42.07±0.004	27.97±0.005	35.96±0.005	19.52±0.001
10	197.12±0.002	27.64±0.004	50.28±0.001	42.87±0.003	96.94±0.008	100.82±0.006	59.42±0.005	31.69±0.007	39.71±0.007	27.71±0.007	34.51±0.003	17.77±0.004
11	185.71±0.007	20.84±0.002	56.73±0.007	40.57±0.008	96.01±0.008	97.72±0.001	59.98±0.003	35.06±0.007	37.34±0.006	27.44±0.005	33.05±0.006	16.02±0.002
12	193.64±0.004	13.84±0.001	63.17±0.002	38.37±0.003	95.05±0.008	94.63±0.005	60.52±0.005	39.16±0.009	35.07±0.008	27.18±0.004	31.60±0.008	14.27±0.007
13	194.13±0.009	6.95±0.006	69.23±0.003	35.99±0.008	93.97±0.009	91.52±0.001	61.07±0.004	43.26±0.009	32.62±0.006	26.91±0.005	30.14±0.005	12.52±0.006

PRX-2

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	236.88±0.005	225.43±0.008	227.09±0.006	234.96±0.001	230.66±0.004	230.16±0.002	234.66±0.002	231.05±0.001	230.36±0.003	239.96±0.001	232.55±0.001	231.96±0.003
1	238.72±0.005	92.74±0.005	113.55±0.005	15.67±0.005	37.30±0.005	58.93±0.005	43.48±0.005	53.78±0.005	81.66±0.006	87.77±0.006	98.07±0.006	118.67±0.005
2	238.56±0.005	79.05±0.003	113.02±0.009	16.39±0.002	36.55±0.007	56.71±0.003	42.31±0.003	51.91±0.004	77.82±0.009	83.71±0.005	93.19±0.008	112.39±0.007
3	239.40±0.005	65.35±0.007	112.48±0.005	17.10±0.005	35.79±0.005	54.48±0.005	41.13±0.005	50.03±0.005	74.06±0.005	79.40±0.005	88.30±0.005	106.10±0.005
4	231.32±0.005	54.32±0.004	111.95±0.002	17.82±0.002	35.04±0.004	52.35±0.008	40.05±0.006	48.25±0.008	70.37±0.001	75.30±0.009	83.51±0.008	99.82±0.003
5	236.08±0.004	51.49±0.004	111.41±0.005	18.53±0.005	34.28±0.005	50.03±0.005	38.78±0.005	46.28±0.005	66.53±0.005	71.03±0.005	78.53±0.005	93.53±0.005

6	238.01±0.003	53.18±0.008	110.88±0.007	19.25±0.004	33.53±0.004	47.81±0.009	37.61±0.007	44.41±0.003	62.77±0.002	66.85±0.007	73.65±0.003	87.25±0.002
7	220.33±0.009	57.56±0.002	110.34±0.005	19.96±0.005	32.77±0.005	45.58±0.005	36.43±0.005	42.53±0.005	59.00±0.005	62.66±0.005	68.77±0.002	81.11±0.004
8	236.93±0.003	64.59±0.007	109.81±0.004	20.68±0.004	32.02±0.007	43.36±0.006	35.26±0.008	40.66±0.003	55.33±0.008	58.53±0.003	63.97±0.003	74.77±0.008
9	238.52±0.008	72.22±0.005	109.27±0.005	21.39±0.005	31.26±0.005	41.13±0.006	34.08±0.005	38.78±0.002	51.47±0.004	54.34±0.008	58.99±0.004	68.52±0.006
10	234.12±0.002	81.79±0.005	108.74±0.009	22.11±0.003	30.51±0.002	38.91±0.003	32.91±0.009	36.91±0.001	47.71±0.007	50.11±0.009	54.11±0.002	62.11±0.004
11	235.71±0.007	91.37±0.004	108.20±0.005	22.82±0.005	29.75±0.005	36.68±0.006	31.73±0.006	35.03±0.006	43.94±0.006	45.94±0.008	49.27±0.007	55.89±0.007
12	214.64±0.004	101.36±0.001	107.67±0.003	23.54±0.002	29.01±0.004	34.53±0.001	30.56±0.009	33.25±0.008	40.40±0.004	41.82±0.008	44.34±0.007	49.54±0.007
13	232.13±0.009	112.82±0.004	107.13±0.005	24.25±0.005	28.24±0.005	32.24±0.006	29.38±0.005	31.28±0.005	36.42±0.006	37.57±0.006	39.45±0.006	43.25±0.006

