# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of Study

Quite a good number of occupations like mining, automobile repairs, paint and battery production in Nigeria today involve metal pollution, and these small operational occupations are often neglected by the government and lack regulatory agencies. Generally, occupational exposure may have accounted for a vast majority of heavy metal poisoning in humans. The automobile workers are involved in operations like automobile servicing, panel beating, spray painting, car battery maintenances as well as welding works which may expose them to an array of heavy metals. Welders are also exposed to toxic fumes which contain certain metals as well as their oxides, gases and intense heat. These automobile workers may be exposed to health and environmental risks more than the general population due to the nature of their occupation or job involving the use of products like leaded gasoline, paints, engine oil and battery fluids. Regrettably in Nigeria, occupational exposure is absolutely unregulated and unmonitored, and these workers do not have access to any occupational health programs. The main pathways of exposure to these metals are through oral, nasal and dermal routes. Other factors that may enhance exposure are poor occupational hygiene, lack of use of personal protective equipment and some other unhealthy practices these artisans engage in. most of these artisans are oblivious of the hazards they are exposed to, and therefore pay little or no attention to possible preventive measures. Although most of these heavy metals are ubiquitous in the environment, in air, water, soil, and as contaminants in foods (Allouche *et al*., 2011; Ibrahim *et al*., 2012), occupational exposure are mostly higher than environmental exposure (Semple, 2005).

Arsenic (Ar), Cadmium (Cd), hexavalent Chromium ($Cr_6$), Lead (Pb) and Mercury (Hg) are listed among the priority metals of public health importance as a result of their high degree of

toxicity (Tchounwou *et al.*, 2012) with no established role in the biological system, and are thus considered non-essential. Of all the toxic metals listed, lead and cadmium have been reported to be of greater public health importance in Nigeria (Orisakwe, 2014). The diverse applications of these metals in industries, agriculture, medicine and technology have contributed immensely to their wide distribution in the environment (Tchounwou, *et al.*, 2012). Occupationally unexposed persons may as well be exposed to these toxic metals from the environment, though not as much as the occupationally exposed. These metals have been used or encountered for decades in various areas, and despite the several adverse effects recorded, there is still a progressive increase in exposure to these metals; from artisanal mining activities, use of leaded petrol or gasoline, inappropriate disposal and burning of wastes particularly in less developed or developing countries like Nigeria (Anyanwu *et al*., 2018). Other metals which are essential for biochemical and physiological functions like Zinc (Zn), Iron (Fe), Copper (Cu), and Selenium (Se), often referred to as trace metals may have adverse effects in excess or deficient states. Generally, heavy metals are toxic chemicals with neurotoxic (Caito and Aschner, 2015), hepatotoxic (Onyeneke and Omokaro, 2016), nephrotoxic (Amah *et al.*, 2014), cardiovascular (Ugbaja *et al*., 2013; Tellez-Plaza *et al*., 2018), endocrine disrupting (Okoli *et al*., 2015) and reproductive effects (Apostoli and Catalani, 2011; El-Beltagy *et al.*, 2015).

The permissible blood levels of cadmium and lead in adults humans are 0.03-0.12µg/dl and <10µg/dl respectively (WHO, 2007), and blood lead and cadmium levels are reliable markers of recent exposure to these toxic metals (Usuda *et al.* 2011), although recently it has been reported that no blood lead level is considered safe in humans. Occupational exposure to cadmium may be from some metals, fumes, pigments and battery productions while that of lead may as well be from production of lead-acid batteries, metals and oxides for paint

pigments, and use of leaded gasoline (Dioka *et al.*, 2004). Alli (2015) reported blood cadmium and lead levels of occupationally exposed persons above the permissible limits.

## 1.2 Statement of the Problem

The diverse adverse health outcomes associated with heavy metals exposure are of great concern and global issue as these toxic metals adversely affect numerous body organs and systems as well as biochemical indices. Regrettably, in many developing countries, Nigeria inclusive, occupational exposure to toxic metals is absolutely unregulated and most times unmonitored (Orisakwe, 2014). Most of these small operational occupations including automobile repairs involve the less educated, and Chikezie *et al*. (2017) suggested the need for enlightenment on the hazards inherent in their job as well as possible ways of protecting the workers from exposure to toxic chemicals. However, there is the possibility of low compliance, and exposure may be inevitable considering the type of work they do. Additionally, chelation therapy (using chelating agents like calcium disodium EDTA, dimercaptosuccinic acid (DMSA) and D-Penicillamine) which is the therapeutic measure employed in cases of heavy metal toxicity has been reported to have their shortfalls, which apart from the cost include; nephrotoxicity, hepatotoxicity, hypersensitivity reactions, gastrointestinal disturbances and depletion of essential metals (Liebelt and Shannon, 1994; Flora and Pachauri, 2010). Thus, cheaper and easily available alternatives with minimal side effects for the alleviation of the toxic effects of these metals may be a better option.

## 1.3 Justification of the Study

Studies have reported diverse adverse health problems including renal failure, cardiovascular diseases, cancer, endocrine disruption and infertility associated with heavy metal exposure (Dioka *et al*., 2004; Orisakwe *et* al., 2007; Onuegbu *et* al., 2011; El-Beltagy *et* al., 2015; Mahmood *et al*., 2015). However, the possible protective potentials of phytonutrients and

vitamins against the toxic effects of these metals are less well documented. As long as the threat of heavy metal poisoning remains prevalent in our society, possibly from unregulated and unavoidable exposure especially in the occupationally exposed as mentioned earlier, the use of dietary supplements including phytonutrients and vitamins as substitutes for the synthetic chelators may prove to be beneficial for the various populations at risk of toxic metal exposure, as these supplements are cost effective, readily available, with minimal or no side effects. Additionally, in heavy metal poisoning where the symptoms are not very specific, and most times undiagnosed, consumption of these products can be a simple way of preventing or managing the adverse health outcomes associated with heavy metal exposure, thereby improving the quality of life and reducing the overall burden in our healthcare system. This study was therefore set to evaluate the levels of lead, cadmium and other biochemical profile in automobile workers, and the subsequent effects of green tea and vitamin C supplementation.

## 1.4 Aim

The aim of this study was to evaluate the levels of some elements, reproductive hormones, oxidative stress markers and lipid profile in male automobile workers, and the subsequent effects of green tea (*Camellia sinensis*) and vitamin C supplementation.

## 1.5 Specific Objectives

1. To assess the levels of some toxic metals (Pb and Cd), reproductive hormones (testosterone, FSH and LH), trace elements (selenium and zinc), oxidative stress markers (MDA & TAC) and lipid profile (total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride) in male automobile workers at Emene, Enugu.

2. To determine the gonadal status and the possible influence of duration of exposure, smoking and alcohol consumption on the measured biochemical parameters in the automobile workers.
3. To assess the relationships between the toxic metals (Pb and Cd) and the biochemical parameters measured in these workers.
4. To determine the effect of supplementation with green tea or vitamin C on blood cadmium and lead levels, and possible alterations in male sex hormones, oxidative stress markers, trace elements and lipid profile in these workers.

## 1.6 Research Questions

1. Are blood lead and cadmium levels elevated in automobile workers?
2. Are there alterations in the levels of male sex hormones, oxidative stress markers, trace elements and lipids in the automobile workers?
3. Are there relationships between the toxic metals and the measured biochemical profile in these workers?
4. Will green tea or vitamin C supplementation affect blood lead and cadmium levels, and possibly improve any alteration in male sex hormones, oxidative stress markers, trace metals and lipid profile in these workers?

## 1.7 Research Hypothesis

**$H_0$:** Blood lead and cadmium levels are not elevated, and the levels of male sex hormones, oxidative stress markers, trace elements and lipid profile are not altered in automobile workers.

**$H_1$:** Blood lead and cadmium levels are elevated, and the levels of male sex hormones, oxidative stress markers, trace elements and lipid profile altered in automobile workers.

**$H_0$:** Blood lead and cadmium are not associated with male sex hormones, oxidative stress markers, trace elements and lipid profile in the automobile workers.

**$H_2$:** Blood lead and cadmium are associated with male sex hormones, oxidative stress markers, trace elements and lipid profile in the automobile workers.

**$H_0$:** Supplementation with green tea or vitamin C will not affect blood cadmium and lead levels or improve any alteration in male sex hormones, oxidative stress markers, trace metals and lipid profile in automobile workers.

**$H_3$:** Supplementation with green tea or vitamin C will reduce blood cadmium and lead levels, and improve any alteration in male sex hormones, oxidative stress markers, trace metals and lipid profile in automobile workers.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Heavy Metals

Heavy metals may be referred to as elements that have ecotoxicological and toxic effects in humans (Duffus, 2002). Human exposure to heavy metals has dramatically increased in the past years as a result of increased use of these metals in various industrial and technological processes (Orisakwe, 2014) with majority of them causing deleterious effects (Mahmood *et al*., 2015). These elements including cadmium (Cd), lead (Pb), mercury (Hg) and arsenic (As) have specific densities of greater than $5g/cm^3$, and the adverse health effects caused by these metals may be attributed to their non-biodegradability (Alissa and Ferns, 2011).

Some of these heavy metals can influence behavior directly by impairing mental and neurological processes, influencing production and utilization of neurotransmitters as well as altering various metabolic processes (White *et al*., 2007). Moreover, accumulation of heavy metals below levels considered non-toxic can still have serious adverse health effects (Lanphear *et al*., 2005). Of all the heavy metals, lead and cadmium appear to be of more public health importance in Nigeria (Orisakwe, 2014).

### 2.1.1 Cadmium (Cd)

Cadmium is a heavy metal of environmental and occupational concern. It is a carcinogenic metal to which humans are exposed through air, water and consumption of foods contaminated with cadmium-containing compounds. Cadmium is frequently utilized in the production of alloys, batteries and pigments (ATSDR, 2008). The major routes of exposure to cadmium are through inhalation, ingestion and skin absorption. Human exposure to cadmium include sources like working in metal industries, smoking, working in cadmium-contaminated work

places, emissions from industrial activities (mining, smelting, manufacturing of pigments, alloys, batteries and stabilizers) (ASTDR, 2008).

### 2.1.2 Lead (Pb)

Lead is a naturally occurring bluish-gray metal in the form of oxides or salts. It is a ubiquitous environmental and industrial pollutant found in air, water, soil and as contaminants in food (Ibrahim *et al*., 2012). Occupational exposure has the highest account of lead poisoning or toxicity. The environmental exposure to lead include ingestion of dust contaminated with lead, inhalation of automobile exhaust from gasoline containing alkyl lead additives, ingestion of lead-based paints and drinking water that passed through leaded pipes (Kumar *et al*., 2011).

Lead has diverse applications in the agricultural, domestic and industrial sectors. It is used in the production of lead-acid batteries, ammunitions, metal products (solder and pipes), toys, food-can soldering and ceramic glazes (Sajitha *et al*., 2010).

Lead is relatively poorly absorbed into the body, but once absorbed, it is slowly excreted, therefore accumulates in the body especially in the bones, and causes various tissue and organ damage (Zhang *et al*., 2012). Adults absorb 35%-50% of lead through drinking water, and the absorption rate for children may be greater than 50% (Tchnounwu *et al*., 2012).

## 2.2 Occupational Health and Exposure to Heavy Metals

In developing countries, like Nigeria, the awareness of occupational health have been hindered as a result of poor data collection, lack of interest of the government as well as weak enforcement of health and safety regulations (Asuzu, 1996). The International Labour Organization (ILO) requires that occupational health and safety services should be developed for workers in all categories of economic activity which will protect workers from various occupational hazards present in almost all occupations. Hence, the inability of any country or organization to provide occupational health and safety services to all categories of workers

contravenes the ILO convention 161 and recommendation 171 of 1985 (International Labour Organization ILO, 2011). Many workers in the automobile field are exposed to various hazards every day on the job. Due to the potential health hazards involved in this automobile repairing and servicing profession, the workers and technicians need to be enlightened in the regulated use, storage and disposal of toxic chemicals as well as in proper safety procedures to promote a safe work environment (Basu *et al*., 2015).

Automobile workers are at increased risk of exposure to various toxic and carcinogenic substances which includes heavy metals. Apart from the leaded gasoline which has been banned worldwide years ago (but still in use in our country Nigeria), there are other different types of mobile oil and grease rich in lead (Garba *et al*., 2013). The battery liquid is one of the main sources of lead and cadmium (Palus *et al*., 2003).

Automobile workers randomly handle these liquid and grease (containing Lead Nathanael) and oils without protective gloves, and some researchers had reported that these chemicals can penetrate the skin (Dongre *et al*., 2011). Moreover, the car body sprays (paints) also contain lead and cadmium (Vitayavirasuk *et al*., 2005) which are toxic, can be inhaled and also penetrates the skin (Hino *et al*., 2008). Automobile processes produce a lot of hazardous chemicals like metal dusts, toxic fumes, silica, polycyclic aromatic hydrocarbons and metals which are released in the workplace with damaging effects on the health of the workers (Ramalangam and Sellappa, 2013).

## 2.3 Epidemiology of Heavy Metal Toxicity

The epidemiology of heavy metal toxicity differs from country to country, and this is dependent on the socio-economic status of the given population as well as age group. In the United Kingdom, there are rare cases of heavy metal toxicity or poisoning even in industries considered to pose threats (Baldwin and Marshall, 1999). In China and South East Asia, the informal electronic recycling industries have assumed a serious occupational hazard due to

heavy metals. Health risks associated with lead poisoning from the use of paints, cosmetics, leaded gasoline and piped water supply in low income countries continue to be a major health problem. Lead poisoning have been reported in more than 2000 children living near smelting plants (Parry, 2009; Watts, 2009). In cases of acute and chronic heavy metal toxicity, encephalopathy is the most common cause of mortality. Among the most vulnerable populations, children are the class mostly affected due to accidental exposure. In these children, lead poisoning or toxicity can cause cognitive and behavioral changes (Hornung *et al*., 2009).

Heavy metal toxicity has remained a serious health issue till date. Cadmium and lead are the common toxic heavy metals in the environment, and are as well of greater public health importance in Nigeria as stated earlier. The developing countries are currently facing serious issues with cadmium and lead pollution problems. Initially, the blood lead level (BLL) thought to cause toxicity in children was 60μg/dl in 1960s, however, this value was lowered to 10μg/dl in 1991. Currently, the Center for Disease Control and Prevention in United States had reported that no blood lead level is safe for children (Hassanien and Elshahawy, 2010).

In Nigeria, Zamfara state to be precise, about 200 children had reportedly died from lead intoxication, and about 18,000 children as well as adults have been affected by lead exposure as a result of informal extraction of gold from lead bearing ores (WHO, 2010).

Blood lead and cadmium levels have been reported to be elevated in occupationally exposed persons when compared to occupationally unexposed (Dioka *et al*., 2004; Orisakwe *et al*., 2007; Onuegbu *et al*., 2011; Basu *et al*., 2015).

### 2.4 Pathophysiology of Heavy Metal Toxicity

Heavy metal-induced toxicity and carcinogenicity mainly involves the induction of oxidative stress (Thuppil and Tannir, 2013) through increased generation of free radicals or reactive oxygen species (ROS) like hydroperoxides, and depletion or lowering of the antioxidant system (Flora *et al*., 2012). The antioxidant system ensures a balance between pro-oxidants and

antioxidants, but heavy metals cause a disruption in this balance leading to an over production of free radicals, and subsequently oxidative stress (Al-Ubaidy *et al*., 2006). Oxidative stress is a precursor of damage to the various body organs. Despite the importance of reactive oxygen species in signaling processes, they cause cellular and tissue damage when in excess.

These free radicals or ROS adversely affect lipid membranes, proteins as well as nucleic acids (Bartosz, 2008). Heavy metals disrupt an array of metabolic processes, they bind to free sulfhydryl groups resulting in the inhibition of glutathione metabolism, numerous enzymes as well as hormone functions (Zahir *et al*., 1999). Glutathione (GSH) is a vital component of the antioxidant defense which reacts with ROS, stabilizes ROS and then becomes oxidized to glutathione disulfide (GSSG). GSSG is reduced back to GSH by glutathione reductase (GR) (Kim *et al*., 2015).

Lead inactivates GSH by binding to its sulfhydryl group resulting in inefficient replenishment of GSH, thus increasing oxidative stress (Hultberg *et al*., 2001). Additionally, lead reduces GSH levels further by blocking or inhibiting the activity of some enzymes like δ-aminolevulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (Ahamed and Siddiqui, 2007). The destabilization of the cellular membrane through lipid peroxidation can lead to anemia (Patrick, 2006), cellular damage and eventually cell death (Halliwell and Chirico, 1993).

Another mechanism of heavy metal toxicity is based on their valency as they can replace divalent cations and trace metals necessary for cellular activity and also components of antioxidant enzymes like GPx, catalase (CAT) and superoxide dismutase (SOD). These trace or essential metals include zinc, copper, and selenium and manganese, and the displacement of these essential metals leads to the inhibition of the antioxidant enzymes thereby increasing the formation of excess ROS which results in oxidative damage on the heart, liver, brain, kidneys, erythrocytes and reproductive system (Ahamed and Siddiqui, 2007).

## 2.5 The Hypothalamus Pituitary-Testicular (HPT) Axis

The HPT axis implies the release of hormones by three glands; hypothalamus, pituitary and testicular glands. The Hypothalamus is located in the brain, which produces and secretes the peptide hormone gonadotrophin releasing hormone (GnRH). GnRH travels to the anterior pituitary gland through the hypophyseal portal system which is the vascular connection between the hypothalamus and the anterior pituitary, and as it reaches the anterior pituitary gland, GnRH binds with receptors on the secretory cells or gonadotropes which synthesize and release luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the general circulation. When LH and FSH are released into the general circulation, they travel to the testes and bind to their receptors on the testes. LH triggers the production of testosterone in the leydig cells while FSH plays a major role in spermatogenesis, testosterone also stimulates intratesticular activity and spermatogenesis in the sertoli cells. These gonadal steroid hormones that are produced act upon reproductive and other target organs in the body and brain, including feedback regulation of the GnRH neurons (Gore, 2010). Some of the hormones of the hypothalamic pituitary testicular axis are further explained in this context. The Hypothalamus Pituitary-Testicular (HPT) Axis diagram is as shown below in figure 2.1.

Below are brief discussions on some male reproductive hormones as seen in figure 2.1.

### i. Testosterone

This hormone is derived from cholesterol like the other steroid hormones (Waterman and Keeney, 1992). The first step in testosterone synthesis is the oxidative cleavage of cholesterol side chain by Cytochrome P450 family 11 subfamily A (CYP11A) enzyme, a mitochondrial cytochrome p450 oxidase, with the loss of 6 carbon atoms to yield pregnenolone. In the next step, two more carbon atoms are removed by the Cytochrome P450 family 17 subfamily A (CYPI7A) enzyme in the endoplasmic reticulum to yield various $C_{19}$ steroids. Additionally, the 3ß-hydroxyl group is oxidized by 3ß-hydroxysteroid dehydrogenase (3ß-HSD) to produce

androstenedione which is further reduced to yield testosterone by 17ß-hydroxysteroid dehydrogenase 17ß-HSD (the rate limiting step). It is produced by the leydig cells in the testis, and is required by the sertoli cells (male generative glands) for spermatogenesis. The number of leydig cells is in turn regulated by LH and FSH, and the amount of testosterone produced by the leydig cells is under the control of LH, which regulates the expression of 17ß-HSD (Payne and O'Shaughnessy, 1996). Some factors that may affect testosterone levels include;

- Age: testosterone levels gradually decline as men age.
- Nutrients: deficiencies of vitamin A (Livera *et al*., 2002) and zinc (Prasad *et al*., 1996) lowers testosterone levels of plasma and serum.
- Exercise may increase testosterone levels in men (Vingren *et al*., 2010).
- Weight loss may result in raised testosterone levels because fat cells synthesize aromatase which is an enzyme that converts testosterone to estradiol (Hakonsen *et al*., 2011) although an association between body mass index (BMI) and testosterone levels is yet to be established (MacDonald *et al*., 2010).

**ii. Luteinizing Hormone (LH)**

LH is a hormone produced by the gonadotropic cells in the anterior pituitary. It is also known as interstitial cell stimulating hormone (ICSH) in men. It stimulates the production of testosterone in the leydig cells (Jiang *et al*., 2014), and acts synergistically with FSH. When testosterone levels are low, GnRH is released by the hypothalamus, stimulating the pituitary gland to release LH, and when testosterone levels increase it acts on the hypothalamus and pituitary through negative feedback to inhibit the release of GnRH and consequently LH. Hence, persistent high levels of LH may indicate a compromised restriction in the normal feedback from the gonads causing pituitary production of both LH and FSH, and may be a sign of testicular failure. On the other hand, low LH levels can result in hypogonadism (failure of

the testicular function) which usually manifests as failure in the production of normal numbers of sperm in males (Gudeloglu and Parekattil, 2013).

### iii Follicle Stimulating Hormone (FSH)

FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and works together with LH in the reproductive system (Silveira *et al*., 2010). In males, it induces sertoli cells to secrete androgen binding globulin (ABG) which is regulated by inhibin's negative feedback on the anterior pituitary. It also plays a role in the initiation of spermatogenesis. Like LH, high levels of FSH may indicate absence of the normal feedback mechanism from the gonads leading to unrestricted production of pituitary FSH. On the other hand, reduced FSH levels can result in hypogonadism which also manifests as failure to produce normal numbers of the sperm (low sperm count).

In other words, testosterone is required for spermatogenesis and other important biological processes. FSH stimulates the sertoli cells to produce androgen binding globulin (ABG) and inhibin. ABG is a protein which binds to testosterone and keeps it within the seminiferous tubules. Inhibin, on the other hand helps to support spermatogenesis and inhibits the production of FSH, LH and GnRH. Increased levels of testosterone and inhibin cause a negative feedback on the pituitary and hypothalamus, resulting in decreased production of LH and FSH. As a result of this, production of testosterone and inhibin is also reduced.