PRX-3

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	192.00±0.005	197.98±0.006	190.25±0.003	189.20±0.005	192.88±0.005	191.40±0.005	190.08±0.004	199.84±0.005	191.52±0.005	197.85±0.008	191.68±0.005	190.25±0.003
1	199.84±0.005	21.22±0.005	36.08±0.003	16.70±0.005	33.18±0.005	62.02±0.006	44.51±0.005	54.81±0.005	85.71±0.005	92.92±0.005	100.13±0.005	121.76±0.005
2	191.68±0.005	21.35±0.005	37.96±0.007	17.35±0.003	32.71±0.006	59.59±0.001	43.27±0.003	52.87±0.004	81.78±0.005	88.52±0.001	95.11±0.008	115.27±0.003
3	191.52±0.005	21.48±0.005	39.84±0.009	17.99±0.005	32.23±0.005	57.15±0.005	42.02±0.005	50.92±0.005	77.62±0.005	83.85±0.005	90.16±0.008	108.77±0.005
4	197.85±0.008	21.61±0.005	41.72±0.003	18.64±0.006	31.76±0.008	54.75±0.002	40.81±0.008	49.00±0.009	73.58±0.001	79.40±0.009	85.15±0.005	102.28±0.006
5	189.20±0.005	21.74±0.005	43.60±0.003	19.28±0.005	31.28±0.005	52.28±0.005	39.53±0.005	47.03±0.005	69.53±0.005	74.78±0.005	80.03±0.005	95.78±0.005
6	192.04±0.005	21.87±0.005	45.48±0.008	19.93±0.009	30.81±0.001	49.85±0.007	38.29±0.009	45.09±0.007	65.49±0.006	70.25±0.004	75.01±0.003	89.29±0.008
7	192.88±0.005	22.00±0.005	47.36±0.006	20.57±0.005	30.33±0.005	47.41±0.005	37.04±0.005	43.14±0.005	61.44±0.005	65.71±0.005	69.99±0.006	82.90±0.009
8	197.72±0.005	22.13±0.005	49.24±0.001	21.22±0.001	29.86±0.002	44.98±0.002	35.80±0.001	41.20±0.001	57.45±0.003	61.25±0.001	65.03±0.001	76.33±0.005
9	191.56±0.005	22.26±0.005	51.12±0.001	21.86±0.005	29.38±0.005	42.54±0.004	34.55±0.005	39.25±0.001	53.40±0.008	56.64±0.004	59.97±0.009	69.93±0.001
10	191.40±0.005	22.39±0.005	52.99±0.009	22.51±0.001	28.91±0.009	40.11±0.001	33.31±0.003	37.31±0.003	49.31±0.009	52.11±0.008	54.91±0.003	63.31±0.003
11	190.25±0.003	22.52±0.005	54.89±0.008	23.15±0.005	28.43±0.005	37.67±0.006	32.06±0.006	35.36±0.006	45.27±0.006	47.78±0.001	49.96±0.005	56.81±0.007
12	190.08±0.004	22.65±0.005	56.75±0.009	23.80±0.003	27.96±0.008	35.31±0.001	30.82±0.008	33.51±0.008	41.39±0.009	43.04±0.007	44.94±0.007	50.32±0.007
13	197.98±0.006	22.78±0.005	58.63±0.009	24.44±0.005	27.48±0.005	32.81±0.006	29.57±0.005	31.47±0.005	37.19±0.006	38.51±0.006	39.83±0.006	43.82±0.005

PRX-4

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	242.20±0.005	245.48±0.005	248.74±0.006	239.68±0.005	226.07±0.005	249.76±0.006	247.72±0.005	244.68±0.006	242.12±0.005	234.61±0.005	232.84±0.005	242.20±0.005
1	232.84±0.005	39.40±0.007	22.12±0.005	17.73±0.005	28.03±0.005	57.90±0.005	45.54±0.005	56.87±0.005	83.65±0.005	90.86±0.005	102.19±0.005	125.88±0.005
2	245.48±0.005	36.57±0.003	23.34±0.005	18.31±0.004	27.91±0.003	55.75±0.004	44.23±0.003	54.79±0.004	79.74±0.002	86.46±0.003	97.02±0.002	119.11±0.003
3	242.12±0.005	33.74±0.004	24.56±0.005	18.88±0.005	27.78±0.005	53.59±0.005	42.91±0.005	52.70±0.005	75.84±0.005	82.07±0.005	91.86±0.005	112.33±0.005
4	248.74±0.006	30.91±0.002	25.78±0.005	19.46±0.009	27.66±0.004	51.43±0.007	41.59±0.005	50.61±0.002	71.93±0.009	77.67±0.004	86.69±0.002	105.56±0.009
5	234.61±0.005	28.08±0.001	27.00±0.005	20.03±0.005	27.53±0.005	49.28±0.005	40.28±0.005	48.53±0.005	68.03±0.005	73.28±0.005	81.53±0.005	98.78±0.005
6	239.68±0.005	25.25±0.001	28.22±0.005	20.61±0.002	27.41±0.007	47.13±0.002	38.97±0.002	46.45±0.008	64.13±0.006	68.89±0.008	76.37±0.006	92.01±0.004
7	244.68±0.006	22.42±0.003	29.44±0.005	21.18±0.005	27.28±0.005	44.97±0.005	37.65±0.005	44.36±0.005	60.22±0.005	64.49±0.005	71.19±0.009	85.22±0.005
8	249.76±0.006	19.59±0.006	30.66±0.005	21.76±0.002	27.16±0.008	42.82±0.007	36.34±0.008	42.28±0.003	56.31±0.008	60.09±0.001	66.03±0.008	78.45±0.002
9	235.39±0.005	16.76±0.008	31.88±0.005	22.33±0.005	27.03±0.005	40.65±0.008	35.02±0.005	40.18±0.009	52.40±0.005	55.69±0.005	60.86±0.005	71.67±0.005
10	226.07±0.005	13.93±0.002	33.10±0.005	22.91±0.002	26.91±0.002	38.51±0.002	33.71±0.004	38.11±0.001	48.51±0.004	51.31±0.009	55.71±0.002	64.91±0.004
11	241.90±0.004	11.10±0.002	34.32±0.005	23.48±0.005	26.78±0.005	36.35±0.005	32.39±0.005	36.02±0.005	44.60±0.005	46.91±0.005	50.54±0.005	58.13±0.005
12	247.72±0.005	8.27±0.004	35.54±0.005	24.06±0.003	26.66±0.004	34.19±0.001	31.07±0.003	33.93±0.007	40.69±0.007	42.51±0.003	45.37±0.009	51.35±0.007
13	243.55±0.003	5.43±0.009	36.76±0.005	24.63±0.005	26.53±0.005	32.04±0.006	29.76±0.005	31.85±0.005	36.80±0.006	38.14±0.006	40.28±0.001	44.59±0.006