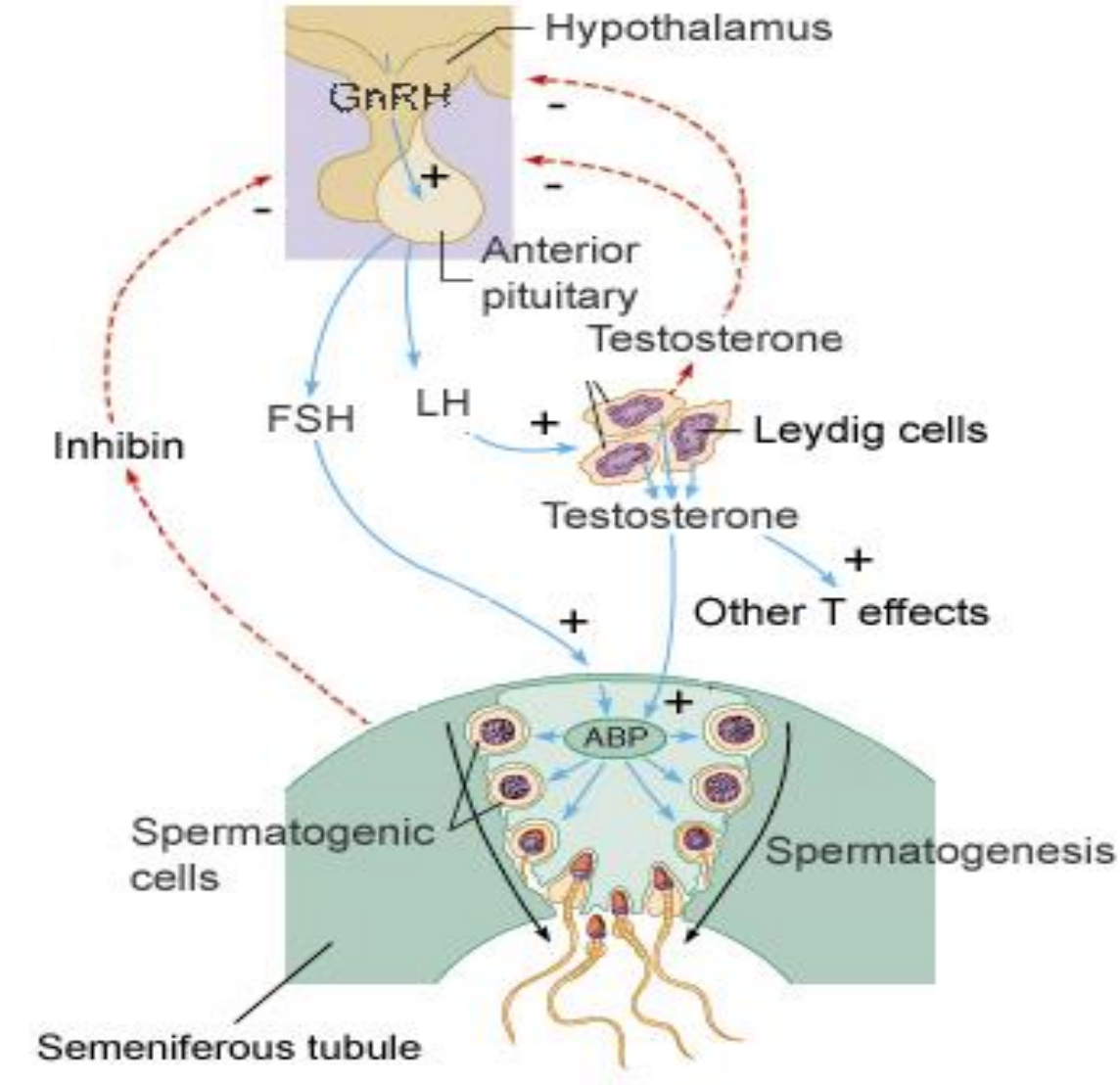


**Figure 2.1 Hypothalamus Pituitary-Testicular (HPT) Axis**

Role of kisspeptin/GPR54 system in human reproductive axis. Frontiers of Hormone Research (Silveira *et al.*, 2010)

## 2.6 Effects of Heavy Metal Exposure

Heavy metals have been reported to adversely affect cellular organelles and components which include cell membranes, lysosomes, nuclei, mitochondria, endoplasmic reticulum and some enzymes involved in metabolic, detoxification and repair processes (Wang and Shi, 2001). These metals have a high degree of toxicity even at lower levels of exposure, and as systemic toxicants they are known to induce multiple organ damage. The various effects on the body are discussed below.

### 2.6.1 Reproductive Effects

Exposure to heavy metals which can act as endocrine disrupting chemicals or substances (Tabb and Blumberg, 2006) have been shown to reduce fertility and reproductive health (Tena-Sempere, 2010). The male reproductive system may be directly or indirectly affected by metals when they target specific reproductive organs or act on the hypothalamus-pituitary-testicular axis interfering with synthesis as well as breakdown of testosterone, luteinizing hormone(LH), follicle stimulating hormone (FSH) (Saiyed *et al*, 2003) or modifying expression levels of hormone receptors (Yeung *et al.* 2011). Figure 2.2 shows the possible effect of endocrine disrupting chemicals on male reproductive function.

The process of spermatogenesis is dependent on functional HPT axis. The hypothalamic kisspeptin-1 (KiSS-1) and its G protein-coupled receptor (GPR54) act as the gatekeepers controlling the secretion of GnRH which regulates the LH, FSH and testicular hormones (testosterone, activin and inhibin B) (Siveira *et al.*, 2010). Any interruption on the hypothalamic circuitry hormonal mediated regulation or on the constituents of the microenvironments in the seminiferous tubules may result in a transient or long term modification of the hormonal feedback circuitry leading to disturbance of spermatogenesis (Tena-Sempere, 2010). This alteration in HPT axis upon exposure to lead has been shown in rats (Sokol *et al.*, 1994).

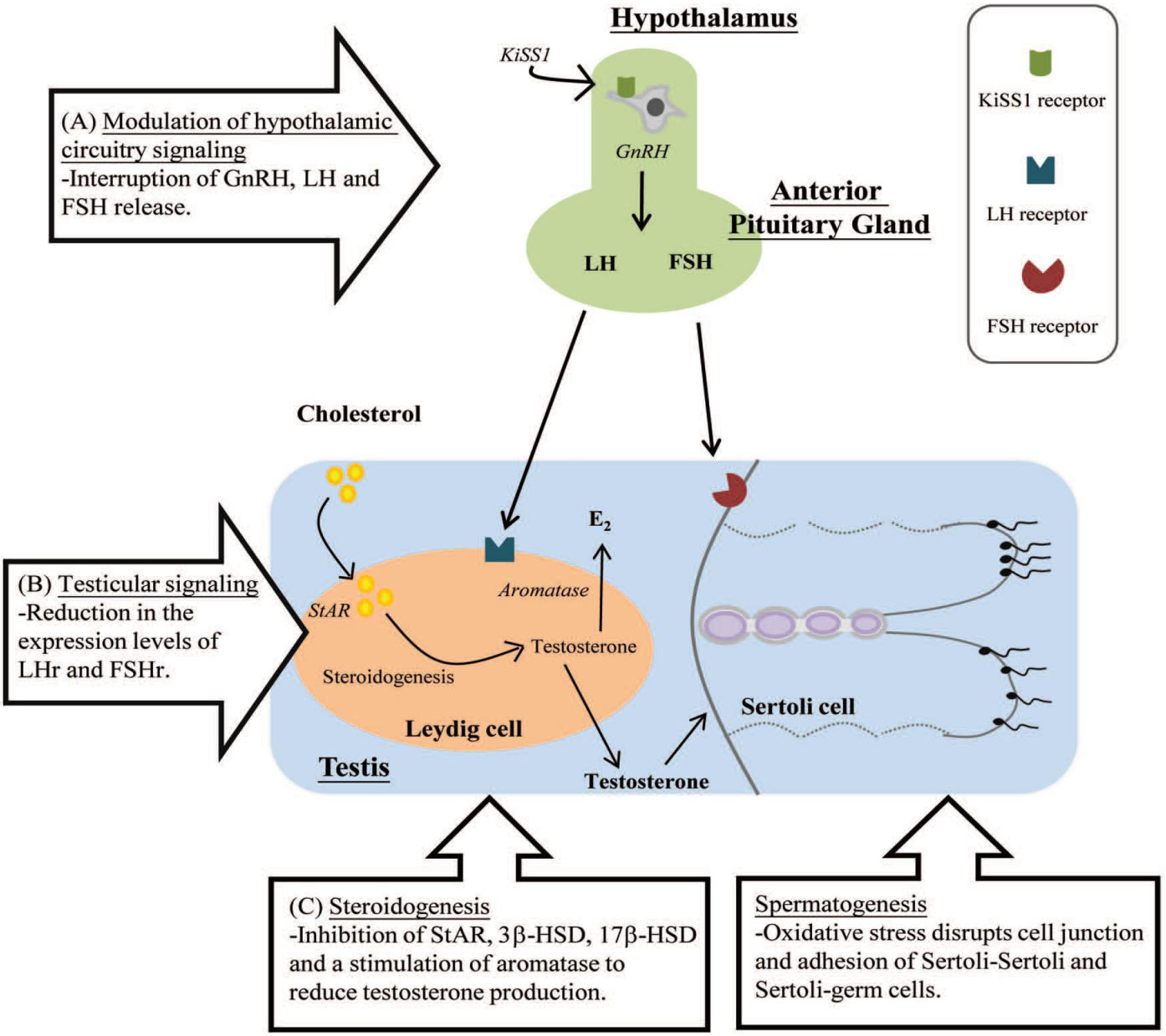


**Figure 2.2 Possible Effects of Endocrine Disrupting Chemicals on Male Reproductive Function.**

Kisspeptin/GPR54 system as potential target for endocrine disruption of reproductive development and function (Tena-Sempere 2010).

Heavy metals can affect the HPT axis through the following mechanisms (Tena-Sempere, 2010);

i. Modulation of the neural circuit in hypothalamus (KiSS-1/GPR54 and HPT axis) leading to the dysregulation of gonadotrophin hormones (FSH/LH) secretion by the pituitary.
ii. Reduction in the expression of gonadotrophin receptors (LHr and FSHr) in the testis.
iii. Interfering with the enzymes (StAR, P450scc, 3β-HSD, 17β-HSD) involved in steroidogenesis and testosterone production.
iv. Disruption of cell-cell interaction and the process of spermatogenesis resulting from oxidative stress.

Some heavy metals like lead and cadmium could adversely affect the male reproductive system either by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis, resulting in impaired semen quality. Several metals especially lead and cadmium are considered reproductive toxicants and/or suspected endocrine disrupting compounds. Jurasovic et al. (2004) reported positive associations between blood cadmium (B-Cd) concentrations and follicle stimulating hormone (FSH) and testosterone levels among men with no occupational exposure. Several studies have reported declines in semen quality associated with both lead (Eibensteiner et al., 2005) and cadmium concentrations in blood (Telisman et al., 2000).

The hormones which play a crucial role in spermatogenesis are used as part of assessment of male reproductive capacity. However, reports of studies on the levels of these hormones in heavy metal exposure have been inconsistent. Kumar, (2004) reported that occupations involving the manufacture or application of bio-accumulative and non-easily degradable compounds, intense exposure to heat and toxic fumes are associated with reproductive

dysfunction, and that lead exposure close to a dose of 40µg/dl might have adverse effects on spermatogenesis and male reproductive endocrine parameters.

Wadi and Ahmad, 2010 demonstrated that plasma LH, FSH and testosterone were not altered by lead administration in mice while Algafari *et al*. 2011 reported normal levels of FSH and testosterone, and an increase in LH in car customizers and welders. Reduced testosterone and elevated LH and FSH levels have also been reported following exposure to lead (Batra *et al*., 2004; Vigeh *et al*., 2011; El-Beltagy *et al*., 2015) and cadmium (Lafuente *et al*., 2002; Obianime and Roberts, 2009; El-Beltagy *et al*., 2015).

The disruption in the levels of these hormones may involve other hormones and/or hormonal feedback other than disruption of testosterone secretion in the HPT axis which may be due to defective regulation of LH at the pituitary level (Tena-sempere, 2010). Degeneration of leydig cells in rats has been shown by histological examination (Saxena *et al*., 1986), suggesting leydig cells as targets for heavy metal toxicity. As a result of the disturbances in the HPT axis induced by heavy metal exposure, inappropriate levels of LH and FSH are released which change the negative feedback loop (Ronis *et al*., 1996) especially at the hypothalamus level, all of which result in reproductive dysfunction. Additionally, the loss of testosterone feedback may be associated with pituitary cell hypertrophy, hyperplasia and eventually pituitary neoplasia (Nyska *et al*., 1998) which could be responsible for the increased LH and FSH.

Heavy metals can affect the semen quality, prostate secretary function and seminal vesicles, the reproductive endocrine function which may lead to loss of fertility, libido or to impotence (Apostoli and Catalani, 2011). Exposure to cadmium lead and arsenic may contribute to the development of prostate cancer (Telisman *et al*., 2007), and lead toxicity reduces fertility in both men and women. It was reported to cause testicular atrophy, reduced testosterone levels and hypo-spermia in male battery workers (Queiroz and Waissmann, 2006).

In humans, protamines protect sperm DNA, and zinc is required for sperm chromatin stability and binds to protamine P2. When lead replaces zinc, the HP2-DNA interaction is interrupted or reduced, resulting to alterations in sperm chromatin condensation and thus reducing fertility (Quintanilla-Vega *et al*., 2000). In women, the effect of heavy metals like lead can manifest as irregular estrus, decreased gestational period, abnormalities in the offspring (Han *et al*., 2000) or spontaneous abortion (Tang and Zhu, 2003). Moreover, animal studies have shown that postnatal exposure to lead during the developmental stage of the hypothalamus alters the development and function of the reproductive system.

### 2.6.2 Cardiovascular Effects

Heavy metals can cause an increase in the acidity of blood, causing the body to draw calcium from the bones in order to restore the pH of blood to normal, these toxic metals also initiate conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn as buffer to the area, resulting to hardening of the arterial walls with gradual blockage of the arteries and osteoporosis (Orisakwe, 2014). Among the cardiovascular abnormalities associated with lead are atherosclerosis, myocarditis, altered heart rate activity and increased ischemic heart disease risk (Varizi, 2008). Lead and cadmium have been reported to alter cholesterol synthesis and transport pathways (Olisekodiaka *et al*., 2012; Ugbaja *et al*., 2013) thereby increasing the risk of atherosclerosis and cardiovascular diseases (Ademuyiwa *et al*., 2005).

Lead up-regulates plasma cholesterol, triglyceride and phospholipid concentrations (Ugbaja *et al*., 2013) resulting in certain changes in the rate of a number of processes including, altered distribution between the plasma and tissues, enhanced absorption of exogenous cholesterol from diet, enhanced synthesis of endogenous cholesterol, decreased cholesterol excretion or decreased transformation of cholesterol to bile acids (Kilic, 1993).

Cebi *et al*., (2011) reported that the mean serum levels of lead were higher in coronary artery disease (CAD) patients. The exact mechanisms of the hypertensive effect of lead exposure are not very clear. However, an inverse relationship between estimated glomerular filtration rate (eGFR) and blood lead level of <5µg/dl has been observed in the general population (Ekong *et al*., 2006). This may indicate that lead-induced reductions in the renal function could play a major role in hypertension. Other possible mechanisms of cardiovascular effects include oxidative stress, impaired or down regulation of nitric oxide (NO) system (Dursun *et al*., 2005) and soluble guanylate cyclase (Farmand *et al*., 2005), inflammation, dysregulation of vasoactive hormones, and aberrations in cellular calcium transport of intracellular calcium distribution (Varizi, 2008).

These mechanisms can result in increased vascular tone and peripheral vascular resistance. Cadmium may as well act on blood pressure through oxidative stress, endothelial dysfunction, partial agonism of calcium channels, increased vasoconstriction, activation of the sympathetic nervous system (Varoni *et al*., 2003), renal tubular damage, sodium retention and water overload (Satarug *et al*., 2006).

### 2.6.3 Renal Effects

Heavy metals are known to be nephrotoxic and can cause proximal tubular damage which may manifest as glycosuria and aminoaciduria, decline in glomerular filtration rate (GFR), hypertension, hyperuricemia, gout and renal failure (Loghman, 1997). Hyperuricemia results from impaired tubular function and alteration in purine metabolism (Alasia, 2010).

The kidneys are the first target organ of heavy metal toxicity because of their ability to reabsorb and accumulate divalent metals, and the extent of renal damage depends on the nature, dose, route as well as duration of exposure. Both acute and chronic cases of heavy metal toxicity cause nephropathies ranging from tubular dysfunctions to severe renal failure and sometimes

to death (Barbier *et al*., 2005). Apart from the proximal tubules, loop of Henle, distal tubule and terminal segments of the nephron where reabsorption occurs may also be affected.

The intestinal absorption of divalent metals like cadmium and lead is facilitated by the divalent metal transporter I (DMT-I) which is located in the erythrocytes, duodenum, liver and proximal convoluted tubular cells. This protein (DMT-I) has a high affinity for reacting with, and storing metals like zinc, cadmium, lead, copper etc. DMT store essential metals such as zinc and copper in the intracellular medium and transfer them to metalloproteins, transcription factors and enzymes. It also plays a role in elimination of reactive oxygen species, cellular repair and regeneration (Sabolic *et al*., 2010; Sabath and Robles-Osorio, 2012).

### 2.6.4 Carcinogenic Effect

Heavy metals including lead and cadmium are potential human carcinogens (International Agency for Research on Cancer IARC, 2012). Studies have reported that exposure to these compounds leads to interruption or disruptions in tumor suppressor gene expression, damage repair processes as well as enzymatic processes involved in metabolism through oxidative stress (Banfalvi, 2011). These carcinogenic metals can disrupt various cellular processes causing DNA damages with both oxidative and non-oxidative mechanisms (Caffo *et al*., 2014). Additionally, heavy metals act as metallohormones that disrupt the endocrine system. Low dose cadmium exposure has been reported to possess estrogen and androgen-like activities by directly binding to estrogen and androgen receptors (Takiguchi and Yoshihara, 2006). The prominence of estrogens in the etiology of breast cancer may suggest that occupational or environmental exposures mimic the effects of estrogens which may be potential risk factors for the disease. Androgens are involved in the normal growth and secretory function of the prostate gland as well as in the development of prostate cancer. Low dose exposure to cadmium has been shown to have androgen-like activity on androgen receptors (Takaguchi and Yoshihara, 2006) and risk factor for development of prostate cancer (Martin *et al*., 2002).

### 2.6.5 Other Effects

Heavy metals can be neurotoxic, fetotoxic, and also adversely affect children as well as the hematological system. Most heavy metals readily cross the placenta to the fetus leading to increased possibility of premature birth and low birth weight (Taylor *et al*., 2015). They can be found in breast milk, and are known to be detrimental to behavior, development and the nervous system. Lead causes anemia by blocking the activity of ferrochelatase, aminolevulinic acid synthase and aminolevulinic acid dehydratase (ALAD). These three enzymes are involved in heme synthesis. Aminolevulinic acid (ALA) is mostly affected, and when inhibited ALA accumulates.

## 2.7 Factors Affecting Health Outcomes of Metal Exposure

Certain factors can influence the health outcomes of human exposure to metals. These include; the chemical form or metal speciation, dose, route, duration of exposure, age, bioavailability as well as distribution and accumulation of metals in various organs. Health status, dietary habits, use of supplements and exposure to other metals (Pizent *et al*., 2012) can also influence response to heavy metal exposure. Metals can interact synergistically, additively or antagonistically affecting each other's absorption, distribution and excretion (Matovic *et al*., 2011). The interaction of toxic and/or essential metals may be important for the final health outcomes of metal exposure. These interactions account for the individual differences in susceptibility to the adverse effects of metals in humans (Telisman, 1999). Genetic polymorphism could be responsible for the individual differences in the susceptibility to heavy metal exposure in persons living in the same environment (Pizent *et al*., 2012).

## 2.8 Trace/Essential Elements

Trace metals, also referred to as essential metals or elements are micronutrients required by the body in small amounts for various metabolic processes. These essential elements are potential

toxicants when present in high concentrations. They include zinc (Zn), selenium (Se), iron (Fe), copper (Cu), manganese (Mn), and others (Prashanth *et al*., 2015). Moreover, insufficient levels or deficient levels of these metals can cause various pathologies. They are needed for proper functioning of living organisms, and can be depleted through various metabolic processes. Some of the trace metals act as co-factors in antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase.

### 2.8.1 Zinc (Zn)

Zinc is an essential trace metal which is involved in various physiological roles. It is required for normal growth and functioning of the immune system, it is also essential for cellular membrane integrity and metabolism (Prasad, 1995). It is also involved in wound healing, DNA synthesis, supports normal growth and development during pregnancy, childhood and adolescence (Maret and Sandstead, 2006). It is present in a wide variety of foods, like beans, whole grains, some sea foods, nuts and dairy products, and a daily intake is needed to maintain adequate levels since the body has no specialized storage system for zinc (Rinc and Gabriel, 2000). Zinc deficiency is characterized by anorexia, grown retardation and impaired immune function. In severe cases, diarrhea, delayed sexual maturation, impotence, hypogonadism in males (Maret and Sandstead, 2006). On the other hand, zinc toxicity may be associated with nausea, vomiting, abdominal cramps, anorexia, diarrhea and headaches.

Many of these symptoms which are associated with other health conditions and therefore are not specific to zinc deficiency or toxicity. A medical examination is necessary to confirm diagnosis, the range is between 50µg/dl and 150µg/dl. Another important aspect of zinc is being a component of the antioxidant enzyme superoxide dismutase (SOD), and as such possess antioxidant properties which reduce heavy metal-induced oxidative stress (Afridi *et al*., 2011).

### 2.8.2 Selenium (Se)

Selenium is a trace metal naturally present in many foods like grains, meat, fish, poultry and eggs (Chun *et al*., 2010), and also available as dietary supplements. It is a constituent of several selenoproteins that play essential roles in reproduction, DNA synthesis, thyroid hormone metabolism and protection from oxidative damage and infection (Sunde *et al*., 2012).

Deficiency of selenium results in certain biochemical changes that might predispose to the development of certain disease conditions like cancer, cardiovascular disease and others. When in excess, it results in hair and nail loss or brittleness, irritability and nervous system abnormalities. Selenium plays an important role in the protection of cells against free radical damage, it is a cofactor of glutathione peroxidase, and thus decreases lipid peroxidation as well as protects the DNA, RNA and proteins from oxidative damage. Other positive health effects of selenium include, reduction in incidence of cancer, protection form cardiovascular diseases (Hatfield *et al*., 2014). Moreover, selenium has similar chemical properties with sulfur, and therefore has a high affinity for many heavy metals like lead and cadmium (Basu *et al*., 2015), and falls within the range of 7µg/dL and 15µg/dL.

## 2.9 Essential Trace Elements in Male Reproduction

Trace metals play important role in reproduction, especially in males, although they are known to exist in the body at low levels. Iron, copper (Tvrda *et al.*, 2014), zinc (Zhao *et al*., 2016) and selenium (Akinloye *et al*., 2005) are necessary in male reproductive health. Nutritional deficiencies of zinc and selenium have been implicated as risk factors involved in the pathogenesis of infertility (Abarikwu, 2013), and approximately 30-40% of infertility cases are caused by male factors (Esteves and Chan, 2015).

### 2.9.1 Zinc and Male Reproduction

Zinc is one of the essential trace minerals needed for the normal functioning of the male reproductive system or process. It is present in high concentrations in the seminal fluid, and could play diverse roles in sperm functional properties as well as a regulatory role in capacitation and acrosome reaction processes (Michailov *et al*., 2014). Zinc is a component of the antioxidant enzyme superoxide dismutase (SOD), and therefore can be protective against oxidative stress (Camejo *et al*., 2011) from exogenous and endogenous sources. Some studies have reported an increase in sperm quality of infertile males following zinc supplementation while other studies had reported otherwise (Camejo *et al*., 2011; Akinloye *et al*., 2011).

Zinc plays a significant role in testosterone production because the zinc-dependent enzyme, I7ß-hydroxysteroid dehydrogenase (I7ß-HSD) is needed to convert androstenedione to testosterone. Maintaining adequate amounts of zinc in diet is therefore of utmost importance for males, as deficiencies could result in reduced libido, low testosterone levels and low sperm counts. Julali *et al*., (2010) reported an increase in testosterone and luteinizing hormone (LH) levels with no change in follicle stimulating hormone (FSH) after oral zinc supplementation in men while Bakheet and Almarshad (2014) reported an increase in both LH and FSH levels after zinc supplementation. Therefore, zinc may also affect the levels of other reproductive hormones like LH and FSH apart from testosterone.

### 2.9.2 Selenium and Male Reproduction

Selenium is a constituent of selenoproteins including glutathione peroxidases (GPx) that protect against oxidative damage to spermatozoa throughout the process of sperm maturation as well as serve as structural components of mature spermatozoa (Ahsan *et al*., 2014). Since selenoproteins participate in sperm structure integrity and maintenance, and can also protect against oxidative DNA damage in human sperm cells (Moslemi and Tavanbakhsh, 2011), dietary selenium should therefore be in optimal quantity so as to maintain adequate

reproductive function in males as well as prevent infertility. Increase ROS reduce fertility because ROS attack spermatozoa membrane decreasing their viability. Therefore, increasing selenium intake enhances antioxidant (GPx) activity, reduces ROS, and consequently enhances male fertility (Klein, 2004).

Selenium has a positive influence on the leydig cells influencing testosterone secretion (Akinloye *et al*., 2005). Its supplementation has also been shown to increase the levels of testosterone, luteinizing hormones as well as follicle stimulating hormone in rats (Sakr *et al*., 2011).

## 2.10 Heavy Metals Interactions with Trace Elements

Heavy metals are antagonistic to trace metals or elements by competing for binding sites on transport and storage proteins, receptors and metalloenzymes. The disruption of balance and metabolism of nutrient elements results in significant alterations in carbohydrate, protein, lipids, neurotransmitters and hormones metabolism (Zalups, 2000). Toxic metals can interfere with essential metals metabolism reducing their concentration or decreasing their bioavailability in an organism (Matovic *et al*., 2011). Lead readily binds to enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) which are highly susceptible to lead toxicity because their functions depend on the presence of the trace metals zinc, copper and selenium respectively. GPx converts hydrogen peroxide ($H_2O_2$) to water and lipid peroxides and to their respective alcohols. Therefore, in the presence of lead, selenium is displaced from GPx leading to the inactivity of GPx, increase $H_2O_2$ and free radical amounts (Ahamed and Siddiqui, 2007). In humans, SOD which has three isoforms (SODI, SOD2 and SOD3) has SOD 3 as the major antioxidant enzyme requiring copper and zinc for its activity. SOD catalyzes the formation of $H_2O_2$ from superoxide radicals and hydrogen ions. Lead displaces copper and zinc leading to reduced SOD activity (Itoh *et al*., 2009).

The disruption of the pro-oxidant and antioxidant balance results in excessive production of ROS leading to oxidative damage of various organs and systems of the body. Workers exposed to lead have been reported to show decreased blood zinc (Dioka *et al*., 2004; Bal *et al*., 2015) and selenium (Basu *et al*., 2015) levels, however, some other researchers have reported no significant difference in zinc and selenium levels in workers occupationally exposed to lead and the control group (Mehdi *et al*., 2000; Arinola and Akinbinu, 2006; Bal *et al*., 2015).

On the other hand, essential metals like zinc and selenium can reduce the absorption and retention of heavy metals preventing their toxic effects. Zinc competes with lead for similar binding sites on a metallothionein-like protein in the gut reducing lead absorption and subsequently decreases its toxicity (Flora *et al*., 1994). Selenium interacts with lead to form inactive selenium-lead complexes thereby reducing the availability of free lead ions in the body (Othman and El Missiry, 1998).

## 2.11 Management of Heavy Metal Toxicity

Heavy metal toxicity can be managed in two different ways which include chelation or dietary strategies.

### 2.11.1 Chelation Therapy

This is the most widely used therapeutic strategy for heavy metal toxicity in order to enhance metal excretion. The drugs used as chelating agents also referred to as chelators include the following; dimercaptosuccinic acid (DMSA), succimer (meso-DMSA), dimercaprol (British Anti-Lewisite (BAL)), D-Penicillamine (DPA) and calcium disodium ethylenediamine tetraacetic acid ($CaNa_2EDTA$) (Kim *et al*., 2015) These synthetic chelators however are associated with various adverse effects including gastrointestinal symptoms, hypertension (Anderson and Aaseth, 2002), depletion of essential metals due to lack of specificity,

hepatotoxicity, nephrotoxicity (Kim et al., 2015) and redistribution of the toxic metals to other organs like brain (Sears, 2013).

Another limiting factor here is the removal of the patient from the source of heavy metal exposure during treatment. In other words, workers occupationally exposed to heavy metals will have to quit their jobs or abstain from work in order to undergo treatment with these chelators. Therefore, the development of safe and efficient strategies against heavy metals like lead and cadmium toxicity is an area of ongoing research.

### 2.11.2 Dietary Strategies

Dietary supplements have been reported to play crucial roles in the alleviation or prevention of cadmium and lead toxicity. Dietary strategies are advantageous because nutritional ingredients can be easily and affordably added to diet with little or no side effects compared to the negative side effects of synthetic chelators (Zhai *et al*., 2015).

Potential dietary strategies for cadmium and lead toxicity include vitamins, edible plants and dietary phytochemicals as well as essential metal supplementation (Zhai *et al*., 2015).

Some studies have shown the important roles of dietary supplements like vitamins (Ugbaja *et al*., 2013), dietary phytochemicals (Nwokocha *et al*., 2012) and essential metals (Amara *et al*., 2008) in protecting against cadmium and lead toxicity compared to the chelation therapy in persons inadvertently exposed to toxic or heavy metals on a daily basis (Monachese *et al*., 2012).

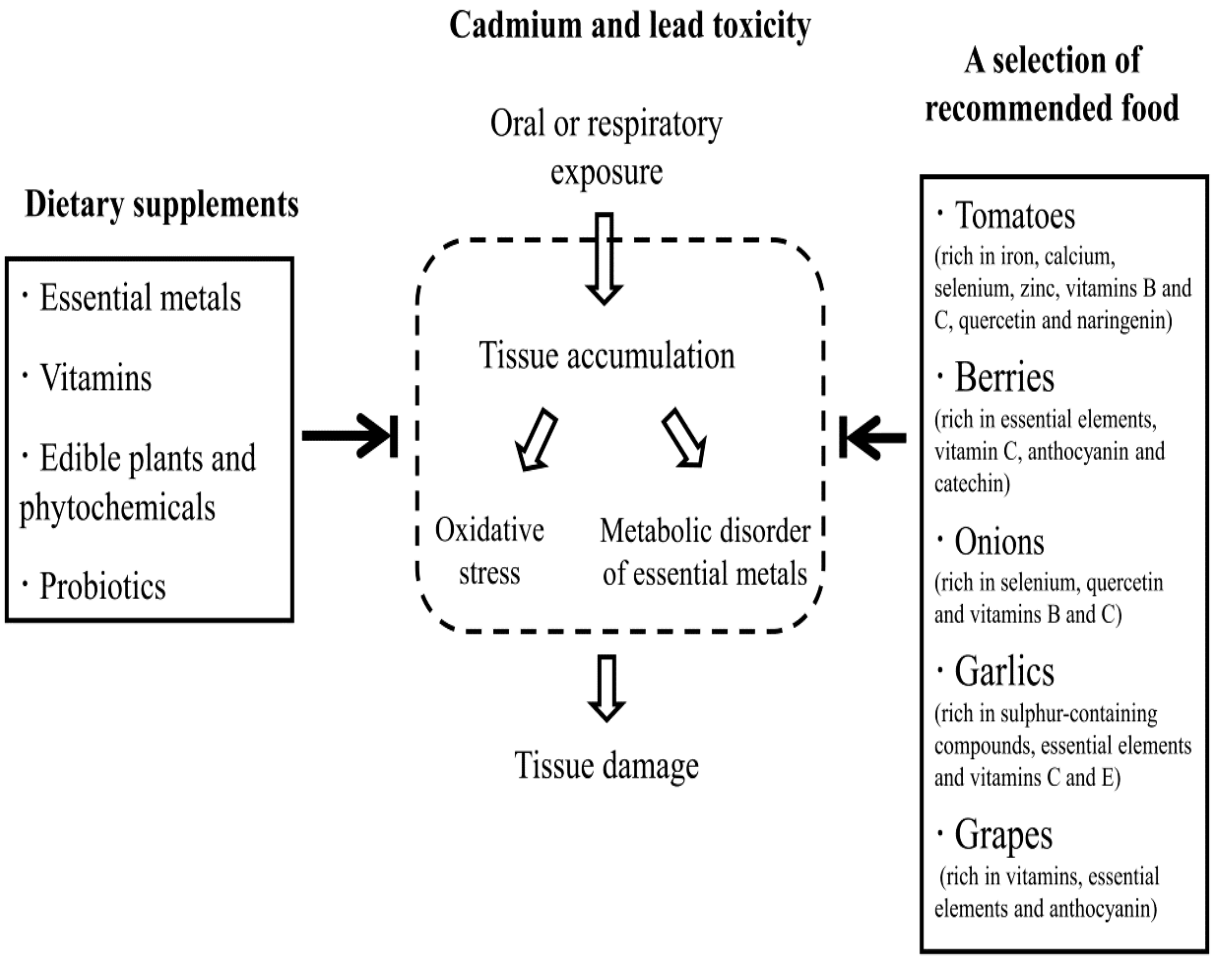


**Figure 2.3 Dietary Strategies for Lead and Cadmium Toxicity**

Dietary supplements and recommended strategy against cadmium and lead toxicity (Zhai et al., 2015)

### i. Vitamins

These are vital nutrients that can easily be obtained from diet. They include vitamins C, B1, B6 and E. Vitamin C alone, and in combination with vitamin E has proved to be effective in protecting against cadmium and lead toxicity (Acharya *et al*., 2008; El-Neweshy and El-Sayed, 2011). Pre-treatment with vitamin E protects against cadmium toxicity as evidenced in hematological values, lipid peroxide concentration and antioxidant defense system in the blood, liver and brain of rats (Nemmiche *et al*., 2007). Vitamin B6 (Pyridoxine) and B1 (thiamine) have been shown to be effective in alleviating the adverse health effects caused by lead toxicity (Gurer and Ercal, 2000; Lee *et al*., 2012).

### ii. Dietary Phytochemicals

These are natural polyphenolic compounds (flavonoids) abundant in fruits, vegetables and other edible plants. They are important dietary sources of vitamins and essential metals, hence, supplementation at adequate levels can enhance the levels of the vitamins and essential metals in the body which in turn reduce the adverse effects of cadmium and lead toxicity (Zhai *et al*., 2015). The protective effects of supplementation with soybean (Perez Diaz *et al*., 2013), garlic (Sadeghi *et al*., 2013), ginger (Reddy *et al*., 2011), green tea (Mehana *et al*., 2012), curry leaves (Mitra *et al*., 2012), grape (Pires *et al*., 2013) and tomato (Nwokocha *et al*., 2012) against cadmium and lead toxicity have been reported.

### iii. Essential Metals

Some studies have shown that supplementation with essential metals can provide protective effects against cadmium and lead toxicity. Studies in both humans and animals have reported that a deficiency in essential metals can lead to greater absorption and toxicity of cadmium and lead (Blaurock-Busch *et al*., 2012). Essential metals reported to alleviate heavy metal toxicity include zinc (Prasanthi *et al*., 2010), selenium (Liu *et al*., 2013), iron (Ryu *et al*., 2004), calcium

(Basha *et al*., 2012) as well as magnesium (Djukic-Cosic *et al*., 2007), as they contribute to the antioxidant defense system by reducing heavy metal-induced oxidative stress and enhancing the antioxidant capacity of the host (Liu *et al*., 2013).

## 2.12 Green Tea (*Camellia sinensis*)

Green tea, from the plant *Camellia sinensis* originated in the areas between India and China, the production which started in China has spread to other countries. The fresh green leaves are steamed and dried to prevent fermentation, thus, maintaining the green colour of the leaves during the drying and rolling processes. It has been shown to possess several health-promoting effects including prevention of cancer (Kavanagh *et al*. 2001) and cardiovascular disease (Sueoka *et al*. 2001), anti-inflammatory (Dona *et al*. 2003), cholesterol-lowering effects (Raederstorff *et al*. 2003), anti-oxidative (Osada *et al*. 2001), metal chelator (Chen *et al*. 2002) and supportive therapy for male infertility associated with oxidative stress (Awoniyi *et al*. 2011).

The beneficial effects of Green tea is attributed to its high catechin content (epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate(EGCG)), minerals, vitamins, polyphenols, flavonols and others (Figueiroa *et al*., 2009). Flavonoids found in Green tea are a group of polyphenolic compounds which play important role in protection against oxidative stress (Babich *et al*., 2005) by scavenging oxygen free radicals, lipid radicals, and prevent lipid peroxidation (Chumg *et al*., 2003). The commonly available green tea contains 6.5-15.4mg EC, 30.9-76.4mg EGC, 3.6-15.9mg ECG and 43.5-83.9mg EGCG per 100ml tea drinks (Henning *et al*., 2003). Consumption of green tea catechins at 800mg has been found reportedly safe in supplemental forms (Chow *et al*., 2006; Nguyen *et al*., 2012). Chacko *et al*. (2010) had earlier reported that green tea contain some trace elements including; zinc selenium, calcium, manganese, and chromium as well as vitamins B, C and E.

Fresh Green Tea

Dried Green Tea

**Figure 2.4 Fresh and Dried leaves of Green Tea (*Camellia sinensis)***

Source: www.bio-botanica.com/product/green-tea-leaf-camellia-sinensis-leaf-extract/

### 2.12.1 Effect on Blood Metals

Green tea has chelating property (El-Shahat *et al*., 2009), and has been shown to aid detoxification of heavy metals by inhibiting their absorption and promoting excretion (Paul, 2008). Green tea catechins have a potential to affect absorption as well as metabolism of metal ions because of the flavonoids which bind with heavy metal ions to form an insoluble complex ionic salt (Mira *et al*., 2002; Idowu, 2017).

Additionally, green tea supplementation or treatment has also been shown to improve some essential metals like selenium, zinc and magnesium levels in both humans (Sulibursk a et al, 2012) and experimental animals. The increased zinc level may be associated with improved antioxidant status from green tea supplementation (Hamdaoui *et al*., 2005). However, Basu *et al*., (2013) reported that serum zinc and selenium levels did not change following green tea supplementation.

### 2.12.2 Effect on Male Reproduction

Various studies have reported both positive and negative effects of green tea on male reproduction especially the hormones. Jassem *et al*., (2008) and Thuppil and Tannir, (2013) reported that green tea administration significantly increased sperm count, mobility and serum testosterone in lead treated rats. This improvement in the reproductive parameters may be due to the action of polyphenols in green tea (Ly *et al*., 2014) which are effective and efficient antioxidants reducing hydrogen peroxide ($H_2O_2$) production and scavenging oxygen free radicals (Fran *et al*., 2000). Green tea attenuated the deleterious effects of cadmium and lead on reproduction of male rats, improved testicular damage, sperm count, testosterone level (El-Beltagy *et al*., 2015; Sha'bani *et al*., 2015), and reduced LH and FSH levels (Jassem *et al*., 2008; Gawish *et al*., 2010).

On the other hand, green tea extract (GTE) polyphenols, especially epigallocatechin gallate (EGCG) have been shown to inhibit testosterone production which may be from direct or

indirect inhibition of P450 side chain cleavage and 17ß-hydroxysteroid dehydrogenase (17β-HSD) which are required for testosterone synthesis (Figueiroa *et al*., 2009). Green tea extract (GTE) was reported by Das and Karmakar, (2015) and Shyamal and Soumendra (2015) to be a potent herbal castrative agent when applied in a specific dose. In these studies, adult male rats treated with green tea leaf extract showed reduced testosterone levels which may be due to decreased activity of the steroidogenic enzymes. A significant increase in LH with no significant change in FSH, decrease in sperm count, serum testosterone level and testicular steroidogenic enzyme activities were recorded in GTE administered group of animals after 26 days (Chandra *et al*., 2011).

### 2.12.3 Effect on Cardiovascular Health

Green tea has been reported to be beneficial to the cardiovascular system by reducing cholesterol (Zheng *et al*., 2011; Zhou *et al*., 2016), coronary heart disease (Pang *et al*., 2015), hypertension (Peng *et al*., 2014) and inflammation (Oz *et al*., 2013). The catechins prevent oxidation process and atherosclerotic plaque formation in coronary heart disease (CHD) (Santesso and Manheimer, 2014). Dyslipidemia or abnormality of lipid metabolism remains one of the major risk factors for the development of cardiovascular disease.

Green tea supplementation has been proven to exert a positive effect on lipid profile by reducing total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and triglyceride (TG) concentrations, and elevating high density lipoprotein cholesterol (HDL-c) concentration (Basu *et al*., 2010; Suliburska *et al*., 2012). The presence of green tea polyphenols in the small intestine may cause reduced absorption of cholesterol from the diet (Frejnagel and Wroblewska, 2010). Nicod *et al*., (2014) however reported the inability of green tea to increase HDL-C levels.

Since green tea catechins influence luminal lipid hydrolysis and intestinal lipid absorption which may interfere with emulsification and hydrolysis, it is therefore suggestive that the

cholesterol lowering effect of catechins may partly be mediated by influencing intestinal lipid absorption (Koo and Noh, 2007). Ge *et al*., (2014) reported that three enzymes, mevalonate kinase, mevalonate diphosphate decarboxylase and farnesyl pyrophosphate synthase in the mevalonate pathway (MVP) of cholesterol biosynthesis can be simultaneously inhibited by green tea polyphenols. Therefore, green tea may function as a regulator of lipid metabolism. Some studies have reported the ability of epigallocatechin gallate (EGCG) to reduce lipid digestion and absorption, and the mechanism could be a function of increasing fecal excretion of lipids (Kim *et al*., 2012; Huang *et al*., 2013).

Additionally, increased body mass index (BMI), which is also a risk factor for cardiovascular disease (Lavie *et al*., 2008; Gregory *et al*., 2017) has been shown to reduce following green tea supplementation (Wang *et al*., 2010; Suliburska *et al*., 2012). The polyphenolic components of green tea are shown to possess anti-obesogenic effect on fat homeostasis by increasing thermogenesis, reducing fat absorption and modification of appetite (Rains *et al*., 2011; Thavanesan, 2011).