PRX-5

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	220.33±0.009	220.33±0.009	223.52±0.008	226.71±0.007	228.13±0.009	225.84±0.005	222.12±0.005	222.27±0.005	221.93±0.003	230.12±0.002	222.64±0.004	229.20±0.005
1	221.93±0.003	36.46±0.001	27.02±0.005	18.76±0.005	35.24±0.005	59.96±0.005	41.42±0.005	56.87±0.005	84.68±0.005	93.95±0.005	104.25±0.005	127.94±0.005
2	223.52±0.008	34.17±0.001	28.77±0.002	19.27±0.003	34.63±0.002	57.67±0.009	40.39±0.004	54.79±0.003	80.70±0.006	89.34±0.008	98.94±0.004	121.03±0.003
3	230.12±0.002	31.88±0.006	30.51±0.005	19.77±0.005	34.01±0.005	55.37±0.005	39.35±0.005	52.70±0.005	76.73±0.005	84.74±0.005	93.64±0.005	114.11±0.005
4	226.71±0.007	29.59±0.009	32.26±0.006	20.28±0.009	33.40±0.007	53.07±0.002	38.31±0.009	50.61±0.007	72.75±0.002	80.13±0.001	88.33±0.003	107.20±0.007
5	222.64±0.004	27.30±0.004	34.00±0.005	20.78±0.005	32.78±0.005	50.78±0.005	37.28±0.005	48.53±0.005	68.78±0.005	75.53±0.005	83.03±0.005	100.28±0.005
6	228.13±0.009	25.01±0.008	35.75±0.008	21.29±0.003	32.17±0.001	48.49±0.001	36.25±0.003	46.45±0.004	64.81±0.003	70.93±0.009	77.73±0.009	93.37±0.009
7	229.20±0.005	22.72±0.003	37.49±0.005	21.79±0.005	31.55±0.005	46.19±0.005	35.21±0.005	44.36±0.005	60.83±0.005	66.32±0.005	72.41±0.007	86.44±0.005
8	225.84±0.005	20.43±0.008	39.24±0.001	22.30±0.006	30.94±0.003	43.90±0.007	34.18±0.007	42.28±0.009	56.85±0.004	61.71±0.007	67.11±0.002	79.53±0.006
9	225.48±0.005	18.14±0.002	40.98±0.005	22.80±0.005	30.32±0.005	41.59±0.006	33.14±0.005	40.18±0.009	52.87±0.005	57.10±0.005	61.80±0.005	72.61±0.005

10	222.12±0.005	15.85±0.001	42.73±0.003	23.31±0.002	29.71±0.004	39.31±0.004	32.11±0.002	38.11±0.002	48.91±0.001	52.51±0.003	56.51±0.0088	65.71±0.007
11	220.74±0.006	13.56±0.009	44.47±0.005	23.81±0.005	29.09±0.005	37.01±0.005	31.07±0.005	36.02±0.005	44.93±0.005	47.90±0.005	51.20±0.005	58.79±0.005
12	222.27±0.005	11.27±0.009	46.22±0.008	24.32±0.009	28.47±0.008	34.71±0.002	30.03±0.003	33.93±0.003	40.95±0.009	43.29±0.007	45.89±0.002	51.87±0.003
13	224.75±0.005	8.98±0.002	47.96±0.005	24.82±0.005	27.86±0.005	32.42±0.005	29.00±0.005	31.85±0.005	36.98±0.005	38.69±0.005	40.59±0.005	44.96±0.005