### 2.12.4 Effect on Oxidative Stress

Green tea is a widely known nutraceutical and an antioxidant. Antioxidants are compounds that protect cells against the deleterious effects of ROS like superoxide, peroxy or hydroxyl radicals, singlet oxygen and peroxynitrite. Oxidative stress is referred to as an imbalance between antioxidants and ROS which results in cellular damage. The catechins present in green tea have been shown to protect against oxidative cell damage by contributing to the antioxidant defense system in addition to antioxidant vitamins (vitamins C and E) and enzymes (superoxide dismutase and catalase (Abdel-Raheim *et al*., 2009).

Green tea supplementation have been reported to increase the total antioxidant capacity (TAC) of plasma or serum (Benzie *et al*., 1999; Sung *et al*., 2000; Suliburska *et al*., 2012; Thomson *et al*., 2012), as well as reduce malondialdehyde (MDA) (Thomson *et al*., 2012; El-Beltagy *et*

*al*., 2015; Arab *et al*., 2016) levels. MDA is one of the final products from lipid peroxidation and a marker of oxidative stress. The anti-oxidative effect of green tea catechins which ameliorates ROS and cellular damage effectively may be from direct (antioxidant) or indirect (increased activity or expression) effect (Chacko *et al*., 2010).

## 2.13 Vitamin C

Vitamin C, also known as ascorbic acid is a water soluble vitamin and antioxidant naturally present in many fruits and vegetables like oranges, watermelon, pineapple, grape fruit, cabbage, spinach, cauliflower and so on. It is also available as a dietary supplement.The recommended daily intake of vitamin C to prevent certain disease conditions like cardiovascular disorders in reported to be between 500mg and 1000mg (Ugwa, 2015). Vitamin C is a vital nutrient and antioxidant that scavenges free radicals, protecting against oxidative damage caused by heavy metals like cadmium (Ji *et al*., 2007), lead (Patrick, 2006) and mercury (Xu *et al*., 2007).

Vitamin C may as well help prevent or delay the development of certain cancers, cardiovascular diseases and other diseases in which oxidative stress plays a causal role, by limiting the damaging effects of free radicals through its antioxidant activity. Vitamin C is one of the essential vitamins in humans and primates since they lack the enzyme L-gulonolactone oxidase required in the last step of its synthetic pathway (Nishikimi and Yaki, 1991).

### 2.13.1 Effect on Male Reproduction

Vitamin C has been reported to be present in many endocrine tissues, and also plays an important role in the regulation of adrenal and gonadal steroidogenesis. Because of its high concentration in the pituitary gland, it has been hypothesized that it may play an important role in the secretion of anterior pituitary hormones including LH and FSH (Okon and Utuk, 2016). Vitamin C plays a vital role in cholesterol synthesis (Sonmez *et al*., 2005), and cholesterol is required for steroid hormone synthesis. Vitamin C is involved in the hydroxylation of steroid

hormone in the adrenal gland as well as conversion of cholesterol into steroid hormone by mediating the rate limiting hydroxylation of side chain (White *et al*., 1978). It can act as a vitaminergic transmitter that activates the release of LH and FSH from the anterior pituitary gland (Karanth *et al*., 2001). The antioxidant property of vitamin C may prevent the adverse effect of toxic metals on the hypothalamus pituitary testicular axis. Obianime and Roberts, (2009) reported the ability of vitamin C supplementation to revert testosterone, LH and FSH secretions to normal in cadmium-induced toxicity in male rats. Supplementation with vitamins maintains the pituitary gonadal axis by keeping the hormonal pattern within normal ranges by reducing the increased hormone levels to normal (Bughdadi, 2014). It has also been shown to raise blood testosterone level through the HPT axis (Ashamu *et al*., 2013). Studies have reported enhanced levels of testosterone (Fernandes *et al*., 2011; Vijayprasad *et al*., 2014; Okon and Utuk, 2016), FSH (Okon and Utuk, 2016) and LH (Fernandes *et al*., 2011), however, Okon and Utuk (2016) at the same time did not report any change in LH levels after vitamin C supplementation.

### 2.13.2 Effect on Blood Metals

Vitamin C had earlier been reported as a possible chelator of toxic metals similar to EDTA (Goyer and Cherian, 1979). It is known to increase urinary elimination of lead and reduces blood, hepatic and renal lead burden in rats. A study on 75 adult smokers receiving 1000mg vitamin C daily for one week indicated a significant reduction in blood lead levels. (Dawson *et al*., 1999). Tandon *et al*., (2001) reported a 34% reduction in lead level of 12 silver refiners after one month daily supplementation of 500mg vitamin C.

Vitamin C supplementation slightly reduced lead levels of 85 human volunteers who consumed drink containing lead (Dawson and Harris, 1997). In another study, two weeks supplementation with 500mg vitamin C reduced blood lead levels in male and female petrol station attendants as well as auto-mechanics (Onunkwor *et al*., 2004). Ugbaja *et al*. (2013) also reported a

decrease in blood lead levels after ascorbate administration in animals. Calabrese *et al*. (1987) however reported that both 500mg and 1000mg vitamin C supplementation for three months in 52 adult male subjects did not significantly affect cadmium, lead and mercury levels in blood and hair samples.

Although vitamin C may possibly affect lead absorption and secretion, its effect may be more obvious in subjects with lower dose exposure with higher vitamin supplementation. In humans exposed to high levels of lead, the reduction in blood lead levels by vitamin C is less significant (Patra *et al*., 2011). Vitamin C supplementation did not significantly reduce blood lead levels of occupationally exposed workers (Lauwerys *et al*., 1983), and also did not alter the blood and sperm levels of lead in the lead treatment rats (Hsu *et al*., 1998). Another study reported that ascorbic acid did not reduce lead burden in the liver, kidney, brain and blood (Patra *et al*., 2001). Patlar *et al*. (2012) reported that supplementation with 300mg vitamin C did not affect blood zinc levels in Taekwando players.

### 2.13.3 Effect on Cardiovascular System

Vitamin C has the ability to inhibit the oxidation of HDL ((Hillstrom, 2003), and preserves the cardioprotective ability of this lipoprotein fraction to prevent atherogenic modification of LDL (Robert *et al*., 2003). It activates the enzyme 7α-hydroxylase which enhances the conversion of plasma cholesterol into bile acid, resulting in decreased serum levels of cholesterol (Eteng *et al*., 2006). Therefore, a deficiency of vitamin C will inhibit this enzyme leading to the block in bile acid synthesis and accumulation of cholesterol in serum, and subsequently increasing the risk of atherosclerosis (Mayes 1996).

Vitamin C supplementation has been reported to reduce serum levels of cholesterol (Eteng *et al*., 2006; Gaur and Dixit, 2012; El Mashad *et al*., 2016), low density lipoprotein cholesterol (Eteng *et al*., 2006; Abdollalzad *et al*., 2009; Gaur and Dixit, 2012; El Mashad *et al*., 2016),

very low density lipoprotein cholesterol (Eteng *et al*., 2006), triglyceride and increased high density lipoprotein cholesterol levels (El Mashad *et al*., 2016).

Abdollahzad *et al*., (2013) and Eteng *et al*., (2006) reported that vitamin C supplementation did not affect the high density lipoprotein cholesterol level. Gaur and Dixit, (2012) also reported that serum levels of HDL-c, VLDL-c and TG did not change after 500mg vitamin C supplementation for one month. Vitamin C supplementation has also been reported to reduce high blood pressure (both systolic and diastolic) (Fernandes *et al*., 2011; Juraschek *et al*., 2012; Shateri *et al*., 2016). Ascorbate increases intracellular concentrations of tetrahydrobiopterin, a co-factor of endothelial nitric oxide synthase (eNOS) which promotes nitric oxide production (Huang *et al*., 2000). Vitamin C therefore, improves the potent vasodilator, nitric oxide (NO) bioactivity (Suematsu *et al*., 2010). Kim *et al*., (2002) however, found no significant change in the mean blood pressures after supplementing with 500mg vitamin C, and body mass index was also reduced after vitamin C supplementation (Huang *et al*., 2005; Johnston *et al*., 2007).

### 2.13.4 Effect on Oxidative Stress

As an antioxidant, vitamin C is capable of ameliorating oxidative stress by increasing the antioxidant activity and reducing lipid peroxidation. It is involved in antioxidant defense, thereby protecting lipid membrane and proteins from oxidative damage. It can act directly by scavenging superoxide, hydroxyl and lipid hydroperoxide radicals, or indirectly by recycling vitamin E (Powers and Jackson, 2008). Malondialdehyde (MDA) and total antioxidant capacity (TAC) are considered biomarkers of oxidative stress. Malondialdehyde is one of the final products of lipid peroxidation, and an increase in free radicals causes over production of MDA (Gawel *et al*., 2004). Total antioxidant capacity (TAC) is used to assess the antioxidant status of biological samples, and can be used to evaluate the antioxidant response against free radicals produced in a particular disease condition (Rubio *et al*., 2016).

Maintenance of adequate antioxidant levels is necessary in order to prevent or manage a number of disease conditions.

Close et al., (2006) reported increased plasma ascorbate concentration on supplementing with 1000mg vitamin C which was sufficient to scavenge free radicals, prevent lipid peroxidation and MDA formation. Tanq *et al*., (2007) as well reported that administration of 250mg vitamins C daily was effective in removing free radicals. Vitamins C supplementation was also reported to reduce malondialdehye levels (Sikka, 2004; Abdollahzad *et al*., 2009; Alkhamees, 2013; Shittu *et al*., 2013) and elevate total antioxidant capacity levels (Ozdem *et al*., 2011; Kamodyova *et al*., 2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Site

The study site is located in Destiny Layout, Emene in Enugu East Local Government Area of Enugu State, Nigeria with geographical coordinates of latitude 6.476 and longitude 7.584. The State had a population of 3,267,837 in 2006, with estimated population of 4,411,119 in 2016 based on the 2006 population census by the National Population Commission and National Bureau of Statistics. The occupational activities within the area include civil service, trading and artisanry. This study was conducted in five different mechanic workshops, and the blood metal analyses and other biochemical analyses were carried out at the facilities of Spring Board Research Centre, Awka and Chemical Pathology Laboratory, Nnamdi Azikiwe University Teaching Hospital, Nnewi respectively.

## 3.2 Sample Size

The sample size formula according to Morris (2008) for sampling a small population is given below.

$$n = \frac{NZ^2pq}{\left[E^2(N-1) + Z^2pq\right]}$$

Where; n = required sample size

N= 64 (population size)

Z=1.96 (95% confidence interval)

p = 0.5 (population proportion)

q = 0.5 (1-p)

E = 0.05 (error margin)

$$n = \frac{64 \times 1.96^2 \times 0.5(0.5)}{0.05^2\,(64-1) + 1.96^2 \times 0.5(0.5)}$$

$$n = \frac{64 \times 3.84 \times 0.25}{0.0025\,(63) + 3.84 \times 0.25}$$

$$n = \frac{61.44}{1.12} = 55$$

Minimum sample size = 55 participants

10% attrition = 5.5

55 + 5.5 = 61 participants

62 participants were enrolled in this study.

### 3.3 Study Participants

A total of one hundred and twenty four (124) male participants aged 18 to 55 years consisting of 62 automobile workers and 62 age-matched control participants were enrolled for the study. The automobile workers were made up of motor mechanics, panel beaters/spray painters, auto electricians and welders while the control group consisted mainly of civil servants and students who were not occupationally exposed to heavy metals.

### 3.4 Study Design

This study was cross sectional with an accompanying intervention among the subset of the study group. The cross sectional study was carried out first, comparing the automobile workers group with the control group while the interventional study was only on the automobile workers who were given a daily supplement of either green tea or vitamin C for a period of two months. Green tea and vitamin C supplements were chosen because of their availability and cost effectiveness.

The aim of the study was first explained to each participant and written informed consent (Appendix I) obtained. A semi-structured questionnaire (Appendix II) was used to obtain socio-demographics and anthropometric data including body mass index (BMI), blood pressure,

nature and duration of job, social habits (smoking and alcohol intake), history of any chronic disease like cardiovascular disease, hypertension, diabetes mellitus, as well as use of vitamin or mineral supplements, fertility or lipid lowering drugs from each consenting participant.

Cross Sectional Study: this part of the study involved 124 male participants assigned into two groups (Automobile workers and Control) of 62 participants each.

Interventional Study: in this part of the study, the 62 automobile workers were randomly assigned into two groups of 31 participants each. The first group received 150mL (containing 527.4mg of Catechins) of commercial green tea, while the second group received 500mg vitamin C tablet daily for a period of two months. A compliance register was provided in order to monitor compliance of the participants while taking the supplements. Of the 31 participants each on green tea and vitamin C supplements, only 25 and 27 participants respectively completed the interventional study.

### 3.5 Sampling Technique

The convenience sampling method was used in this study because only the participants who gave consent and were willing to participate in the study were recruited.

### 3.6 Inclusion Criteria

Apparently healthy consenting male participants aged 18 to 55 years who have worked in the automobile workshop for six months or more were included in the study.

### 3.7 Exclusion Criteria

Participants with any history of chronic disease like diabetes, heart disease, hypertension, or on any vitamin or mineral supplement, fertility or lipid lowering drugs as well as obese individuals (with BMI >30kg/$m^2$) were excluded from the study.

### 3.8 Ethical Consideration

Ethical clearance was sought and obtained from Nnamdi Azikiwe University Teaching Hospital Research Ethics Committee (NAUTHREC) with approval number NAUTH/CS/66/Vol. 10/ 20/2017/021 (Appendix III).

### 3.9 Gonadal Status Classification

The participants were classified into two groups based on their serum levels of testosterone, follicle stimulating hormone and luteinizing hormone. Participants with normal levels of testosterone (2.5-10.0ng/mL), follicle stimulating hormone (1.0-14.0IU/L) and luteinizing hormone (0.7-7.4IU/L) were classified as eugonadal whereas those with normal testosterone and high levels of FSH and LH were classified as compensated hypogonadism (Ventimiglia *et al*., 2018).

### 3.10 Source of Supplements

The green tea (Lipton Brand) bags were manufactured by Unilever, Nigeria with batch number 16252 and NAFDAC Registration number B1-8866, and vitamin C tablets (500mg) manufactured by Mason Natural USA with batch number 16914J.

### 3.11 Preparation and Serving of Supplements (Green tea and Vitamin C)

Two green tea bags (containing 1.6g each of dried green tea leaves) were soaked in 150mL of freshly boiled water for five minutes. Both the green tea and vitamin C (500mg) supplements were given to the participants daily at their respective workshops except on Sundays (work-free days).

### 3.12 Phytochemical Analysis of Green Tea

The phytochemical constituents of green tea was determined using the method as described by Kelly and Nelson (2014) as stated below.

Principle

This is based on separation of components of samples based on their partition co-efficient. The sample solution injected into the gas chromatograph enters a gas stream (mobile phase) which transports the sample into a column, and the quantity of each component is measured by the detector as they exit the column.

Extraction of Phytochemicals

10g of dried green tea leaves was weighed and transferred into a beaker, 100mL of ethanol was added and allowed to incubate at room temperature for 3 hours. After the reaction time, the reaction product was transferred to a separator funnel, and the tubes washed with 20mL of ethanol. The extracts were washed three times with 10mL of 10% v/v ethanol aqueous solution. The solvent was evaporated, and the final sample solubilized in 1000µL of dichloromethane from which 200µL was transferred to a vial for gas chromatography (GC) analysis.

Quantification by Gas Chromatography-Flame Ionization Detector (GC-FID)

The analysis of phytochemicals was performed on a BUCK M910 Gas Chromatography (USA) equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15µm X 250µm X 0.15µm) was used. The injector temperature was 280$^{O}$C with spitless injection of 2µL of sample and a linear velocity of 30cms$^{-1}$, with Helium 5.0pa.s was the carrier gas with a flow rate of 40mL/min. The oven operated initially at 200$^{O}$C, it was then heated to 330 $^{O}$C at a rate of 3$^{O}$C/minute and kept at this temperature for 5 minutes. The detector operated at a temperature of 320 $^{O}$C. The phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The phytochemical constituents of green tea are expressed in mg/g as shown in the table below.

**Table 3.1 Phytochemical Constituents of Lipton Green Tea (mg/g)**

| Constituents | Concentration (mg/g) |
|---|---|
| Catechin | 2.60 |
| Epicatechin (EC) | 14.30 |
| Epigallocatechin gallate (EGCG) | 63.40 |
| Epigallocatechin (EGC) | 76.30 |
| Epicatechin gallate (ECG) | 8.20 |
| Oxalate | 2.20 |
| Caffeine | 24.00 |
| Saponin | 1.27 |
| Tannin | 1.70 |
| Total Catechins = | 164.8mg/g of green tea leaves |

EGCG (the most active green tea catechin) = 63.4mg/g of green tea leaves.

2 green tea bags (3.2g of dried green tea leaves) therefore contain 202.9mg EGCG which is within the recommended mean daily intake of 90-300mg/day EGCG for effective biological functions according to the European Food Safety Authority (EFSA, 2018).

### 3.13 Measurements of Weight and Height

These were measured following standard procedure, and were used for body mass index (BMI) calculation.

#### 3.13.1 Weight Measurements

The scale was set at the zero mark in order to ensure accurate measurements using the body weight scale (Hana Scale, Model 9811b). The participants were asked to remove any heavy items from their pockets as well as heavy clothing like jackets or shoes. The participants were then asked to step on the scale, look directly forward, and stay still on the scale, and the reading was then taken.

#### 3.13.2 Height Measurements

The participants were asked to remove their shoes, stand erect with the back against the vertical stand the Stadiometer (Model HM01), and head directly underneath the dropdown measuring device prior to taking the measurement. The measuring device was then lowered until it gently rests on top of the head, and reading taken.

#### 3.13.3 BMI Calculation

This calculation was carried out by dividing the body weight of participant by the body height, as shown in the formula;

BMI ($kg/m^2$) = Weight (in kilogram)/Height$^2$ (in meter)

### 3.14 Blood Pressure Measurements

The blood pressure (systolic and diastolic) of the participants were measured by a trained and qualified Nurse using the ausculatory method with Aneroid Sphygmomanometer as described by Pickering *et al.*, (2005). The blood pressures of the participants were measured in the

morning before the commencement of the day's activity at baseline, and after one and two months of supplementation.

Participant Preparation

The participants were instructed to remove all clothing covering the location of cuff placement. They were comfortably seated and relaxed for about five minutes, with back support, uncrossed legs, and feet perfectly resting on the floor. They were also instructed not to talk during the measurement procedure.

Measurement

An appropriate blood pressure cuff was placed over the brachial artery of the left arm about 3cm above the antecubital fossa to minimize noise produced when the stethoscope touches the cuff. The stethoscope was then placed over the brachial artery, and cuff inflated to above the systolic pressure. As it was gradually deflated, korotkoff sounds were detected by the stethoscope and monitored until it completely disappeared. The needle pointer on the aneroid sphygmomanometer scale where the first clear tapping sound appeared was recorded as the systolic blood pressure while the point where the sound disappeared was recorded as the diastolic blood pressure. The readings were taken twice and the average recorded as the participant's blood pressure.

### 3.15 Sample Collection and Biochemical Analyses

After an overnight fast, 8milliliters (mL) of blood samples was collected from the participants between 8.00am and 10.30am by standard venipuncture method as described by Lewis *et al*. (2006) for the measurement of biochemical parameters. The blood samples were collected prior to intervention (at baseline) as well as after one and two months of supplementation. 3mL of blood was dispensed into $K_2$-EDTA vacutainer tubes for blood metals (lead, cadmium) and trace elements (selenium, zinc) analyses and stored at $4^{O}C$ while 5mL of blood was dispensed

into plain vacutainer tubes, centrifuged at 3000rpm for five minutes and the sera obtained were stored in aliquots at -20$^{O}$C for serum testosterone, follicle stimulating hormone, luteinizing hormone, malondialdehyde, total antioxidant capacity, total cholesterol, high density lipoprotein and triglyceride analyses. All samples were analyzed within two weeks of collection.

## 3.16 Methods

### 3.16.1 Determination of Lead Level

Blood lead was analysed using Varian AA240 Atomic Absorption Spectrophotometer (AAS) (USA) according to the method of American Public Health Association (APHA) 1995.

Principle

The working principle of AAS is based on the sample being aspirated into the flame and atomized when the AAS light beam from the lead hollow cathode lamp is directed through the flame into the monochromator and unto the detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of lead in the sample.

Procedure

The blood samples were first digested according to the method described by Adrian, (1973). 1ml of nitric acid was added to 1ml of blood sample, mixed properly, boiled at 100$^{O}$C for 30 minutes, made up to 10ml with deionized water, and lead concentration measured using atomic absorption spectrophotometer.

Preparation of reference solutions

A series of standard lead solutions in the optimum concentration range were prepared by diluting lead stock solution with water containing 1.5ml concentrated nitric acid per liter. The blank was prepared using the reagents, and a calibration curve for lead was prepared by plotting the absorbance of standard against the concentrations.

### 3.16.2 Determination of Cadmium Level

Blood cadmium was analysed using Varian AA240 Atomic Absorption Spectrophotometer (AAS) (USA) according to the method of American Public Health Association (APHA) 1995.

Principle

The working principle of AAS is based on the sample being aspirated into the flame and atomized when the AAS light beam from the cadmium hollow cathode lamp is directed through the flame into the monochromator and unto the detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of cadmium in the sample.

Procedure

The blood samples were first digested according to the method described by Adrian, (1973). 1ml of nitric acid was added to 1ml of blood sample, mixed properly, boiled at $100^{O}C$ for 30 minutes, made up to 10ml with deionized water, and cadmium concentration measured using atomic absorption spectrophotometer.

Preparation of reference solutions

A series of standard cadmium solutions in the optimum concentration range were prepared by diluting cadmium stock solution with water containing 1.5ml concentrated nitric acid per liter. The blank was prepared using the reagents, and a calibration curve for cadmium was prepared by plotting the absorbance of standard against the concentrations.

### 3.16.3 Determination of Selenium Level

Blood cadmium was analysed using Varian AA240 Atomic Absorption Spectrophotometer (AAS) (USA) according to the method of American Public Health Association (APHA) 1995.

Principle

The working principle of AAS is based on the sample being aspirated into the flame and atomized when the AAS light beam from the selenium hollow cathode lamp is directed through the flame into the monochromator and unto the detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of selenium in the sample.

Procedure

The blood samples were first digested according to the method described by Adrian, (1973). 1ml of nitric acid was added to 1ml of blood sample, mixed properly, boiled at $100^{O}C$ for 30 minutes, made up to 10ml with deionized water, and selenium concentration measured using atomic absorption spectrophotometer.

Preparation of reference solutions

A series of standard selenium solutions in the optimum concentration range were prepared by diluting cadmium stock solution with water containing 1.5ml concentrated nitric acid per liter. The blank was prepared using the reagents, and a calibration curve for selenium was prepared by plotting the absorbance of standard against the concentrations.

### 3.16.4 Determination of Zinc Level

Blood zinc was analysed using Varian AA240 Atomic Absorption Spectrophotometer (AAS) (USA) according to the method of American Public Health Association (APHA) 1995.

Principle

The working principle of AAS is based on the sample being aspirated into the flame and atomized when the AAS light beam from the zinc hollow cathode lamp is directed through the flame into the monochromator and unto the detector that measures the amount of light absorbed

by the atomized element in the flame. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of zinc in the sample.

Procedure

The blood samples were first digested according to the method described by Adrian, (1973). 1ml of nitric acid was added to 1ml of blood sample, mixed properly, boiled at $100^{O}C$ for 30 minutes, made up to 10ml with deionized water, and zinc concentration measured using atomic absorption spectrophotometer.

Preparation of reference solutions

A series of standard zinc solutions in the optimum concentration range were prepared by diluting zinc stock solution with water containing 1.5ml concentrated nitric acid per liter. The blank was prepared using the reagents, and a calibration curve for zinc was prepared by plotting the absorbance of standard against the concentrations.

### 3.16.5 Determination of Testosterone Level (Monobind Inc, USA)

The serum testosterone level was estimated by enzyme-linked immunosorbent assay (ELISA) according to the method described by Tateiki *et al*., 1977.

Principle

This is based on solid phase enzyme linked immunosorbent assay (ELISA). Upon mixing of the biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competitive reaction results between the native antigen and enzyme-antigen conjugate for a limited number of antibody binding sites.

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

$Ab_{Btn}$; Biotinylated Antibody (constant quantity)

Ag; Native Antigen (variable quantity)

$^{Enz}Ag$; Enzyme-Antigen Conjugate (constant quantity)

$AgAb_{Btn}$; Antigen-Antibody Complex

$^{Enz}AgAb_{Btn}$; Enzyme-Antigen Conjugate-Antibody Complex

$k_a$; Rate constant of association

$k_{-a}$; Rate constant of disassociation

$k = k_a / k_{-a}$; Equilibrium constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs, this affects the separation of the antibody bound fraction after decantation or aspiration.

$$AgAb_{Btn} + {}^{Enz}AgAb_{Btn-} + Streptavidin_{cw} \longrightarrow \text{Immobilized complex}$$

$Streptavidin_{cw}$; Streptavidin immobilized on well

Immobilized complex; Sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration (standards) a dose response curve can be generated, from which the antigen concentration of the unknown can be ascertained.

Procedure

All reagents, standards, serum and control samples were brought to room temperature before proceeding with the assay. 10µl each of standard, serum and control samples were pipetted into the wells, 50µl of the working testosterone enzyme reagent solution was also added to all the wells, mixed by swirling and incubated at room temperature for 60 minutes. After incubation, the contents of the microplate were discarded by decantation, washed three times manually using the wash buffer (20ml of wash concentrate in 1000ml of distilled water) and blotted on absorbent paper. 100µl of the working substrate solution was then added to all the wells, incubated at room temperature for 15 minutes. 50µl of the stop solution was then added to each

well, mixed gently, and the absorbance in each well read at 450nm within 30 minutes in Mindray 96A microplate reader.

### 3.16.6 Determination of Follicle Stimulating Hormone Level (Monobind Inc, USA)

The serum FSH level was estimated using ELISA according to the method described by Odell and Parlow, 1981.

Principle

This is based on solid phase enzyme-linked immunosorbent assay (ELISA). The microplate wells are coated with a monoclonal antibody directed towards a unique antigenic site on a FSH molecule. The immobilization takes place during the assay at the surface of the microplate well through the interaction of streptavidin coated on the well and the exogenously added biotinylated monoclonal anti-FSH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and a serum containing the native antigen, reaction occurs between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex.

$^{Enz}Ab_{(p)} + Ag_{FSH} + {}^{Btn}Ab_{(m)} \qquad {}^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$

$^{Btn}Ab_{(m)}$; Biotinylated Monoclonal Antibody (excess quantity)

$Ag_{FSH}$; Native Antigen (variable quantity)

$^{Enz}Ab_{(p)}$; Enzyme-labelled Antibody (excess quantity)

$^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$; Antigen-Antibodies Sandwich Complex

$k_a$; Rate constant of association

$k_{-a}$; Rate constant of disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

$^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{cw} \longrightarrow$ Immobilized complex

$Streptavidin_{cw}$; Streptavidin immobilized on well

Immobilized complex; Antibodies-Antigen Sandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of the unknown can be ascertained.

Procedure

All reagents, standards, serum and control samples were brought to room temperature before proceeding with the assay. 50µl each of serum and control samples were pipetted into the wells, 100 µl of FSH-enzyme reagent solution was then added to the wells, mixed by swirling and incubated at room temperature for 60 minutes. After incubation, the contents of the microplate wells were discarded by decantation, washed three times manually using the wash buffer (20ml of wash concentrate in 1000ml of distilled water), and blotted on absorbent paper. 100µl of the working substrate solution was added to all the wells, incubated at room temperature for 15 minutes. 50µl of the stop solution was then added to each well, mixed gently, and the absorbance in each well read at 450nm within 30 minutes in Mindray 96A microplate reader.

### 3.16.7 Determination of Luteinizing Hormone Level (Monobind Inc, USA)

The serum LH level was estimated using ELISA according to the method described by Kosasa, 1981.

Principle

This is based on solid phase enzyme-linked immunosorbent assay (ELISA). The microplate wells are coated with a monoclonal antibody directed towards a unique antigenic site on a LH molecule. The immobilization takes place during the assay at the surface of the microplate well through the interaction of streptavidin coated on the well and the exogenously added

biotinylated monoclonal anti-LH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and a serum containing the native antigen, reaction occurs between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex.

$$^{Enz}Ab_{(p)} + Ag_{LH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$; Biotinylated Monoclonal Antibody (excess quantity)

$Ag_{LH}$; Native Antigen (variable quantity)

$^{Enz}Ab_{(p)}$; Enzyme-labelled Antibody (excess quantity)

$^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)}$; Antigen-Antibodies Sandwich Complex

$k_a$; Rate constant of association

$k_{-a}$; Rate constant of disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

$$^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)} + Streptavidin_{cw} \longrightarrow \text{Immobilized complex}$$

$Streptavidin_{cw}$; Streptavidin immobilized on well

Immobilized complex; Antibodies-Antigen Sandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of the unknown can be ascertained.

Procedure

All reagents, standards, serum and control samples were brought to room temperature before proceeding with the assay. 50µl each of serum and control samples were pipetted into the wells, 100 µl of LH-enzyme reagent solution was then added to the wells, mixed by swirling and

incubated at room temperature for 60 minutes. After incubation, the contents of the microplate wells were discarded by decantation, washed three times manually using the wash buffer (20ml of wash concentrate in 1000ml of distilled water), and blotted on absorbent paper. 100µl of the working substrate solution was added to all the wells, incubated at room temperature for 15 minutes. 50µl of the stop solution was then added to each well, mixed gently, and the absorbance in each well read at 450nm within 30 minutes in Mindray 96A microplate reader.

### 3.16.8 Determination of Malondialdehyde Level

This was determined using the colorimetric method described by Gutteridge and Wilkins (1982).

Principle

MDA is a product of lipid peroxidation, and when heated with 2-thiobarbituric acid (TBA) under alkaline condition forms a pink coloured complex which has absorption maximum at 532nm. The intensity of the colour generated is directly proportional to the concentration of MDA in the sample.

Procedure

1ml of 1% TBA dissolved in alkaline medium (0.05M sodium hydroxide) was added to 100µl of serum in a test tube, these were mixed thoroughly after which 1ml of glacial acetic acid was added, shaken, incubated at $100^{O}C$ for 15 minutes and allowed to cool. Turbidity was removed by centrifuging at 3000rpm for 10 minutes, and the absorbance of the supernatant read at 532nm. The blank was prepared by adding the same volume of TBA and glacial acetic acid to 100µl of distilled water. The level of MDA in serum was expressed in µmol/l using the molar extinction co-efficient for MDA ($1.56 \text{ X } 10^5 \text{ M}^{-1}\text{cm}^{-1}$).

Calculation

$\text{MDA } (\mu\text{mol/l}) = \text{Absorbance} \times 1000000/E_{532}$

Where; $E_{532}$ = Molar Extinction Co-efficient for MDA

### 3.16.9 Determination of Total Antioxidant Capacity Level

This was estimated using the Ferric Reducing Ability of Plasma (FRAP) method as described by Benzie and Strain, (1996).

Principle

At low pH, antioxidant power causes the reduction of ferric tripyridyl triazine (FeIIITPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorbance at 593nm in mixture (test) with those containing ferrous ion in known concentration (standard).

Procedure

Firstly, a working reagent containing acetate buffer (pH 3.6), ferric chloride and TPTZ in the ratio of 10:1:1 respectively was prepared. 1.8ml each of the working reagent was added to 60µl each of serum, standard and distilled water in test tubes labelled as sample, standard and blank respectively. The content of each tube was mixed thoroughly, incubated at $37^{O}C$ for 10 minutes, and the resulting blue coloured solution was read at 593nm. The standard solution contains 1000µmol/l of ferrous sulphate.

Calculation

$$\text{TAC (µmol/l)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (1000)}$$

### 3.16.10 Determination of Total Cholesterol Level (Randox Diagnostics, UK)

This was estimated by enzymatic colorimetric method as described by Roeschlaw *et al*., (1974).

Principle

Cholesterol is determined after enzymatic hydrolysis and oxidation, the indicator quinoneimine is formed from hydrogen peroxide ($H_2O_2$) and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol Ester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{cholesterol}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure

1000µl of cholesterol reagent was added to 10µl each of the serum, standard, control and distilled water in test tubes, and labelled respectively as test, standard, control and blank. The content of each tube was mixed and incubated at room temperature for 10 minutes, and the absorbances were measured against the reagent blank at 500nm.

Calculation

$$\text{Total Cholesterol (mmol/l)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (5.27)}$$

### 3.16.11 Determination of High Density Lipoprotein Cholesterol Level (Randox Diagnostics, UK)

HDL-C was estimated by precipitation and enzymatic colorimetric reaction according to the method described by Grove, (1979).

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant is determined.

Procedure

500µl of the diluted precipitant and 200µl of sample, standard, control and distilled water were added into appropriate test tubes, mixed, incubated and centrifuged at room temperature for 10

minutes. 1000µl of cholesterol reagent was added to 10µl each of the supernatant, mixed and incubated at room temperature for 10 minutes. The absorbance was measured at 500nm against reagent blank.

Calculation

$$\text{HDL-C (mmol/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (1.3)}$$

### 3.16.12 Determination of Triglyceride Level (Randox Diagnostics, UK)

This was estimated by enzymatic colorimetric reaction according to the method described by Fossati and Prencipe (1982).

Principle

TG measurements are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from $H_2O_2$, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow[\text{oxidase}]{\text{glycerol phosphate}} \text{Dihydroxyacetone Phosphate} + H_2O$$

$$2H_2O_2 + \text{4-Aminophenazone} + \text{4-Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + \text{HCL} + 4H_2O$$

Principle

1000µl of the TG reagent was added to 10µl each of serum, standard, control and distilled water, mixed and incubated at room temperature for 10 minutes. Absorbance was measured against the reagent blank at 500nm.

Calculation

$$TG\ (mmol/L) = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (2.15)}$$

### 3.16.13 Determination of Low Density Lipoprotein Cholesterol Level

The LDL-C was estimated by calculation according to the formula given by Friedewald *et al*., (1972).

Calculation

$$LDL\text{-}C\ (mmol/L) = \text{Total Cholesterol} - HDLC - 0.456 \times TG$$

### 3.16.14 Determination of Very Low Density Lipoprotein Cholesterol Level

The VLDL-C was estimated by calculation according to the formula given by Friedewald *et al*., (1972).

Calculation

$$VLDL\text{-}C\ (mmol/L) = 0.456 \times TG$$

## 3.17 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 was used for statistical analysis. Data were checked for normality, and expressed as mean ± standard deviation and median. Chi-Square was used to assess the relationships between categorical variables, the independent student's t-test or Man Whitney U test was used to assess the mean differences between two independent variables, and Wilcoxon test used to assess two related variables. Pearson's correlation co-efficient was used to assess the level of association between two variables, Kruskal wallis test was used to compare the mean differences among groups, and pairwise comparison used to assess the inter group variability. The level of significance was considered at $p<0.05$.

### 3.18 Quality Control

Pooled sera and blood from apparently healthy male individuals were used as controls, the control samples were placed at intervals and analyzed alongside the other samples during biochemical assay. After each run (within and between day), the values of the control samples were recorded, from which the means and standard deviations (SD) were obtained for calculation of co-efficient of variation (CV= SD/mean x 100) of each biochemical parameter for the determination of intra and inter assay precision of biochemical measurements. The tables below show the means, standard deviations and co-efficient of variations of the control samples for within (intra) and between (inter) assay.

**Table 3.2 CV of Control Samples for Intra (Within) Assay Precision (at Baseline).**

| Parameters | Mean ± SD | CV (%) |
|---|---|---|
| Testosterone (ng/mL) | 7.41 ± 0.27 | 3.6 |
| FSH (IU/L) | 9.03 ± 0.17 | 1.9 |
| LH (IU/L) | 5.06 ± 0.13 | 2.6 |
| MDA (µmol/L) | 1.64 ± 0.04 | 2.4 |
| TAC (µmol/L) | 903.06 ± 6.07 | 0.7 |
| TC (mmol/L) | 4.62 ± 0.13 | 2.8 |
| HDLC (mmol/L) | 1.30 ± 0.02 | 1.5 |
| TG (mmol/L) | 0.91 ± 0.01 | 1.1 |
| Cadmium (µg/dL) | 0.22 ± 0.01 | 4.5 |
| Lead (µg/dL) | 5.09 ± 0.17 | 3.3 |
| Selenium (µg/dL) | 10.76 ± 0.17 | 1.6 |
| Zinc (µg/dL) | 102.49 ± 1.58 | 1.5 |

FSH- Follicle Stimulating Hormone. LH- Luteinizing Hormone. MDA- Malondialdehyde. TAC- Total Antioxidant Capacity. TC- Total Cholesterol. HDL- High Density Lipoprotein. TG- Triglyceride.

**Table 3.3 CV of Control Samples for Intra (Within) Assay Precision (at One Month Intervention).**

| Parameters | Mean ± SD | CV (%) |
|---|---|---|
| Testosterone (ng/mL) | 7.29 ± 0.20 | 2.7 |
| FSH (IU/L) | 9.07 ± 0.14 | 1.5 |
| LH (IU/L) | 5.07 ± 0.13 | 2.6 |
| MDA (µmol/L) | 1.68 ± 0.03 | 1.8 |
| TAC (µmol/L) | 908.48 ± 15.36 | 1.7 |
| TC (mmol/L) | 4.62 ± 0.12 | 2.6 |
| HDLC (mmol/L) | 1.30 ± 0.01 | 0.8 |
| TG (mmol/L) | 0.92 ± 0.02 | 2.2 |
| Cadmium (µg/dL) | 0.22 ± 0.01 | 4.5 |
| Lead (µg/dL) | 5.08 ± 0.18 | 3.3 |
| Selenium (µg/dL) | 10.92 ± 0.12 | 1.1 |
| Zinc (µg/dL) | 101.22 ± 1.07 | 1.1 |

FSH- Follicle Stimulating Hormone. LH- Luteinizing Hormone. MDA- Malondialdehyde. TAC- Total Antioxidant Capacity. TC- Total Cholesterol. HDL- High Density Lipoprotein. TG- Triglyceride.

**Table 3.4 CV of Control Samples for Intra (Within) Assay Precision (at Two Months Intervention).**

| Parameters | Mean ± SD | CV (%) |
|---|---|---|
| Testosterone (ng/mL) | 7.34 ± 0.25 | 3.4 |
| FSH (IU/L) | 9.08 ± 0.15 | 1.7 |
| LH (IU/L) | 5.09 ± 0.15 | 2.9 |
| MDA (µmol/L) | 1.67 ± 0.03 | 1.8 |
| TAC (µmol/L) | 901.45 ± 7.15 | 0.8 |
| TC (mmol/L) | 4.65 ± 0.12 | 2.6 |
| HDLC (mmol/L) | 1.29 ± 0.02 | 1.6 |
| TG (mmol/L) | 0.92 ± 0.02 | 2.2 |
| Cadmium (µg/dL) | 0.21 ± 0.01 | 4.8 |
| Lead (µg/dL) | 5.09 ± 0.17 | 3.3 |
| Selenium (µg/dL) | 10.75 ± 0.19 | 1.8 |
| Zinc (µg/dL) | 102.00 ± 1.11 | 1.1 |

FSH- Follicle Stimulating Hormone. LH- Luteinizing Hormone. MDA- Malondialdehyde. TAC- Total Antioxidant Capacity. TC- Total Cholesterol. HDL- High Density Lipoprotein. TG- Triglyceride.

**Table 3.5 CV of Control Samples for Inter (Between) Assay Precision.**

| Parameters | Mean ± SD | CV (%) |
|---|---|---|
| Testosterone (ng/mL) | 7.35 ± 0.24 | 3.3 |
| FSH (IU/L) | 9.06 ± 0.15 | 1.7 |
| LH (IU/L) | 5.07 ± 0.14 | 2.8 |
| MDA (µmol/L) | 1.66 ± 0.03 | 1.8 |
| TAC (µmol/L) | 904.33 ± 9.53 | 1.1 |
| TC (mmol/L) | 4.63 ± 0.12 | 2.6 |
| HDLC (mmol/L) | 1.30 ± 0.02 | 1.5 |
| TG (mmol/L) | 0.92 ± 0.02 | 2.2 |
| Cadmium (µg/dL) | 0.22 ± 0.01 | 4.5 |
| Lead (µg/dL) | 5.09 ± 0.17 | 3.3 |
| Selenium (µg/dL) | 10.81 ± 0.16 | 1.5 |
| Zinc (µg/dL) | 101.90 ± 1.25 | 1.2 |

FSH- Follicle Stimulating Hormone. LH- Luteinizing Hormone. MDA- Malondialdehyde. TAC- Total Antioxidant Capacity. TC- Total Cholesterol. HDL- High Density Lipoprotein. TG- Triglyceride.

# CHAPTER FOUR

# RESULTS

The results from this study are summarized and represented in the tables below.

## 4.1 Demographics and Social Habits of Automobile Workers and Controls.

In table 4.1, there were no significant associations in the marital status, smoking and alcohol consuming habits between the automobile workers and control group ($p=0.718$, $p=0.849$ and $p=0.169$ respectively), however, automobile workers had lower educational level than the controls ($p<0.0001$).

## 4.2 Age, Anthropometric Parameters and Blood Pressure Values in Automobile Workers and Controls.

There was no significant difference in the ages of the automobile workers and control group ($p = 0.445$). The mean values of weight, height and body mass index (BMI) in automobile workers did not differ significantly from that of the control group ($p = 0.850$, $p = 0.366$ and 0.224 respectively) whereas the mean values of systolic and diastolic blood pressures were significantly higher in the automobile workers when compared with the control group ($p = 0.013$ and 0.010 respectively) as shown in table 4.2.

**Table 4.1: Demographics and Social Habits of Automobile Workers and controls**

| Characteristics | AMW (n = 62) | Controls (n = 62) | $\chi^2$ | p-value |
|---|---|---|---|---|
| **Marital Status** | | | | |
| Married | 29 (46.8%) | 27 (43.5%) | 0.130 | 0.718 |
| Single | 33 (53.2%) | 35 (56.5%) | | |
| **Educational Level** | | | | |
| Tertiary | 0 (0%) | 30 (48.8%) | 44.552 | 0.0001* |
| Secondary | 43 (69.4%) | 29 (46.8%) | | |
| Primary | 17 (27.4%) | 3 (4.8%) | | |
| No formal Education | 2 (3.2%) | 0 (0%) | | |
| **Alcohol** | | | | |
| Yes | 47 (75.8%) | 40 (64.5%) | 1.888 | 0.169 |
| No | 15 (24.2%) | 22 (35.5%) | | |
| **Smoking** | | | | |
| Yes | 21 (33.9%) | 20 (32.3%) | 0.036 | 0.849 |
| No | 41 (66.1%) | 42 (67.7%) | | |

AMW - Automobile Workers. * - Significant.