PRX-6

Weeks	***CONT.***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***	***0.5***	***0.6***	***0.7***	***0.8***	***0.9***	***1***
0	250.10±0.005	250.10±0.005	251.58±0.005	254.04±0.006	253.54±0.005	257.02±0.005	252.68±0.005	251.76±0.006	250.34±0.005	254.82±0.005	256.30±0.005	285.78±0.005
1	250.34±0.005	39.40±0.004	31.25±0.002	19.79±0.005	38.33±0.005	63.05±0.005	42.45±0.005	28.71±0.002	55.84±0.005	82.62±0.005	89.83±0.005	99.10±0.005
2	251.58±0.005	36.57±0.008	33.41±0.007	20.23±0.001	37.51±0.003	60.54±0.008	41.35±0.002	26.78±0.006	53.83±0.004	78.78±0.009	85.50±0.002	94.14±0.003
3	254.82±0.005	33.74±0.004	35.57±0.004	20.66±0.005	36.68±0.005	58.04±0.005	40.24±0.005	24.85±0.003	51.81±0.005	74.95±0.005	81.18±0.005	89.19±0.005
4	254.04±0.006	30.91±0.007	37.73±0.006	21.10±0.006	35.86±0.007	55.53±0.003	39.13±0.008	22.92±0.007	49.79±0.009	71.11±0.002	76.85±0.004	84.23±0.009
5	256.30±0.005	28.08±0.001	39.89±0.008	21.53±0.005	35.03±0.005	53.03±0.005	38.03±0.005	20.99±0.009	47.78±0.005	67.28±0.005	72.53±0.005	79.28±0.005
6	253.54±0.005	25.25±0.009	42.05±0.001	21.97±0.009	34.21±0.009	50.53±0.006	36.93±0.007	19.06±0.001	45.77±0.006	63.45±0.004	68.21±0.001	74.33±0.004
7	285.78±0.005	22.42±0.007	44.21±0.004	22.40±0.005	33.38±0.005	48.02±0.005	35.82±0.005	17.13±0.003	43.75±0.005	59.61±0.005	63.88±0.005	69.37±0.002
8	257.02±0.005	19.59±0.004	46.37±0.006	22.84±0.004	32.56±0.006	45.52±0.002	34.72±0.009	15.20±0.007	41.74±0.002	55.77±0.003	59.55±0.001	64.41±0.007
9	252.61±0.005	16.76±0.006	48.53±0.002	23.27±0.005	31.73±0.005	43.00±0.005	33.61±0.005	13.27±0.009	39.72±0.007	51.93±0.005	55.22±0.005	59.45±0.005
10	252.68±0.005	13.93±0.003	50.69±0.007	23.71±0.007	30.91±0.003	40.51±0.001	32.51±0.002	11.34±0.001	37.71±0.001	48.11±0.007	50.91±0.003	54.51±0.001
11	250.68±0.006	11.10±0.001	52.85±0.009	24.14±0.005	30.08±0.005	38.00±0.005	31.40±0.005	22.92±0.001	35.69±0.005	44.27±0.005	46.58±0.005	49.55±0.005
12	251.76±0.006	8.27±0.002	55.01±0.003	24.58±0.002	29.25±0.005	35.49±0.003	30.29±0.002	24.85±0.004	33.67±0.003	40.43±0.009	42.25±0.002	44.59±0.009
13	253.23±0.005	5.43±0.009	57.17±0.001	25.01±0.005	28.43±0.005	32.99±0.005	29.19±0.005	26.78±0.002	31.66±0.005	36.60±0.005	37.93±0.005	39.64±0.005

APPENDIX XIX

GLUTATHIONE SYSTEM

Glutathione Reductase

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	21.51±0.006	21.90±0.005	21.16±0.001	21.81±0.003	21.50±0.003	23.77±0.002	23.37±0.001	22.66±0.002	24.46±0.002	21.09±0.005	21.16±0.002	21.46±0.003
1	21.79±0.004	22.12±0.005	17.73±0.005	48.34±0.007	30.03±0.001	34.28±0.005	34.37±0.007	48.17±0.005	37.67±0.005	21.22±0.005	38.33±0.005	27.00±0.005
2	21.95±0.003	23.34±0.005	18.31±0.006	43.29±0.005	27.56±0.009	28.80±0.004	32.37±0.002	44.91±0.003	35.23±0.004	21.35±0.005	37.51±0.008	26.95±0.001
3	21.40±0.006	24.56±0.005	18.88±0.005	38.25±0.002	25.09±0.007	23.31±0.005	30.37±0.006	41.63±0.005	32.80±0.005	21.48±0.005	36.68±0.005	26.89±0.005
4	22.65±0.008	25.78±0.005	19.46±0.001	33.20±0.005	22.60±0.001	17.83±0.006	28.37±0.009	38.35±0.007	30.36±0.008	21.61±0.005	35.86±0.003	26.84±0.009
5	22.28±0.001	27.00±0.005	20.03±0.005	28.16±0.009	20.15±0.009	12.34±0.005	26.37±0.004	35.08±0.005	27.97±0.004	21.74±0.005	35.03±0.005	26.78±0.005
6	21.25±0.009	28.22±0.005	20.61±0.009	23.11±0.005	17.68±0.002	6.85±0.009	24.37±0.008	31.81±0.004	25.49±0.009	21.87±0.005	34.21±0.003	26.73±0.007
7	22.42±0.003	29.44±0.005	21.18±0.005	29.20±0.001	15.21±0.003	27.69±0.005	22.37±0.002	28.53±0.008	23.05±0.005	22.00±0.005	33.38±0.005	26.67±0.005
8	21.32±0.002	30.66±0.005	21.76±0.002	24.89±0.004	12.74±0.004	25.84±0.005	20.37±0.009	25.21±0.008	20.53±0.007	22.13±0.005	32.56±0.006	26.62±0.001
9	21.91±0.006	31.88±0.005	22.33±0.005	20.79±0.009	10.27±0.008	23.99±0.005	18.37±0.004	22.00±0.007	18.09±0.001	22.26±0.005	31.73±0.005	26.56±0.005
10	21.99±0.004	33.10±0.005	22.96±0.006	16.53±0.001	7.80±0.001	22.14±0.005	16.37±0.002	17.10±0.007	15.77±0.007	22.39±0.005	30.91±0.002	26.51±0.003
11	21.70±0.008	34.32±0.005	23.48±0.005	12.43±0.005	19.68±0.008	20.29±0.005	14.37±0.007	15.43±0.005	13.31±0.002	22.52±0.005	30.08±0.005	26.45±0.005
12	21.79±0.004	35.54±0.005	24.06±0.003	8.25±0.003	13.96±0.005	18.44±0.005	12.36±0.009	12.16±0.001	10.88±0.008	22.65±0.005	29.25±0.005	26.40±0.003
13	21.81±0.001	36.76±0.005	24.63±0.005	7.67±0.004	7.67±0.005	39.83±0.005	10.69±0.001	35.08±0.005	8.44±0.003	22.78±0.005	28.43±0.005	26.34±0.005