**Table 4.2: Anthropometric Parameters and Blood Pressure Values in Automobile Workers and Controls.**

| Parameters | AMW (n = 62) | Controls (n = 62) | t | p-value |
|---|---|---|---|---|
| Age (years) | 32.81 ± 10.71 | 31.39 ± 9.88 | 0.77 | 0.445 |
| **Anthropometric Parameters** | | | | |
| Weight (kg) | 74.40 ± 14.79 | 73.89 ± 15.50 | 0.19 | 0.850 |
| Height (m) | 1.75 ± 0.08 | 1.76 ± 0.09 | 0.91 | 0.366 |
| BMI (kg/m2) | 24.20 ± 3.33 | 23.51 ± 3.01 | 1.22 | 0.224 |
| **Blood Pressure** | | | | |
| SBP (mmHg) | 123.79 ± 10.97 | 119.03 ± 9.91 | 2.54 | 0.013* |
| DBP (mmHg) | 81.94 ± 8.17 | 78.31 ± 7.18 | 2.63 | 0.010* |

* - Significant. AMW - Automobile Workers. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure.

## 4.3 Levels of Elements and Oxidative Stress Markers in Automobile Workers and Controls.

The mean blood levels of lead, cadmium and serum malondialdehyde were significantly higher in the automobile workers (48.18 ± 9.91µg/dl, 2.40 ± 0.26 µg/dl and 2.32 ± 0.74µmol/l respectively) when compared to the control group (13.67 ± 2.36µg/dl, 0.14 ± 0.02 µg/dl and 1.79 ± 0.30µmol/l respectively). The mean blood levels of selenium, zinc and serum total antioxidant capacity were significantly lower in the automobile workers (6.81 ± 1.03µg/dl, 99.15 ± 15.40 µg/dl and 725.30 ± 128.73µmol/l respectively) when compared with the control group (15.38 ± 2.02µg/dl, 110.01 ± 24.31 µg/dl and 807.01 ± 106.81µmol/l respectively) as shown in table 4.3.

## 4.4 Sex Hormones and Lipid Profile Levels in Automobile Workers and Controls.

The mean serum level of testosterone was significantly lower in the automobile workers when compared with the control group ($p = 0.0001$). The mean serum levels of follicle stimulating hormone, luteinizing hormone, total cholesterol, low density lipoprotein, very low density lipoprotein and triglyceride were significantly higher in the automobile workers when compared to the control group ($p = 0.0001$, $p = 0.0001$, $p = 0.0001$, $p = 0.0001$, $p = 0.008$, $p = 0.011$ respectively). However, the mean serum levels of high density lipoprotein cholesterol in both automobile workers (1.19 ± 0.08mmol/l) and control group (1.20 ± 0.09mmol/l) were not significantly different ($p = 0.263$) as shown in table 4.4.

**Table 4.3: Levels of Elements and Oxidative Stress Markers in Automobile Workers and Controls.**

| Parameters | AMW (n = 62) | Controls (n = 62) | t | p-value |
|---|---|---|---|---|
| **Elements** | | | | |
| Lead (µg/dL) | 48.18 ± 9.91 | 13.67 ± 2.36 | 13.34 | 0.0001* |
| Cadmium (µg/dL) | 2.40 ± 0.26 | 0.14 ± 0.02 | 8.52 | 0.0001* |
| Selenium (µg/dL) | 6.81 ± 1.03 | 15.38 ± 2.02 | 13.54 | 0.0001* |
| Zinc (µg/dL) | 99.15 ± 15.40 | 110.01 ± 24.31 | 2.97 | 0.004* |
| **Oxidative Stress Markers** | | | | |
| MDA (µmol/L) | 2.32 ± 0.74 | 1.79 ± 0.30 | 3.47 | 0.001* |
| TAC (µmol/L) | 725.30 ± 128.73 | 807.01 ± 106.81 | 3.84 | 0.0001* |

AMW - Automobile Workers. * - Significant. MDA – Malondialdehyde. TAC - Total Antioxidant Capacity.

**Table 4.4: Sex Hormones and Lipid Profile Levels in Automobile Workers and Controls.**

| Parameters | AMW (n = 62) | Controls (n = 62) | t | p-value |
|---|---|---|---|---|
| **Sex Hormones** | | | | |
| Testosterone (ng/mL) | 6.85 ± 1.91 | 8.17 ± 2.00 | -3.74 | 0.0001* |
| FSH (IU/L) | 12.10 ± 3.12 | 9.13 ± 2.20 | 5.31 | 0.0001* |
| LH (IU/L) | 7.16 ± 2.18 | 5.75 ± 1.00 | 4.62 | 0.0001* |
| **Lipid Profile** | | | | |
| TC (mmol/L) | 5.05 ± 0.87 | 4.29 ± 0.62 | 5.55 | 0.0001* |
| HDLC (mmol/L) | 1.19 ± 0.08 | 1.20 ± 0.09 | 1.13 | 0.263 |
| LDLC (mmol/L) | 3.43 ± 0.73 | 2.73 ± 0.54 | 5.89 | 0.0001* |
| VLDLC (mmol/L) | 0.43 ± 0.08 | 0.37 ± 0.04 | 2.70 | 0.008* |
| TG (mmol/L) | 0.95 ± 0.17 | 0.83 ± 0.09 | 2.59 | 0.011* |

AMW - Automobile Workers. * - Significant. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL-High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG Triglyceride.

## 4.5 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Levels in Automobile Workers based on Profession.

Among the different professional groups of automobile workers; mechanics, panel beaters/spray painters and autoelectricians/welders, there were no significant differences in the mean values of body mass index ($p = 0.411$), systolic blood pressure ($p = 0.610$) and diastolic blood pressure ($p = 0.903$). Additionally, there were no significant differences in the mean levels of lead ($p = 0.906$), cadmium ($p = 0.185$), selenium ($p = 0.344$), zinc ($p = 0.797$), malondialdehyde ($p = 0.518$) and total antioxidant capacity ($p = 0.923$) among the different groups of automobile workers as shown in table 4.5.

## 4.6 Sex Hormones and Lipid Profile Levels in Automobile Workers based on Profession.

There were no significant differences in the mean levels of testosterone ($p = 0.730$), follicle stimulating hormone ($p = 0.978$), luteinizing hormone ($p = 0.296$), total cholesterol ($p = 0.630$), high density lipoprotein ($p = 0.654$), low density lipoprotein ($p = 0.675$), very low density lipoprotein ($p = 0.552$) and triglyceride ($p = 0.552$) among the different professional groups of automobile workers (mechanics, panel beaters/spray painters and autoelectricians/welders) as shown in table 4.6.

**Table 4.5: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW based on Profession.**

Data expressed as Mean ± SD (Median)

| Parameters | Mechanics (n = 41) | SP/PB (n = 11) | AE/Welders (n = 10) | H | p-value |
|---|---|---|---|---|---|
| BMI ($kg/m^2$) | 24.10 ± 3.35 (23.70) | 24.01 ± 3.56 (23.26) | 25.26 ± 3.08 (25.50) | 1.778 | 0.411 |
| **Blood Pressure** | | | | | |
| SBP (mmHg) | 124.76 ± 10.84 (125.00) | 120.91 ± 12.81 (120.00) | 123.00 ± 9.78 (122.50) | 0.998 | 0.610 |
| DBP (mmHg) | 82.07 ± 7.66 (80.00) | 80.91 ± 10.09 (80.00) | 82.50 ± 7.55 (80.00) | 0.203 | 0.903 |
| **Elements** | | | | | |
| Lead (µg/dL) | 49.34 ± 10.30 (55.24) | 47.21 ± 10.55 (41.92) | 44.48 ± 7.60 (37.78) | 0.198 | 0.906 |
| Cadmium (µg/dL) | 2.74 ± 0.32 (2.20) | 1.96 ± 0.61 (1.50) | 1.46 ± 0.65 (0.18) | 3.372 | 0.185 |
| Selenium (µg/dL) | 7.17 ± 1.60 (6.90) | 6.00 ± 1.53 (4.64) | 6.20 ± 1.17 (6.93) | 2.135 | 0.344 |
| Zinc (µg/dL) | 98.79 ± 13.17 (99.81) | 98.44 ± 22.74 (110.00) | 101.38 ± 15.99 (109.29) | 0.455 | 0.797 |
| **Oxidative Stress Markers** | | | | | |
| MDA (µmol/L) | 2.35 ± 0.51 (2.10) | 2.21 ± 0.68 (1.85) | 2.33 ± 0.74 (1.86) | 1.314 | 0.518 |
| TAC (µmol/L) | 720.90 ± 139.35 (711.00) | 730.00 ± 68.06 (723.00) | 738.30 ± 143.40 (776.50) | 0.080 | 0.923 |

AMW - Automobile Workers. SP/PB - Spray Painters/Panel Beaters. AE - Auto Electricians. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA – Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics - Kruskal-Wallis Test (H)

**Table 4.6: Sex Hormones and Lipid Profile Levels in AMW based on Profession.**

Data expressed as Mean ± SD (Median)

| Parameters | Mechanics (n = 41) | SP/PB (n = 11) | AE/Welders (n = 10) | H | p-value |
|---|---|---|---|---|---|
| **Sex Hormones** | | | | | |
| Testo (ng/mL) | 6.86 ± 1.99 (6.56) | 7.07 ± 1.57 (7.06) | 6.54 ± 1.97 (6.00) | 0.629 | 0.730 |
| FSH (IU/L) | 12.20 ± 3.39 (11.18) | 11.96 ± 3.04 (12.23) | 11.84 ± 3.01 (11.03) | 0.044 | 0.978 |
| LH (IU/L) | 7.43 ± 2.29 (6.66) | 6.77 ± 1.98 (5.41) | 7.19 ± 1.69 (6.83) | 2.432 | 0.296 |
| **Lipid Profile** | | | | | |
| TC (mmol/L) | 5.06 ± 0.84 (5.07) | 4.83 ± 0.89 (4.84) | 5.22 ± 1.00 (4.74) | 0.923 | 0.630 |
| HDLC (mmol/L) | 1.19 ± 0.07 (1.19) | 1.19 ± 0.10 (1.21) | 1.17 ± 0.10 (1.19) | 0.849 | 0.654 |
| LDLC (mmol/L) | 3.43 ± 0.74 (3.48) | 3.23 ± 0.82 (3.36) | 3.60 ± 0.86 (3.24) | 0.785 | 0.675 |
| VLDLC (mmol/L) | 0.44 ± 0.07 (0.39) | 0.40 ± 0.08 (0.35) | 0.46 ± 0.10 (0.39) | 1.189 | 0.552 |
| TG (mmol/L) | 0.96 ± 0.16 (0.86) | 0.87 ± 0.17 (0.76) | 1.00 ± 0.22 (0.66) | 1.189 | 0.552 |

AMW = Automobile Workers. SP = Spray Painters. PB = Panel Beaters. AE = Auto Electricians. Testo – Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG – Triglyceride. Test Statistics - Kruskal-Wallis Test (H)

## 4.7 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Automobile Workers based on Duration of Exposure.

In table 4.7, there were no significant differences in the body mass index of the automobile workers with respect to duration of exposure ($p = 0.139$). The mean levels of systolic and diastolic blood pressures, lead, cadmium and malondialdehyde increased while the mean levels of selenium, zinc and total antioxidant capacity decreased in these workers with increased duration of exposure.

The mean systolic and diastolic blood pressure values were significantly lower in automobile workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$), but not significantly different from 6-10 years of exposure ($p>0.05$). These values were also significantly lower in 6-10 years compared with ≥16 years ($p<0.05$), but not significantly different from 11-15 years of exposure ($p>0.05$).

The mean lead and cadmium levels were significantly lower in automobile workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years, 6-10 years was also significantly lower than ≥16 years and 11-15 years of exposure ($p<0.05$).

The mean selenium level was significantly higher in automobile workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$).

The mean zinc level was significantly higher in workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$), but not significantly different from 6-10 years of exposure ($p>0.05$), but not significantly different from 11-15 years of exposure ($p>0.05$).

The mean malondialdehyde level was significantly lower in workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years whereas the mean levels of total antioxidant capacity were significantly higher in workers exposed for ≤5 years and 6-10 years when compared with those exposed for ≥16 years ($p<0.05$).

**Table 4.7: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW based on Duration of Exposure.**

Data expressed as Mean ± SD (Median)

| Parameters | ≤5 years (n = 19) | 6-10 years (n = 13) | 11-15 years (n = 10) | ≥16 years (n = 20) | H | p-value |
|---|---|---|---|---|---|---|
| BMI ($kg/m^2$) | 22.74 ± 3.17 (20.90) | 24.61 ± 3.41 (24.62) | 25.01 ± 2.72 (24.65) | 24.93 ± 3.47 (24.90) | 5.497 | 0.139 |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 114.47 ± 7.62 (110.00) [a,b] | 120.38 ± 9.00 (120.00) [a] | 128.00 ± 8.88 (127.50) | 132.75 ± 7.34 (135.00) | 30.948 | 0.0001* |
| DBP (mmHg) | 76.58 ± 6.02 (75.00) [a,b] | 78.08 ± 6.63 (80.00) [a,] | 85.50 ± 7.62 (85.00) | 87.75 ± 6.58 (90.00) | 23.769 | 0.0001* |
| **Elements** | | | | | | |
| Lead (µg/dL) | 24.40 ± 3.38 (25.26) [a,b] | 42.59 ± 4.25 (38.78) [a,b] | 66.01 ± 3.09 (64.62) | 65.48 ± 0.46 (66.65) | 50.300 | 0.0001* |
| Cadmium (µg/dL) | 0.58 ± 0.18 (0.16) [a,b] | 1.62 ± 0.37 (1.80) [a,b] | 3.42 ± 0.57 (3.82) | 4.11 ± 0.41 (4.89) | 30.229 | 0.0001* |
| Selenium (µg/dL) | 9.70 ± 1.40 (10.20) [a,b] | 6.74 ± 1.23 (7.35) | 4.91 ± 0.97 (3.92) | 5.05 ± 0.95 (4.21) | 25.084 | 0.0001* |
| Zinc (µg/dL) | 110.86 ± 8.66 (111.60) [a,b] | 102.11 ± 13.28 (101.10) | 92.44 ± 9.48 (90.10) | 89.45 ± 16.45 (90.80) | 25.069 | 0.0001* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 1.56 ± 0.42 (1.49) [a,b] | 2.22 ± 0.76 (2.00) | 2.91 ± 1.34 (2.56) | 2.81 ± 1.35 (2.34) | 20.411 | 0.0001* |
| TAC (µmol/L) | 805.90 ± 141.83(758.00) [a] | 759.30 ± 76.31(776.00) [a] | 712.80 ± 104.30(705.50) | 632.80 ± 94.61(635.00) | 18.004 | 0.0001* |

* - Significant. AMW - Automobile Workers. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics - Kruskal-Wallis Test (H)

Pairwise Comparison:

a = $p<0.05$ when compared with ≥16years. b = $p<0.05$ when compared with 11-15 years. c = $p<0.05$ when compared with 6-10 years

## 4.8 Sex Hormones and Lipid Profile Levels in AMW workers based on Duration of Exposure.

In table 4.8, the mean level of testosterone decreased while the mean levels of follicle stimulating hormone, total cholesterol, low density lipoprotein, very low density lipoprotein and triglyceride increased in the automobile workers with increased duration of exposure. However, there were no significant differences in the mean levels of luteinizing hormone and high density lipoprotein in these workers with respect to duration of exposure ($p = 0.699$ and $p = 0.515$ respectively).

The mean level of testosterone was significantly higher in automobile workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$), the mean level at 6-10 years was also significantly higher than ≥16 years ($p<0.05$) but not significantly different from 11-15 years of exposure ($p>0.05$).

The mean level of follicle stimulating hormone was significantly lower in workers exposed for ≤5 years when compared with those exposed for ≥16 years ($p<0.05$) whereas the mean total cholesterol levels at ≤5 years of exposure was significantly lower when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$).

The mean levels of total cholesterol, low density lipoprotein, very low density lipoprotein and triglyceride were only significantly lower in workers exposed for ≤5 years when compared with those exposed for ≥16 years and 11-15 years ($p<0.05$).

None of the parameters were significantly different 11-15 years f exposure compared with ≥16 years of exposure ($p>0.05$

**Table 4.8: Sex Hormones and Lipid Profile Levels in Automobile Workers based on Duration of Exposure.**

Data expressed as Mean ± SD (Median)

| Parameters | ≤5 years (n = 19) | 6-10 years (n = 13) | 11-15 years (n = 10) | ≥16 years (n = 20) | H | p-value |
|---|---|---|---|---|---|---|
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 8.84 ± 1.10 (9.18) [a,b] | 7.44 ± 1.02 (7.67) [a] | 6.26 ± 0.99 (6.00) | 4.87 ± 0.98 (4.55) | 44.451 | 0.0001* |
| FSH (iu/L) | 10.67 ± 1.39 (10.34) [a] | 11.13 ± 1.02 (10.84) | 11.86 ± 2.17 (10.63) | 14.21 ± 2.00 (14.01) | 8.640 | 0.034* |
| LH (iu/L) | 6.62 ± 0.66 (6.26) | 6.42 ± 0.48 (6.51) | 7.17 ± 1.21 (6.36) | 8.15 ± 1.46 (7.84) | 1.428 | 0.699 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 4.38 ± 0.66 (4.27) [a,b] | 4.84 ± 0.59 (4.63) | 5.55 ± 0.69 (5.70) | 5.56 ± 0.83 (5.25) | 23.493 | 0.0001* |
| HDLC (mmol/L) | 1.18 ± 0.08 (1.18) | 1.18 ± 0.08 (1.20) | 1.22 ± 0.06 (1.23) | 1.18 ± 0.09 (1.20) | 2.289 | 0.515 |
| LDLC (mmol/L) | 2.86 ± 0.64 (2.71) [a,b] | 3.27 ± 0.51 (3.14) | 3.83 ± 0.57 (3.18) | 3.86 ± 0.74 (3.57) | 22.462 | 0.0001* |
| VLDLC(mmol/L) | 0.35 ± 0.02 (0.35) [a,b] | 0.39 ± 0.07 (0.35) | 0.50 ± 0.08 (0.45) | 0.51 ± 0.09 (0.46) | 18.083 | 0.0001* |
| TG (mmol/L) | 0.76 ± 0.05 (0.76) [a,b] | 0.76 ± 0.14 (0.76) | 1.10 ± 0.18 (0.99) | 1.12 ± 0.20 (1.01) | 18.083 | 0.0001* |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG – Triglyceride.

Test Statistics – Krukal-Walis Test (H)

Pairwise Comparison:

a = $p<0.05$ when compared with ≥16years. b = $p<0.05$ when compared with 11-15 years. c = $p<0.05$ when compared with 6-10 years

### 4.9 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal AMW, Compensated Hypogonadic AMW and Eugonadal Controls.

In table 4.9, there were no significant differences in the mean values of body mass indexes in the eugonadal automobile workers, compensated hypogonadic automobile workers and eugonadal controls ($p = 0.560$).

The mean values of systolic and diastolic blood pressures in the eugonadal AMW were not significantly different from the eugonadal control. These values were however significantly higher in the compensated hypogonadic AMW (128.44 ± 10.12mmHg and 87.19 ± 7.52mmHg respectively) compared with eugonadal AMW (122.17 ± 10.89mmHg and 80.11 ± 7.64mmHg respectively) and eugonadal control (119.03 ± 9.91mmHg and 78.31 ± 7.18mmHg respectively).

The mean levels of lead, cadmium and malondialdehyde were significantly higher in both eugonadal AMW (45.49 ± 9.60µg/dl, 2.20 ± 0.31µg/dl and 2.19 ± 0.49µmol/l respectively) and compensated hypogonadic AMW (55.91 ± 10.04µg/dl, 2.99 ± 0.51µg/dl and 2.69 ± 0.73µmol/l respectively) when compared with eugonadal (13.67 ± 3.60µg/dl, 0.14 ± 0.02µg/dl and 1.79 ± 0.20µmol/l respectively), but were not significantly different in compensated hypogonadic AMW when compared with eugonadal AMW ($p > 0.05$).

Additionally, the mean levels of selenium, zinc and total antioxidant capacity were significantly lower in both eugonadal AMW (7.17 ± 1.05µg/dl, 100.76 ± 15.13µg/dl and 740.11 ± 124.15µmol/l respectively) and compensated hypogonadic AMW (5.76 ± 1.38µg/dl, 94.51 ± 15.73µg/dl and 682.60 ± 136.15µmol/l respectively) when compared with eugonadal (15.38 ± 2.02µg/dl, 110.01 ± 24.31µg/dl and 807.01 ± 106.81µmol/l respectively), but were not significantly different in compensated hypogonadic AMW when compared with eugonadal AMW ($p > 0.05$)..