Glutathione Peroxidase

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	130.88±0.003	130.87±0.001	130.05±0.001	130.93±0.003	129.14±0.002	131.35±0.001	129.56±0.002	130.77±0.008	130.12±0.005	131.96±0.004	130.24±0.005	131.03±0.001
1	130.77±0.007	89.69±0.001	63.53±0.009	44.37±0.005	47.60±0.005	36.80±0.004	30.09±0.005	21.22±0.005	14.27±0.001	16.70±0.005	19.36±0.003	15.20±0.002
2	129.86±0.005	82.80±0.007	61.23±0.005	35.92±0.005	46.15±0.008	31.11±0.005	29.83±0.007	21.35±0.005	16.02±0.008	17.35±0.002	18.92±0.005	13.27±0.004
3	129.56±0.009	75.90±0.005	58.94±0.003	28.90±0.001	44.69±0.005	25.67±0.002	29.56±0.005	21.48±0.005	30.02±0.003	17.99±0.005	18.49±0.007	11.34±0.009
4	129.45±0.005	69.01±0.002	56.64±0.005	26.82±0.005	43.23±0.003	22.71±0.001	29.30±0.003	21.61±0.005	28.27±0.009	18.64±0.007	18.05±0.005	9.41±0.001
5	131.35±0.007	62.11±0.005	54.35±0.006	27.57±0.009	41.78±0.005	21.71±0.002	29.03±0.005	21.74±0.005	26.52±0.001	19.28±0.005	17.62±0.002	7.48±0.007

6	130.24±0.005	55.22±0.003	52.05±0.004	30.17±0.005	40.33±0.002	22.14±0.006	28.77±0.006	21.87±0.005	24.77±0.007	19.93±0.009	17.18±0.005	6.08±0.001
7	129.14±0.002	48.32±0.005	49.75±0.009	33.82±0.004	38.87±0.005	23.50±0.006	28.50±0.005	22.00±0.005	23.02±0.006	20.57±0.005	16.75±0.008	5.43±0.008
8	131.03±0.005	41.43±0.009	47.46±0.003	38.93±0.001	37.42±0.009	25.67±0.005	28.24±0.002	22.13±0.005	21.27±0.004	21.22±0.003	18.26±0.001	5.32±0.009
9	130.93±0.008	34.53±0.005	45.16±0.008	44.12±0.005	35.96±0.005	28.47±0.002	27.97±0.005	22.26±0.005	19.52±0.009	21.86±0.005	18.76±0.005	5.65±0.002
10	130.12±0.005	27.64±0.003	42.87±0.003	50.28±0.001	34.51±0.002	31.69±0.007	27.71±0.009	22.39±0.005	17.77±0.002	22.51±0.007	19.27±0.006	6.28±0.009
11	130.05±0.002	20.76±0.009	40.57±0.008	56.72±0.007	33.05±0.005	35.06±0.007	27.44±0.005	22.52±0.005	16.02±0.001	23.15±0.005	19.77±0.005	7.14±0.002
12	131.96±0.003	13.83±0.005	38.28±0.003	63.17±0.002	31.59±0.006	39.16±0.009	27.18±0.002	22.65±0.005	14.00±0.007	23.80±0.001	20.28±0.003	8.29±0.001
13	130.87±0.001	6.94±0.001	35.98±0.008	52.82±0.005	30.13±0.003	25.67±0.002	26.91±0.005	22.78±0.005	12.52±0.003	24.44±0.005	20.78±0.005	9.54±0.004