**Table 4.9: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal AMW, Compensated Hypogonadic AMW and Eugonadal Controls.** Data expressed as Mean ± SD (Median)

| Parameters | Eugonadal AMW (n = 46) | Comp. Hypogonadic AMW (n = 16) | Eugonadal Controls (n = 60) | H | p value |
|---|---|---|---|---|---|
| BMI ($kg/m^2$) | 24.16 ± 3.41 (24.08) | 23.32 ± 3.17 (23.96) | 23.51 ± 3.01 (23.24) | 1.161 | 0.560 |
| **Blood Pressure** | | | | | |
| SBP (mmHg) | 122.17 ± 10.89 (120.00) [b] | 128.44 ± 10.12 (130.00) [a] | 119.03 ± 9.91 (117.50) | 9.856 | 0.007* |
| DBP (mmHg) | 80.11 ± 7.64 (80.00) [b] | 87.19 ± 7.52 (87.50) [a] | 78.31 ± 7.18 (80.00) | 14.500 | 0.001* |
| **Elements** | | | | | |
| Lead (µg/dL) | 45.49 ± 9.60 (45.47) [a] | 55.91 ± 10.04 (40.00) [a] | 13.67 ± 3.60 (13.60) | 81.627 | 0.0001* |
| Cadmium (µg/dL) | 2.20 ± 0.31 (1.80) [a] | 2.99 ± 0.51 (2.63) [a] | 0.14 ± 0.02 (0.01) | 59.110 | 0.0001* |
| Selenium (µg/dL) | 7.17 ± 1.05 (7.43) [a] | 5.76 ± 1.38 (4.41) [a] | 15.38 ± 2.02 (15.92) | 73.603 | 0.0001* |
| Zinc (µg/dL) | 100.76 ± 15.13 (100.75) [a] | 94.51 ± 15.73 (90.00) [a] | 110.01 ± 24.31 (106.60) | 6.640 | 0.031* |
| **Oxidative Stress Markers** | | | | | |
| MDA (µmol/L) | 2.19 ± 0.49 (2.00) [a] | 2.69 ± 0.73 (2.10) [a] | 1.79 ± 0.20 (1.77) | 8.620 | 0.013* |
| TAC (µmol/L) | 740.11 ± 124.15 (748.00) [a] | 682.60 ± 136.15 (669.50) [a] | 807.01 ± 106.81 (805.00) | 20.700 | 0.0001* |

Test Statistics – Krukal-Walis Test (H)

Pairwise Comparison:

a = $p<0.05$ when compared with Eugonadal Automobile Workers.

b = $p<0.05$ when compared with Compensated Hypogonadic Automobile Workers

## 4.10 Sex Hormones and Lipid Profile Levels in Eugonadal AMW, Compensated hypogonadic AMW and Eugonadal Control.

In table 4.10, the mean levels of testosterone were significantly lower while the mean serum levels of follicle stimulating hormone were significantly higher in both eugonadal automobile workers and compensated hypogonadic AMW when compared with the eugonadal control. The mean level of testosterone was also significantly lower while follicle stimulating hormone and luteinizing hormone levels were significantly higher in compensated hypogonadic AMW when compared with eugonadal AMW and eugonadal control.

The mean levels of total cholesterol and low density lipoprotein cholesterol were significantly higher in both eugonadal AMW (5.01 ± 0.88mmol/l and 3.40 ± 0.78mmol/l respectively) and compensated hypogonadic AMW (5.15 ± 0.85mmol/l and 3.49 ± 0.75mmol/l respectively) when compared to the eugonadal control (4.29 ± 0.62mmol/l and 2.73 ± 0.54mmol/l respectively), however, these levels were not significantly different in compensated hypogonadic AMW compared with the eugonadal AMW.

There were no significant differences in the mean levels of high density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride in eugonadal AMW, compensated hypogonadic AMW and eugonadal control ($p > 0.05$).

**Table 4.10: Sex Hormones and Lipid Profile Levels in Eugonadal AMW, Compensated Hypogonadic AMW and Eugonadal Controls.**
Data expressed as Mean ± SD (Median)

| Parameters | Eugonadal AMW (n = 46) | Comp. Hypogonadic AMW (n = 16) | Eugonadal Controls (n = 60) | H | p-value |
|---|---|---|---|---|---|
| **Sex Hormones** | | | | | |
| Testo (ng/mL) | 7.26 ± 0.84 (7.02) [a,b] | 5.67 ± 1.06 (0.02) [a] | 8.17 ± 1.00 (9.07) | 18.989 | 0.0001* |
| FSH (IU/L) | 10.35 ± 1.03 (10.28) [a,b] | 17.13 ± 1.06 (16.95) [a] | 9.13 ± 1.25 (8.41) | 47.677 | 0.0001* |
| LH (IU/L) | 6.07 ± 0.45 (6.02) [b] | 10.29 ± 0.83 (10.85) [a] | 5.75 ± 0.50 (5.61) | 44.008 | 0.0001* |
| **Lipid Profile** | | | | | |
| TC (mmol/L) | 5.01 ± 0.88 (4.86) [a] | 5.15 ± 0.85 (5.16) [a] | 4.29 ± 0.62 (4.18) | 24.591 | 0.0001* |
| HDLC (mmol/L) | 1.21 ± 0.08 (1.19) | 1.18 ± 0.08 (1.19) | 1.20 ± 0.09 (1.20) | 2.582 | 0.275 |
| LDLC (mmol/L) | 3.40 ± 0.78 (3.35) [a] | 3.49 ± 0.75 (3.46) [a] | 2.73 ± 0.54 (2.59) | 27.580 | 0.0001* |
| VLDLC (mmol/L) | 0.43 ± 0.08 (0.38) | 0.46 ± 0.08 (0.39) | 0.37 ± 0.04 (0.39) | 4.581 | 0.101 |
| TG (mmol/L) | 0.93 ± 0.16 (0.83) | 1.00 ± 0.17 (0.86) | 0.87 ± 0.08 (0.85) | 4.279 | 0.118 |

* - Significant. AMW = Automobile Workers. Testo - Testosterone. FSH - Follicle Stimulating Hormone.
LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG - Triglyceride

Test Statistics – Krukal-Walis Test (H)

Pairwise Comparison:

a = $p<0.05$ when compared with Eugonadal Automobile Workers.

b = $p<0.05$ when compared with Compensated Hypogonadic Automobile Workers

### 4.11 Blood Pressure, Elements and Oxidative Stress Markers in AMW with respect to Smoking Habit.

The mean values of body mass index, systolic and diastolic blood pressures were significantly higher in the automobile workers who smoke when compared with the automobile workers who do not smoke ($p = 0.010$, $p = 0.0001$ and $p = 0.002$ respectively). Additionally, the mean levels of lead, cadmium and malondialdehyde were significantly higher in the automobile workers who smoke compared with the automobile workers who do not smoke ($p = 0.0001$, $p = 0.0001$ and $p = 0.003$ respectively) whereas the mean levels of selenium, zinc and total antioxidant capacity were significantly lower in the automobile workers who smoke when compared with the workers who do not smoke ($p = 0.008$, $p = 0.001$ and $p = 0.033$ respectively) as shown in table 4.11.

### 4.12 Sex Hormones and Lipid Profile Levels in AMW with respect to Smoking Habit.

The mean level of testosterone was significantly lower in the automobile workers who smoke when compared with the non-smokers ($p = 0.001$), however, the mean serum levels of follicle stimulating hormone, luteinizing hormone and high density lipoprotein cholesterol in the automobile workers who smoke were not significantly different from the workers who do not smoke ($p = 0.743$, $p = 0.537$ and $p = 0.988$ respectively). The mean serum levels of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein and triglyceride were significantly higher in the automobile workers who smoke when compared with the automobile workers who do not smoke ($p = 0.010$, $p = 0.013$, $p = 0.009$ and $p = 0.009$ respectively) as shown in table 4.12.

**Table 4.11: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW with respect to Smoking Habit.**

Data expressed as Mean ± SD (Median)

| Parameters | Smoking (n = 21) | Non-Smoking (n = 41) | Z | p-value |
|---|---|---|---|---|
| BMI ($Kg/m^2$) | 25.72 ± 3.26 (25.71) | 23.42 ± 3.12 (23.26) | -2.582 | 0.010* |
| **Blood Pressure** | | | | |
| SBP (mmHg) | 132.38 ± 7.68 (135.00) | 119.39 ± 9.76 (120.00) | -4.475 | 0.0001* |
| DBP (mmHg) | 86.43 ± 7.44 (85.00) | 79.63 ± 7.61 (80.00) | -3.044 | 0.002* |
| **Elements** | | | | |
| Lead (µg/dL) | 59.49 ± 5.46 (63.88) | 36.34 ± 9.39 (34.46) | -4.202 | 0.0001* |
| Cadmium (µg/dL) | 4.80 ± 0.21 (4.96) | 1.17 ± 0.19 (0.30) | -6.168 | 0.0001* |
| Selenium (µg/dL) | 5.44 ± 1.23 (4.00) | 7.50 ± 1.57 (7.51) | -2.670 | 0.008* |
| Zinc (µg/dL) | 91.49 ± 12.15 (90.50) | 103.07 ± 15.54 (109.87) | -3.258 | 0.001* |
| **Oxidative Stress Markers** | | | | |
| MDA (µmol/L) | 2.78 ± 0.60 (2.57) | 2.08 ± 0.53 (1.82) | -2.938 | 0.003* |
| TAC (µmol/L) | 670.11 ± 108.44 (651.00) | 753.60 ± 130.28 (752.00) | -2.127 | 0.033* |

* - Significant. AMW - Automobile Workers. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics – Man Whitney U Test (Z)

**Table 4.12: Sex Hormones and Lipid Profile Levels in AMW with respect to Smoking Habit.** Data expressed as Mean ± SD (Median)

| Parameters | Smoking (n = 21) | Non-Smoking (n = 41) | Z | p-value |
|---|---|---|---|---|
| **Sex Hormones** | | | | |
| Testo (ng/mL) | 5.73 ± 0.78 (5.31) | 7.42 ± 0.97 (8.12) | -3.324 | 0.001* |
| FSH (IU/L) | 12.24 ± 2.18 (11.12) | 12.03 ± 1.63 (12.01) | -0.327 | 0.743 |
| LH (IU/L) | 7.34 ± 1.39 (6.02) | 7.24 ± 0.99 (6.85) | -0.617 | 0.537 |
| **Lipid Profile** | | | | |
| TC (mmol/L) | 5.46 ± 0.87 (5.25) | 4.83 ± 0.80 (4.84) | -2.571 | 0.010* |
| HDLC (mmol/L) | 1.19 ± 0.08 (1.18) | 1.18 ± 0.09 (1.20) | -0.015 | 0.988 |
| LDLC (mmol/L) | 3.78 ± 0.78 (3.51) | 3.24 ± 0.70 (3.28) | -2.477 | 0.013* |
| VLDLC (mmol/L) | 0.48 ± 0.08 (0.44) | 0.35 ± 0.07 (0.37) | -2.606 | 0.009* |
| TG (mmol/L) | 1.06 ± 0.17 (0.97) | 0.89 ± 0.16 (0.76) | -2.606 | 0.009* |

* - Significant. AMW = Automobile Workers. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. TG - Triglyceride VLDL - Very Low Density Lipoprotein. Test Statistics – Man Whitney U Test (Z)

## 4.13 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW with respect to Alcohol Consumption.

The mean diastolic blood pressure and lead levels were significantly higher in alcohol consuming automobile workers when compared with the non-alcohol consuming workers ($p = 0.043$ and $p = 0.018$ respectively).

The mean values of BMI and systolic blood pressure, levels of cadmium, selenium, zinc, malondialdehyde and total antioxidant capacity were not significantly different in alcohol consuming automobile workers when compared with the non-alcohol consuming automobile workers ($p>0.05$) as shown in table 4.13.

## 4.14 Sex Hormones and Lipid Profile levels in AMW with respect to Alcohol Consumption.

The mean level of testosterone was significantly lower in alcohol consuming automobile workers when compared with the non-alcohol consuming automobile workers ($p = 0.001$). There were no significant differences in the mean levels of follicle stimulating hormone, luteinizing hormone, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride in both groups ($p>0.05$) as shown in table 4.14.

**Table 4.13: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW with respect to Alcohol Consumption.**

Data expressed as Mean ± SD (Median)

| Parameters | Alcohol Consuming (n = 47) | Non-Alcohohol Consuming (n = 15) | Z | p-value |
|---|---|---|---|---|
| BMI ($Kg/m^2$) | 25.72 ± 3.26 (24.45) | 23.43 ± 3.12 (21.94) | -0.872 | 0.383 |
| **Blood Pressure** | | | | |
| SBP (mmHg) | 125.11 ± 11.30 (130.45) | 119.67 ± 8.96 (120.00) | -1.751 | 0.080 |
| DBP (mmHg) | 83.09 ± 7.41 (80.00) | 78.33 ± 9.57 (75.00) | -2.025 | 0.043* |
| **Elements** | | | | |
| Lead (µg/dL) | 51.36 ± 9.41 (55.48) | 38.21 ± 10.07 (30.62) | -2.367 | 0.018* |
| Cadmium (µg/dL) | 2.65 ± 0.30 (2.20) | 1.60 ± 0.50 (0.30) | -1.424 | 0.154 |
| Selenium (µg/dL) | 6.47 ± 1.45 (5.92) | 7.86 ± 1.71 (7.51) | -1.553 | 0.120 |
| Zinc (µg/dL) | 98.20 ± 16.02 (101.10) | 102.12 ± 13.34 (110.00) | -0.904 | 0.366 |
| **Oxidative Stress Markers** | | | | |
| MDA (µmol/L) | 2.46 ± 0.63 (2.10) | 1.87 ± 0.24 (1.82) | -1.504 | 0.133 |
| TAC (µmol/L) | 711.10 ± 108.44 (713.00) | 769.71 ± 146.84 (752.00) | -2.551 | 0.285 |

* - Significant. AMW - Automobile Workers. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics – Man Whitney U Test (Z)

**Table 4.14: Sex Hormones and Lipid Profile levels in AMW with respect to Alcohol Consumption.** Data expressed as Mean ± SD (Median)

| Parameters | Alcohol Consuming (n = 47) | Non-Alcohohol Consuming (n = 15) | Z | p-value |
|---|---|---|---|---|
| **Sex Hormones** | | | | |
| Testo (ng/mL) | 6.42 ± 0.94 (6.10) | 8.18 ± 0.69 (8.20) | -3.189 | 0.001* |
| FSH (IU/L) | 12.31 ± 1.96 (11.79) | 11.46 ± 1.48 (10.34) | -0.542 | 0.588 |
| LH (IU/L) | 7.39 ± 1.15 (6.91) | 6.90 ± 1.07 (6.24) | -1.134 | 0.257 |
| **Lipid Profile** | | | | |
| TC (mmol/L) | 5.14 ± 0.85 (5.07) | 4.75 ± 0.88 (4.63) | -1.771 | 0.077 |
| HDLC (mmol/L) | 1.19 ± 0.08 (1.19) | 1.18 ± 0.08 (1.18) | -0.396 | 0.692 |
| LDLC (mmol/L) | 3.50 ± 0.76 (3.43) | 3.18 ± 0.76 (3.06) | -1.454 | 0.122 |
| VLDLC (mmol/L) | 0.45 ± 0.08 (0.39) | 0.39 ± 0.07 (0.35) | -1.828 | 0.067 |
| TG (mmol/L) | 0.98 ± 0.17 (0.86) | 0.85 ± 0.15 (0.76) | -1.828 | 0.067 |

* - Significant. AMW = Automobile Workers. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. TG – Triglyceride. VLDL - Very Low Density Lipoprotein. Test Statistics – Man Whitney U Test (Z)

### 4.15 Correlation of blood Lead with Blood Pressure, Trace Elements, Oxidative Stress Markers, Sex Hormones and Lipid Profile in Automobile Workers and Controls.

In table 4.15, there was a significant positive correlation of blood lead with systolic and diastolic blood pressures (r = 0.650 and r = 0.576 respectively), malondialdehyde (r = 0.603), follicle stimulating hormone (r = 0.303), luteinizing hormone (r = 0.282), total cholesterol (r = 0.609), low density lipoprotein cholesterol (r = 0.590), very low density lipoprotein cholesterol (r = 0.458) and triglyceride (r = 0.461) in the automobile workers ($p<0.05$). There was also a significant negative correlation of blood lead with selenium (r = -0.708), zinc (r = -0.611), total antioxidant capacity (r = -0.516) and testosterone (r = -0.693), with no significant correlation with high density lipoprotein cholesterol (r = 0.062) in the automobile workers.

None of the measured parameters correlated significantly with blood lead in the control group ($p>0.05$).

### 4.16 Correlation of blood Cadmium with Blood Pressure, Trace Elements, Oxidative Stress Markers, Sex Hormones and Lipid Profile in Automobile Workers and Control.

In table 4.16, there was a significant positive correlation of blood cadmium with systolic and diastolic blood pressures (r = 0.725 and r = 0.506 respectively), malondialdehyde (r = 0.295), total cholesterol (r = 0.397), low density lipoprotein cholesterol (r = 0.391), very low density lipoprotein cholesterol (r = 0.265) and triglyceride (r = 0.268) in the automobile workers ($p<0.05$). There was also a significant negative correlation of blood cadmium with selenium (r = -0.421), zinc (r = -0.391), total antioxidant capacity (r = -0.399) and testosterone (r = -0.544), with no significant correlations with follicle stimulating hormone (r = 0.133), luteinizing hormone (r = 0.144) and high density lipoprotein cholesterol (r = 0.037) in the automobile workers. None of the measured parameters correlated significantly with blood cadmium in the control group ($p>0.05$).

**Table 4.15: Correlation of blood Lead with Blood Pressure, Trace Elements, Oxidative Stress Markers, Sex Hormones and Lipid Profile in AMW and Controls.**

| Parameters | Automobile Workers | | Controls | |
|---|---|---|---|---|
| | Lead (r) | p-value | Lead (r) | p-value |
| SBP (mmHg) | 0.650 | 0.0001* | 0.125 | 0.331 |
| DBP (mmHg) | 0.576 | 0.0001* | 0.082 | 0.526 |
| Selenium (µg/dL) | -0.708 | 0.0001* | 0.079 | 0.541 |
| Zinc (µg/dL) | -0.611 | 0.0001* | 0.213 | 0.051 |
| MDA (µmol/L) | 0.603 | 0.0001* | 0.029 | 0.821 |
| TAC (µmol/L) | -0.516 | 0.0001* | 0.122 | 0.345 |
| Testo (ng/mL) | -0.693 | 0.0001* | 0.074 | 0.566 |
| FSH (IU/L) | 0.303 | 0.017* | 0.114 | 0.378 |
| LH (IU/L) | 0.282 | 0.026* | 0.073 | 0.571 |
| TC (mmol/L) | 0.609 | 0.0001* | 0.123 | 0.340 |
| HDLC (mmol/L) | 0.062 | 0.630 | 0.014 | 0.911 |
| LDLC (mmol/L) | 0.590 | 0.0001* | 0.146 | 0.258 |
| VLDLC (mmol/L) | 0.458 | 0.0001* | 0.043 | 0.742 |
| TG (mmol/L) | 0.461 | 0.0001* | 0.060 | 0.641 |

* - Significant. AMW - Automobile Workers. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG - Triglyceride

**Table 4.16: Correlation of blood Cadmium with Blood Pressure, Trace Elements, Oxidative Stress Markers, Sex Hormones and Lipid Profile in AMW and Controls.**

| Parameters | Automobile Workers | | Controls | |
|---|---|---|---|---|
| | Cadmium (r) | p-value | Cadmium (r) | p-value |
| SBP (mmHg) | 0.725 | 0.0001* | 0.041 | 0.750 |
| DBP (mmHg) | 0.506 | 0.0001* | 0.001 | 0.955 |
| Selenium (µg/dL) | -0.421 | 0.001* | 0.050 | 0.697 |
| Zinc (µg/dL) | -0.391 | 0.002* | 0.050 | 0.701 |
| MDA (µmol/L) | 0.295 | 0.020* | 0.109 | 0.398 |
| TAC (µmol/L) | -0.399 | 0.001* | 0.016 | 0.902 |
| Testo (ng/mL) | -0.544 | 0.0001* | 0.118 | 0.362 |
| FSH (IU/L) | 0.133 | 0.302 | 0.032 | 0.804 |
| LH (IU/L) | 0.144 | 0.263 | 0.031 | 0.811 |
| TC (mmol/L) | 0.397 | 0.001* | 0.175 | 0.174 |
| HDLC (mmol/L) | 0.037 | 0.777 | 0.217 | 0.090 |
| LDLC (mmol/L) | 0.391 | 0.002* | 0.119 | 0.355 |
| VLDLC (mmol/L) | 0.265 | 0.038* | 0.235 | 0.056 |
| TG (mmol/L) | 0.268 | 0.035* | 0.232 | 0.058 |

* - Significant. AMW - Automobile Workers. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL-High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG - Triglyceride

## 4.17 BMI, Blood Pressure and Levels of Elements and Oxidative Stress Markers in Automobile Workers at different stages of Green Tea Supplementation.

In table 4.17, the mean values of body mass index, systolic and diastolic blood pressures as well as lead, cadmium, and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of green tea intake. However, the decrease in the mean values of body mass index, systolic blood pressure, diastolic blood pressure and cadmium level, and the increase in the mean zinc level were only significant at two months of green tea intake compared to their values at baseline and one month ($p<0.05$) whereas the decrease in the mean levels of lead and malondialdehyde, and the increase in the mean levels of selenium and total antioxidant capacity were significant at one month and two months of green tea intake compared with the baseline value as well as at two months compared with one month ($p<0.05$).

## 4.18 Sex Hormones and Lipid Profile levels in Automobile Workers at different stages of Green Tea Supplementation.

In table 4.18, the mean levels of testosterone and high density lipoprotein increased progressively while the mean levels of follicle stimulating hormone, luteinizing hormone, total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride decreased progressively from baseline to two months of green tea intake. However, the increase in the mean levels of testosterone, and the decrease in the mean levels of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride were significant at one month and two months of green tea intake compared with the baseline value as well as at two months compared with one month.

The decrease in the mean level of FSH was significant at two months of green tea intake compared to their values at baseline and one month whereas the decrease in LH level was significant at two months of green tea intake compared with the baseline value ($p<0.05$).