Total glutathione

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	9.77±0.005	9.76±0.005	9.80±0.005	9.54±0.005	9.98±0.005	9.86±0.005	9.92±0.005	9.80±0.005	9.57±0.002	9.94±0.008	9.61±0.009	9.83±0.004
1	9.80±0.005	23.04±0.003	31.53±0.009	38.02±0.009	28.01±0.005	30.55±0.001	15.67±0.005	28.99±0.006	50.91±0.005	67.62±0.005	89.52±0.005	99.32±0.005
2	9.83±0.005	24.58±0.009	33.73±0.003	40.71±0.008	28.04±0.005	31.72±0.006	16.39±0.007	29.75±0.001	53.01±0.008	67.67±0.005	89.59±0.005	99.39±0.005
3	9.86±0.005	26.13±0.006	35.92±0.008	43.40±0.008	28.07±0.005	32.90±0.001	17.10±0.005	30.50±0.006	54.79±0.009	67.72±0.005	89.66±0.005	99.46±0.005
4	9.89±0.005	27.68±0.002	38.12±0.002	46.09±0.007	28.10±0.005	34.07±0.003	17.82±0.009	31.26±0.001	56.75±0.001	67.77±0.005	89.73±0.005	99.53±0.005
5	9.92±0.005	29.22±0.009	40.31±0.007	48.78±0.007	28.13±0.005	35.25±0.001	18.53±0.005	32.01±0.006	58.67±0.002	67.82±0.005	89.80±0.005	99.60±0.005
6	9.94±0.008	30.77±0.005	42.51±0.001	51.47±0.006	28.16±0.005	36.28±0.006	19.25±0.004	32.68±0.005	60.64±0.001	67.87±0.005	89.86±0.005	99.66±0.005
7	9.98±0.005	32.32±0.002	44.70±0.006	54.16±0.006	28.19±0.005	37.46±0.006	19.96±0.005	33.54±0.004	62.54±0.004	67.92±0.005	89.94±0.005	99.74±0.005
8	9.61±0.009	33.86±0.008	46.90±0.005	56.85±0.005	28.22±0.005	38.69±0.009	20.68±0.006	34.29±0.004	64.50±0.002	67.97±0.005	90.00±0.005	99.80±0.007
9	9.54±0.005	35.41±0.005	49.09±0.005	59.54±0.005	28.25±0.005	40.01±0.003	21.39±0.005	35.06±0.004	66.46±0.001	68.01±0.005	90.08±0.005	99.88±0.005
10	9.57±0.002	36.96±0.001	51.28±0.009	62.23±0.004	28.28±0.005	41.11±0.007	22.11±0.002	35.78±0.002	68.41±0.003	68.07±0.005	90.14±0.005	99.94±0.009
11	9.80±0.005	38.50±0.008	53.48±0.004	64.92±0.004	28.31±0.005	42.30±0.001	22.82±0.005	36.56±0.004	70.35±0.008	68.11±0.005	90.21±0.005	100.02±0.003
12	9.83±0.004	40.05±0.004	55.67±0.008	67.61±0.003	28.34±0.005	43.48±0.004	23.54±0.003	37.31±0.007	72.35±0.001	68.17±0.005	90.29±0.005	100.09±0.005
13	9.76±0.005	41.60±0.001	57.87±0.003	70.30±0.003	28.37±0.005	44.65±0.001	24.25±0.005	38.08±0.006	74.19±0.009	68.22±0.005	90.36±0.005	100.16±0.005

Oxidized glutathione

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	11.76±0.005	12.75±0.003	11.76±0.005	11.70±0.005	11.36±0.006	11.08±0.005	11.52±0.005	11.56±0.005	11.80±0.005	11.67±0.006	11.63±0.005	11.83±0.005
1	11.63±0.005	13.50±0.006	18.76±0.005	14.96±0.005	18.72±0.005	20.06±0.005	23.02±0.005	26.73±0.005	30.90±0.005	35.13±0.005	40.55±0.005	31.25±0.009
2	11.70±0.005	14.25±0.001	19.27±0.008	14.99±0.005	18.77±0.005	20.09±0.005	24.25±0.004	26.80±0.005	30.91±0.005	35.20±0.005	40.56±0.005	33.41±0.002
3	11.67±0.006	15.00±0.002	19.77±0.005	15.02±0.005	18.82±0.005	20.12±0.005	25.47±0.005	26.87±0.005	30.92±0.005	35.27±0.005	40.57±0.005	35.57±0.007
4	11.36±0.006	15.75±0.009	20.28±0.003	15.05±0.005	18.87±0.005	20.15±0.005	26.70±0.008	26.94±0.005	30.92±0.005	35.34±0.005	40.57±0.005	37.73±0.004
5	11.83±0.005	16.50±0.002	20.78±0.005	15.08±0.005	18.92±0.005	20.18±0.005	27.92±0.005	27.01±0.005	30.94±0.005	35.41±0.005	40.59±0.005	39.89±0.006
6	11.08±0.005	17.25±0.004	21.29±0.004	15.11±0.005	18.97±0.005	20.21±0.005	29.15±0.001	27.08±0.005	30.95±0.005	35.48±0.005	40.60±0.005	42.05±0.008
7	11.55±0.005	18.00±0.001	21.79±0.005	15.14±0.005	19.02±0.005	20.24±0.005	30.37±0.005	27.15±0.005	30.95±0.005	35.55±0.005	40.60±0.005	44.21±0.009
8	11.52±0.005	18.89±0.003	22.30±0.006	15.17±0.005	19.07±0.005	20.27±0.005	31.60±0.004	27.22±0.005	30.97±0.005	35.62±0.005	40.62±0.005	46.37±0.002
9	11.69±0.005	26.28±0.004	22.80±0.005	15.20±0.005	19.12±0.005	20.30±0.005	32.82±0.005	27.29±0.005	30.98±0.005	35.69±0.005	40.63±0.005	48.53±0.006
10	11.56±0.005	20.25±0.004	23.34±0.001	15.23±0.005	19.17±0.005	20.33±0.005	34.05±0.008	27.36±0.005	30.98±0.005	35.76±0.005	40.63±0.005	50.69±0.001
11	11.63±0.005	21.00±0.004	23.81±0.005	15.26±0.005	19.22±0.005	20.36±0.005	35.27±0.005	27.43±0.005	31.00±0.005	35.83±0.005	40.65±0.005	52.85±0.003
12	11.80±0.005	21.75±0.009	24.32±0.002	15.29±0.005	19.27±0.005	20.39±0.005	36.50±0.001	27.50±0.005	31.01±0.005	35.90±0.005	40.66±0.005	55.01±0.003
13	11.97±0.005	22.50±0.001	24.82±0.005	15.32±0.005	19.32±0.005	20.42±0.005	37.72±0.005	27.57±0.005	31.02±0.005	35.97±0.005	40.67±0.004	57.17±0.007

Reduced Glutathione

Weeks	CONT.	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0	69.76±0.005	72.98±0.005	74.96±0.003	70.16±0.001	71.38±0.001	69.22±0.003	73.60±0.002	74.76±0.003	68.23±0.001	73.72±0.002	70.01±0.006	68.00±0.001
1	71.63±0.005	9.54±0.003	12.77±0.004	23.06±0.004	9.29±0.002	10.48±0.006	7.35±0.006	2.26±0.001	20.01±0.004	32.49±0.008	48.97±0.004	68.07±0.005
2	70.70±0.005	10.33±0.009	14.46±0.003	25.72±0.003	9.27±0.007	11.63±0.001	7.86±0.009	2.94±0.006	22.10±0.003	32.47±0.004	49.03±0.009	65.98±0.005
3	73.67±0.006	11.13±0.006	16.15±0.003	28.38±0.003	9.25±0.003	12.77±0.006	8.37±0.007	3.63±0.001	23.87±0.004	32.45±0.006	49.09±0.001	63.89±0.005
4	68.36±0.006	11.93±0.002	17.84±0.002	31.04±0.002	9.23±0.006	13.91±0.008	8.88±0.002	4.31±0.006	25.82±0.006	32.43±0.003	49.16±0.003	61.80±0.005
5	70.83±0.005	12.72±0.009	19.53±0.002	33.70±0.002	9.21±0.009	15.06±0.006	9.39±0.008	5.00±0.001	27.72±0.007	32.41±0.007	49.21±0.007	59.71±0.005
6	69.08±0.005	13.52±0.005	21.22±0.001	36.36±0.001	9.19±0.001	16.07±0.001	9.90±0.001	5.69±0.003	29.68±0.006	32.39±0.001	49.26±0.008	57.61±0.005
7	71.55±0.005	14.32±0.002	22.91±0.001	39.02±0.001	9.17±0.007	17.22±0.001	10.41±0.009	6.38±0.009	31.58±0.009	32.37±0.009	49.34±0.001	55.53±0.005
8	71.52±0.005	14.97±0.005	24.60±0.005	41.68±0.005	9.15±0.004	18.42±0.004	10.92±0.006	7.06±0.009	33.52±0.007	32.35±0.002	49.38±0.004	53.43±0.007
9	70.69±0.005	9.13±0.001	26.29±0.003	44.34±0.001	9.13±0.009	19.70±0.008	11.43±0.004	7.76±0.005	35.47±0.006	32.32±0.008	49.45±0.006	51.35±0.005

10	73.56±0.005	16.71±0.001	27.94±0.008	46.99±0.009	9.11±0.003	20.78±0.002	11.94±0.007	8.41±0.007	37.42±0.008	32.31±0.003	49.51±0.003	49.25±0.009
11	68.63±0.005	17.50±0.008	29.66±0.009	49.65±0.009	9.09±0.008	21.93±0.006	12.45±0.003	9.12±0.009	39.35±0.003	32.28±0.006	49.56±0.007	47.17±0.008
12	72.80±0.005	18.30±0.004	31.35±0.008	52.31±0.008	9.07±0.002	23.08±0.009	12.96±0.004	9.80±0.005	41.33±0.006	32.27±0.002	49.63±0.009	45.08±0.005
13	69.97±0.005	19.10±0.001	33.04±0.008	54.97±0.008	9.05±0.006	24.22±0.006	13.47±0.009	10.51±0.001	43.17±0.004	32.25±0.009	49.69±0.001	42.99±0.005

APPENDIX XX

THIOREDUXINE SYSTEM

Thioreduxine

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	14.96±0.001	14.52±0.001	14.39±0.006	14.81±0.005	14.75±0.008	15.57±0.007	15.46±0.009	15.85±0.009	14.32±0.005	14.33±0.009	14.11±0.009	13.19±0.005
1	14.77±0.005	14.27±0.003	23.04±0.003	21.98±0.001	25.16±0.007	24.10±0.005	28.39±0.001	28.65±0.003	21.45±0.007	29.25±0.005	29.67±0.009	36.59±0.001
2	14.69±0.006	16.02±0.007	24.58±0.009	23.44±0.006	26.87±0.005	25.73±0.002	28.90±0.006	29.23±0.005	22.87±0.005	30.08±0.001	30.72±0.009	41.08±0.005
3	14.80±0.005	17.77±0.008	26.13±0.006	24.91±0.002	28.58±0.004	27.36±0.002	29.70±0.008	30.10±0.007	24.30±0.002	30.90±0.005	31.70±0.003	35.57±0.005
4	14.62±0.009	19.52±0.004	27.68±0.002	26.37±0.007	30.29±0.002	28.98±0.007	30.22±0.006	30.69±0.005	25.72±0.005	31.63±0.003	32.57±0.001	39.17±0.001
5	14.33±0.005	21.27±0.006	29.22±0.009	27.84±0.003	32.00±0.001	30.61±0.005	30.93±0.008	31.47±0.007	27.15±0.004	32.45±0.007	33.53±0.005	42.76±0.005
6	14.75±0.008	23.02±0.001	30.77±0.005	29.30±0.008	33.70±0.009	32.24±0.002	31.54±0.008	32.15±0.007	28.57±0.005	33.38±0.006	34.59±0.008	46.36±0.004
7	14.96±0.005	24.77±0.001	32.32±0.002	30.77±0.004	35.41±0.008	33.87±0.006	32.08±0.001	32.76±0.004	30.00±0.002	34.11±0.008	35.47±0.006	49.95±0.005
8	14.08±0.002	26.52±0.004	33.86±0.008	32.23±0.009	37.12±0.006	35.49±0.007	32.78±0.003	33.49±0.005	31.42±0.005	35.01±0.005	36.54±0.002	35.47±0.005
9	14.09±0.005	28.27±0.002	35.41±0.005	33.70±0.005	38.83±0.005	37.12±0.005	33.29±0.001	34.12±0.008	32.85±0.006	35.848±0.008	37.48±0.006	38.35±0.003
10	14.91±0.001	30.02±0.007	36.96±0.001	35.17±0.005	40.54±0.003	38.75±0.002	34.013±0.009	34.92±0.009	34.27±0.005	36.66±0.008	38.47±0.003	41.22±0.005
11	14.12±0.006	31.77±0.003	38.50±0.008	36.63±0.006	42.25±0.002	40.38±0.001	34.43±0.005	35.39±0.004	35.70±0.001	37.50±0.005	39.42±0.004	44.10±0.007
12	14.14±0.006	33.52±0.008	40.05±0.004	38.10±0.001	43.96±0.005	42.00±0.007	35.15±0.006	36.18±0.005	37.12±0.005	38.15±0.007	40.18±0.009	46.97±0.005
13	14.85±0.005	35.27±0.004	41.60±0.001	39.56±0.007	45.66±0.009	43.63±0.005	35.85±0.008	36.95±0.007	38.55±0.009	39.15±0.005	41.35±0.003	49.85±0.004

Thioreduxine Reductase

Weeks	*CONT.*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
0	91.49±0.005	90.98±0.008	90.78±0.004	91.59±0.005	90.39±0.005	91.49±0.005	90.69±0.003	90.37±0.007	90.18±0.001	91.29±0.005	90.79±0.005	90.57±0.006
1	90.69±0.003	21.19±0.005	24.76±0.005	33.09±0.005	27.14±0.005	22.72±0.004	8.55±0.005	41.29±0.005	31.56±0.003	27.16±0.003	26.58±0.005	37.12±0.005
2	91.48±0.001	22.93±0.007	22.76±0.003	30.46±0.004	29.33±0.001	20.48±0.001	2.94±0.002	39.09±0.005	30.18±0.005	24.79±0.005	24.94±0.009	35.70±0.008
3	91.89±0.005	24.66±0.005	20.75±0.005	27.82±0.005	31.51±0.005	18.23±0.008	4.08±0.006	36.89±0.005	28.81±0.008	22.43±0.001	23.29±0.005	34.27±0.005
4	90.57±0.006	26.40±0.002	18.75±0.001	25.19±0.008	33.70±0.006	15.99±0.005	8.77±0.009	34.69±0.005	27.43±0.005	20.06±0.005	21.65±0.007	32.85±0.002
5	91.49±0.005	28.13±0.005	16.74±0.005	22.55±0.005	35.88±0.005	13.75±0.001	14.19±0.001	32.49±0.005	26.06±0.007	17.70±0.006	20.00±0.005	31.42±0.005

6	90.79±0.005	29.87±0.006	14.74±0.007	19.92±0.006	38.07±0.003	11.50±0.008	19.87±0.004	30.29±0.005	24.68±0.005	15.33±0.005	18.36±0.001	30.00±0.006
7	90.39±0.005	31.60±0.005	36.79±0.005	17.28±0.005	27.14±0.005	9.44±0.009	25.55±0.005	28.09±0.005	23.31±0.006	12.97±0.003	29.87±0.005	28.57±0.005
8	91.29±0.005	33.34±0.003	38.80±0.009	14.65±0.007	24.96±0.004	7.47±0.004	31.24±0.003	25.89±0.005	21.93±0.005	10.60±0.005	24.94±0.004	27.15±0.004
9	91.59±0.005	35.07±0.005	28.77±0.005	27.82±0.005	22.77±0.005	5.77±0.007	36.92±0.005	39.09±0.005	20.56±0.001	34.25±0.005	20.00±0.005	25.72±0.004
10	90.18±0.001	36.81±0.001	26.77±0.001	22.55±0.005	20.59±0.006	4.79±0.006	25.55±0.005	41.29±0.005	19.18±0.004	31.89±0.009	15.07±0.007	24.29±0.001
11	90.78±0.004	35.07±0.005	24.76±0.005	17.28±0.005	18.40±0.005	5.96±0.009	14.18±0.005	43.49±0.005	17.80±0.008	29.52±0.005	10.13±0.005	22.87±0.004
12	90.37±0.008	33.34±0.004	22.76±0.004	12.01±0.005	16.22±0.001	7.70±0.004	4.08±0.009	28.09±0.005	16.42±0.004	27.16±0.003	38.10±0.009	21.44±0.007
13	90.98±0.008	31.60±0.005	20.75±0.005	6.74±0.005	14.03±0.005	9.43±0.009	8.55±0.004	25.89±0.005	15.07±0.006	24.79±0.005	29.87±0.005	20.02±0.004