**Table 4.17: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW at different stages of Green Tea Supplementation.** Data expressed as Mean ± SD (Median)

| Parameters | A<br>Baseline<br>(n = 25) | B<br>One month<br>(n = 25) | C<br>Two months<br>(n = 25) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| BMI ($kg/m^2$) | 24.82 ± 3.64 (25.78) | 24.72 ± 3.56 (25.53) | 24.29 ± 3.55 (25.03) | 0.276 | 0.0001* | 0.001* |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 123.75 ± 10.33 (127.50) | 122.32 ± 8.97 (120.00) | 120.36 ± 8.60 (120.00) | 0.241 | 0.034* | 0.012* |
| DBP (mmHg) | 82.14 ± 6.73 (80.00) | 81.25 ± 5.87 (80.00) | 78.57 ± 6.36 (80.00) | 0.311 | 0.006* | 0.007* |
| **Elements** | | | | | | |
| Lead (µg/dL) | 45.34 ± 10.29 (38.37) | 43.79 ± 9.92 (39.44) | 40.46 ± 9.08 (35.48) | 0.030* | 0.001* | 0.013* |
| Cadmium (µg/dL) | 2.57 ± 0.41 (2.00) | 2.38 ± 0.38 (2.00) | 2.19 ± 0.34 (1.93) | 0.128 | 0.003* | 0.006* |
| Selenium (µg/dL) | 6.47 ± 1.54 (5.81) | 7.01 ± 1.58 (6.55) | 7.65 ± 1.61 (7.30) | 0.004* | 0.0001* | 0.0001* |
| Zinc (µg/dL) | 101.94 ± 15.91 (109.80) | 101.80 ± 15.08 (108.95) | 102.87 ± 14.56 (108.44) | 0.707 | 0.001* | 0.009* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.09 ± 0.48 (2.02) | 1.94 ± 0.44 (1.92) | 1.80 ± 0.38 (1.71) | 0.0001* | 0.0001* | 0.0001* |
| TAC (µmol/L) | 725.11 ± 138.54 (752.00) | 844.30 ± 120.71 (830.50) | 1026.40 ± 159.50 (972.00) | 0.0001* | 0.0001* | 0.0001* |

Test Statistics – Wilcoxon Test

**Table 4.18: Sex Hormones and Lipid Profile Levels in Automobile Workers at different stages of Green Tea Supplementation.**

Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n = 25)** | **One month (n = 25)** | **Two months (n = 25)** | **B/A** | **C/A** | **C/B** |
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 7.09 ± 0.97 (6.77) | 7.32 ± 1.01 (7.23) | 7.54 ± 0.99 (7.74) | 0.037* | 0.007* | 0.010* |
| FSH (IU/L) | 11.31 ± 1.62 (10.23) | 11.21 ± 1.57 (10.12) | 10.77 ± 1.52 (9.59) | 0.172 | 0.014* | 0.010* |
| LH (IU/L) | 6.84 ± 0.97 (6.38) | 6.65 ± 1.00 (5.90) | 6.28 ± 0.99 (5.94) | 0.241 | 0.046* | 0.076 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 5.13 ± 0.85 (4.94) | 4.62 ± 0.67 (4.45) | 4.23 ± 0.70 (4.09) | 0.0001* | 0.0001* | 0.0001* |
| HDLC (mmol/L) | 1.17 ± 0.08 (1.18) | 1.18 ± 0.07 (1.18) | 1.19 ± 0.06 (1.20) | 0.202 | 0.031* | 0.029* |
| LDLC (mmol/L) | 3 .55 ± 0.77 (3.39) | 3.07 ± 0.65 (2.87) | 2.71 ± 0.68 (2.60) | 0.0001* | 0.0001* | 0.0001* |
| VLDLC (mmol/L) | 0.40 ± 0.06 (0.37) | 0.37 ± 0.06 (0.35) | 0.33 ± 0.05 (0.33) | 0.008* | 0.002* | 0.023* |
| TG (mmol/L) | 0.90 ± 0.14 (0.86) | 0.81 ± 0.11 (0.78) | 0.73 ± 0.10 (0.72) | 0.006* | 0.0001* | 0.006* |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG – Triglyceride Test Statistics – Wilcoxon Test

### 4.19 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.19, there were no significant differences in the values of body mass index and cadmium levels in the automobile workers at different stages of vitamin C supplementation ($p>0.05$). The mean values of systolic and diastolic blood pressures as well as lead and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean values of systolic and diastolic blood pressures, lead level, and the increase in the mean zinc level were significant at two months of vitamin C intake compared to their values at baseline and one month ($p<0.05$) whereas the decrease in the mean level of malondialdehyde, and the increase in the mean levels of selenium and total antioxidant capacity were significant at one month and two months of vitamin C intake compared with the baseline value as well as at two months compared with one month ($p<0.05$).

**Table 4.19: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in AMW at different stages of Vitamin C Supplementation.** Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n = 27)** | **One month (n = 27)** | **Two months (n = 27)** | **B/A** | **C/A** | **C/B** |
| BMI ($kg/m^2$) | 23.49 ± 2.82 (23.26) | 23.31 ± 2.64 (23.26) | 23.31 ± 2.52 (23.23) | 0.821 | 0.957 | 0.527 |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 123.79 ± 12.44 (120.00) | 123.10 ± 10.39 (120.00) | 121.55 ± 9.46 (120.00) | 0.285 | 0.040* | 0.049* |
| DBP (mmHg) | 81.72 ± 9.84 (80.00) | 80.69 ± 7.04 (80.00) | 78.10 ± 7.61 (80.00) | 0.368 | 0.001* | 0.001* |
| **Elements** | | | | | | |
| Lead (µg/dL) | 50.68 ± 9.66 (57.00) | 50.15 ± 9.86 (55.20) | 47.97 ± 9.66 (51.76) | 0.265 | 0.001* | 0.004* |
| Cadmium (µg/dL) | 2.30 ± 0.38 (2.00) | 2.36 ± 0.40 (1.95) | 2.26 ± 0.40 (1.93) | 0.895 | 0.501 | 0.350 |
| Selenium (µg/dL) | 6.81 ± 1.51 (5.92) | 6.92 ± 1.48 (5.51) | 7.67 ± 1.51 (7.80) | 0.008* | 0.0001* | 0.0001* |
| Zinc (µg/dL) | 96.89 ± 14.61 (95.60) | 97.49 ± 14.44 (93.88) | 100.00 ± 14.62 (97.80) | 0.144 | 0.0001* | 0.0001* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.39 ± 0.57 (2.00) | 2.32 ± 0.55 (2.00) | 2.15 ± 0.56 (1.79) | 0.006* | 0.0001* | 0.0001* |
| TAC (µmol/L) | 718.10 ± 124.70 (708.00) | 809.20 ± 104.59 (792.00) | 998.20 ± 175.43 (986.00) | 0.0001* | 0.0001* | 0.0001* |

* - Significant. AMW - Automobile Workers. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA - Malondialdehyde.TAC - Total Antioxidant Capacity. Test Statistics – Wilcoxon Test

## 4.20 Sex Hormones and Lipid Profile Levels in Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.20, there were no significant differences in the mean levels of testosterone, follicle stimulating hormone and luteinizing hormone in the automobile workers at different stages of vitamin C supplementation ($p>0.05$). The mean levels of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride decreased progressively while the mean level of high density lipoprotein cholesterol increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean levels of total cholesterol, low density lipoprotein cholesterol, very low density cholesterol and triglyceride were significant at one month and two months of vitamin C intake compared with the baseline values as well as at two months compared with one month ($p<0.05$) whereas the increase in the mean high density lipoprotein cholesterol level was only significant at two months of vitamin C intake compared to their values at baseline and one month ($p<0.05$).

**Table 4.20: Sex Hormones and Lipid Profile Levels in Automobile Workers at different stages of Vitamin C Supplementation.**

Data expressed as Mean ± SD (Median)

| Parameters | A Baseline (n = 27) | B One month (n = 27) | C Two months (n = 27) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 6.51 ± 0.99 (6.56) | 6.60 ± 0.84 (6.34) | 6.93 ± 0.89 (6.57) | 0.751 | 0.125 | 0.310 |
| FSH (IU/L) | 13.22 ± 1.94 (12.69) | 12.39 ± 2.04 (12.70) | 12.31 ± 2.07 (12.67) | 0.581 | 0.315 | 0.320 |
| LH (IU/L) | 7.61 ± 1.16 (6.94) | 7.53 ± 0.88 (7.10) | 7.49 ± 0.77 (7.01) | 0.482 | 0.393 | 0.364 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 4.95 ± 0.86 (5.04) | 4.78 ± 0.83 (4.99) | 4.52 ± 0.76 (4.40) | 0.0001* | 0.0001* | 0.0001* |
| HDLC (mmol/L) | 1.21 ± 0.07 (1.22) | 1.21 ± 0.07 (1.23) | 1.23 ± 0.06 (1.24) | 0.225 | 0.017* | 0.018* |
| LDLC (mmol/L) | 3.29 ± 0.71 (3.40) | 3.14 ± 0.71 (3.30) | 2.90 ± 0.65 (2.94) | 0.0001* | 0.0001* | 0.0001* |
| VLDLC (mmol/L) | 0.45 ± 0.08 (0.39) | 0.42 ± 0.06 (0.40) | 0.39 ± 0.07 (0.35) | 0.008* | 0.002* | 0.023* |
| TG (mmol/L) | 0.98 ± 0.18 (0.86) | 0.93 ± 0.14 (0.87) | 0.86 ± 0.14 (0.76) | 0.006* | 0.0001* | 0.006* |

* - Significant. Testo - Testosterone. SH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. TG – Triglyceride VLDL - Very Low Density Lipoprotein. Test Statistics – Wilcoxon Test

### 4.21 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal Automobile Workers at different stages of Green Tea Supplementation.

In table 4.21, the mean values of body mass index, systolic and diastolic blood pressures as well as lead, cadmium and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of green tea intake in the eugonadal automobile workers. However, the decrease in the mean values of body mass index, systolic blood pressure, diastolic blood pressure and lead levesl, and the increase in the mean zinc level were only significant at two months of green tea intake compared to their values at baseline and one month ($p<0.05$) whereas the decrease in the mean level of malondialdehyde, and the increase in the mean levels of selenium and total antioxidant capacity were significant at one month and two months of green tea intake compared with the baseline value as well as at two months compared with one month ($p<0.05$). The decrease in the mean cadmium level was however significant at two months of green tea intake compared with the values at baseline and one month.

**Table 4.21: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal AMW at different stages of Green Tea Supplementation.** Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n = 18)** | **One month (n = 18)** | **Two months (n = 18)** | **B/A** | **C/A** | **C/B** |
| BMI ($kg/m^2$) | 24.71 ± 3.66 (25.78) | 24.69 ± 3.66 (25.53) | 24.29 ± 3.66 (25.03) | 0.861 | 0.009* | 0.001* |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 122.95 ± 10.54 (125.00) | 120.91 ± 9.08 (120.00) | 120.36 ± 8.60 (120.00) | 0.133 | 0.035* | 0.046* |
| DBP (mmHg) | 80.45 ± 5.75 (80.00) | 80.23 ± 5.87 (80.00) | 77.27 ± 6.12 (80.00) | 0.808 | 0.038* | 0.016* |
| **Elements** | | | | | | |
| Lead (µg/dL) | 42.32 ± 4.16 (36.21) | 40.80 ± 4.07 (37.40) | 37.61 ± 3.69 (32.22) | 0.048* | 0.006* | 0.001* |
| Cadmium (µg/dL) | 2.33 ± 0.45 (1.65) | 2.08 ± 0.39 (1.95) | 1.99 ± 0.38 (1.73) | 0.102 | 0.030* | 0.102 |
| Selenium (µg/dL) | 6.90 ± 0.66 (7.21) | 7.58 ± 0.67 (7.61) | 7.65 ± 1.61 (8.08) | 0.002* | 0.001* | 0.004* |
| Zinc (µg/dL) | 102.24 ± 16.30 (110.00) | 102.25 ± 16.09 (109.34) | 103.52 ± 14.56 (109.14) | 0.897 | 0.014* | 0.048* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.09 ± 0.48 (1.87) | 1.94 ± 0.44 (1.66) | 1.80 ± 0.38 (1.52) | 0.0001* | 0.0001* | 0.0001* |
| TAC (µmol/L) | 725.11 ± 138.54 (752.00) | 844.30 ± 120.71 (857.00) | 1026.40 ± 159.50 (1058.00) | 0.0001* | 0.0001* | 0.0001* |

* - Significant. AMW - Automobile Workers. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics – Wilcoxon Test

## 4.22 Sex Hormones and Lipid Profile Levels in Eugonadal Automobile Workers at different stages of Green Tea Supplementation.

In table 4.22, there were no significant differences in the mean levels of testosterone, follicle stimulating hormone and luteinizing hormone in the eugonadal automobile workers at different stages of green tea supplementation ($p>0.05$).

The mean levels of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride decreased progressively while the mean level of high density lipoprotein cholesterol increased progressively from baseline to two months of green tea intake. However, the decrease in the mean levels of total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride were significant at one month and two months of green tea intake compared with the baseline values as well as at two months compared with one month ($p<0.05$) while the increase in the mean level of high density lipoprotein cholesterol was significant at two months of green tea intake compared to their values at baseline and one month ($p<0.05$).

**Table 4.22: Sex Hormones and Lipid Profile Levels in Eugonadal Automobile Workers at different stages of Green Tea Supplementation.**

Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n = 18)** | **One month (n = 18)** | **Two months (n = 18)** | **B/A** | **C/A** | **C/B** |
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 7.31 ± 0.86 (7.02) | 7.56 ± 0.93 (7.52) | 7.61 ± 0.87 (7.81) | 0.062 | 0.051 | 0.054 |
| FSH (IU/L) | 9.99 ± 0.96 (9.50) | 9.90 ± 0.94 (9.26) | 9.59 ± 1.02 (8.91) | 0.322 | 0.101 | 0.074 |
| LH (IU/L) | 6.05 ± 0.42 (6.02) | 5.83 ± 0.42 (5.85) | 5.67 ± 0.58 (5.72) | 0.284 | 0.390 | 0.357 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 5.03 ± 0.79 (4.84) | 4.57 ± 0.66 (4.41) | 4.22 ± 0.75 (4.07) | 0.0001* | 0.0001* | 0.0001* |
| HDLC (mmol/L) | 1.17 ± 0.09 (1.18) | 1.18 ± 0.07 (1.18) | 1.19 ± 0.07 (1.21) | 0.469 | 0.014* | 0.042* |
| LDLC (mmol/L) | 3.47 ± 0.72 (3.32) | 3.03 ± 0.66 (2.87) | 2.71 ± 0.73 (2.55) | 0.0001* | 0.0001* | 0.0001* |
| VLDLC (mmol/L) | 0.39 ± 0.04 (0.37) | 0.36 ± 0.05 (0.35) | 0.32 ± 0.04 (0.30) | 0.028* | 0.001* | 0.011* |
| TG (mmol/L) | 0.88 ± 0.11 (0.86) | 0.77 ± 0.09 (0.76) | 0.69 ± 0.09 (0.66) | 0.005* | 0.0001* | 0.013* |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TG - Triglyceride TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. Test Statistics – Wilcoxon Test

## 4.23 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Automobile Workers with Compensated Hypogonadism at different stages of Green Tea Supplementation.

In table 4.23, the mean values of body mass index, systolic and diastolic blood pressures as well as lead, cadmium, and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of green tea intake in compensated hypogonadic automobile workers. However, the decrease in the mean value of body mass index and cadmium level, and the increase in the mean levels of zinc and selenium were significant at two months of green tea intake compared to their values at baseline and one month ($p<0.05$).

The decrease in the mean values of systolic and diastolic blood pressures, lead and malondialdehyde levels were significant at two months of green tea intake compared with their values at baseline and one month. Additionally, the increase in the mean level of total antioxidant capacity was significant at one month and two months of green tea intake compared with the baseline value.

**Table 4.23: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Automobile Workers with Compensated Hypogonadism at different stages of Green Tea Supplementation.** Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n =7)** | **One month (n = 7)** | **Two months (n = 7)** | **B/A** | **C/A** | **C/B** |
| BMI ($kg/m^2$) | 25.22 ± 3.87 (24.68) | 24.83 ± 3.48 (24.34) | 24.29 ± 3.43 (23.63) | 0.068 | 0.018* | 0.017* |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 126.67 ± 9.83 (130.00) | 126.50 ± 6.89 (130.00) | 124.00 ± 5.48 (130.00) | 0.705 | 0.046* | 0.408 |
| DBP (mmHg) | 88.33 ± 6.83 (90.00) | 85.00 ± 4.47 (85.00) | 83.33 ± 5.16 (85.00) | 0.096 | 0.020* | 0.157 |
| **Elements** | | | | | | |
| Lead (µg/dL) | 56.41 ± 11.32 (63.14) | 53.76 ± 10.14 (58.70) | 50.91 ± 9.48 (52.70) | 0.176 | 0.042* | 0.397 |
| Cadmium (µg/dL) | 3.45 ± 0.99 (4.10) | 3.45 ± 0.96 (4.30) | 2.90 ± 0.82 (3.29) | 0.516 | 0.018* | 0.020* |
| Selenium (µg/dL) | 4.93 ± 1.30 (3.95) | 4.94 ± 1.23 (3.95) | 5.71 ± 1.16 (5.16) | 0.865 | 0.018* | 0.018* |
| Zinc (µg/dL) | 100.84 ± 11.31 (97.40) | 100.55 ± 11.67 (98.00) | 101.96 ± 11.07 (98.60) | 0.735 | 0.018* | 0.028* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.89 ± 0.81 (2.20) | 2.79 ± 0.69 (2.05) | 2.59 ± 0.68 (2.01) | 0.397 | 0.020* | 0.090 |
| TAC (µmol/L) | 645.20 ± 141.91 (752.00) | 764.21 ± 100.96 (802.00) | 917.81 ± 58.56 (940.00) | 0.018* | 0.017* | 0.018* |

* - Significant. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity. Test Statistics – Wilcoxon Test

### 4.24 Sex Hormones and Lipid Profile Levels in Compensated Hypogonadic Automobile Workers at different stages of Green Tea Supplementation.

In table 4.24, the mean levels of testosterone and high density lipoprotein cholesterol increased progressively while the mean levels of follicle stimulating hormone, luteinizing hormone, total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride decreased progressively from baseline to two months of green tea intake in the compensated hypogonadic automobile workers. However, the increase in the mean levels of testosterone and high density lipoprotein cholesterol, and the decrease in the mean levels of very low density lipoprotein cholesterol and triglyceride were significant at two months of green tea intake compared to their values at baseline ($p<0.05$).

Additionaly, the decrease in the mean levels of follicle stimulating and luteinizing hormones were significant at two months of green tea intake compared with their values at baseline and one month whereas the decrease in total cholesterol and low density lipoprotein cholesterol were significant at one month and two months of green tea intake compared with their values at baseline as well as at two months compared with one month.

**Table 4.24: Sex Hormones and Lipid Profile Levels in Compensated Hypogonadic Automobile Workers at different stages of Green Tea Supplementation.** Data expressed as Mean ± SD (Median)

| Parameters | A<br>Baseline<br>(n = 7) | B<br>One month<br>(n = 7) | C<br>Two months<br>(n = 7) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 6.28 ± 1.13 (5.89) | 6.53 ± 1.15 (5.21) | 7.71 ± 0.87 (5.40) | 0.113 | 0.035* | 0.059 |
| FSH (IU/L) | 16.16 ± 1.14 (16.44) | 15.99 ± 0.92 (15.99) | 15.07 ± 0.99 (14.63) | 0.176 | 0.018* | 0.020* |
| LH (IU/L) | 9.76 ± 1.02 (10.23) | 9.64 ± 1.10 (10.50) | 8.52 ± 1.30 (9.97) | 0.175 | 0.028* | 0.042* |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 5.50 ± 1.04(5.25) | 4.82 ± 0.69(4.64) | 4.27 ± 0.46 (4.06) | 0.018* | 0.017* | 0.018* |
| HDLC (mmol/L) | 1.17 ± 0.09 (1.16) | 1.18 ± 0.07 (1.18) | 1.19 ± 0.07 (1.20) | 0.141 | 0.036* | 0.336 |
| LDLC (mmol/L) | 3.88 ± 0.91 (0.35) | 3.24 ± 0.65 (2.83) | 2.69 ± 0.46 (2.62) | 0.018* | 0.015* | 0.018* |
| VLDLC (mmol/L) | 0.46 ± 0.10 (0.35) | 0.40 ± 0.08 (0.31) | 0.39 ± 0.06 (0.37) | 0.248 | 0.048* | 0.166 |
| TG (mmol/L) | 1.00 ± 0.16 (0.76) | 0.95 ± 0.14 (0.86) | 0.85 ± 0.13 (0.86) | 0.169 | 0.047* | 0.111 |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TG - Triglyceride TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. Test Statistics – Wilcoxon Test

### 4.25 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.25, there were no significant differences in the values of body mass index and cadmium levels in the eugonadal automobile workers at different stages of vitamin C supplementation ($p>0.05$).

The mean values of systolic and diastolic blood pressures as well as lead and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean values of systolic blood and diastolic blood pressures were significant at two months of vitamin C intake compared with their values at baseline.

The decrease in the mean lead level, and the increase in the mean zinc level were significant at two months of vitamin C intake compared with their values at baseline and one month ($p<0.05$).

The increase in the mean selenium and total antioxidant capacity levels as well as the decrease in the mean level of malondialdehyde were significant at one month and two months of vitamin C intake compared with their baseline values as well as at two months compared with one month ($p<0.05$).

**Table 4.25: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Eugonadal Automobile Workers at different stages of Vitamin C Supplementation.** Data expressed as Mean ± SD (Median)

| Parameters | A<br>Baseline<br>(n =19) | B<br>One month<br>(n = 19) | C<br>Two months<br>(n = 19) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| BMI ($kg/m^2$) | 23.34 ± 2.92 (23.26) | 23.30 ± 2.90 (23.26) | 23.34 ± 2.80 (23.23) | 0.900 | 0.831 | 0.397 |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 120.79 ± 12.50 (120.00) | 120.53 ± 10.53 (120.00) | 118.21 ± 11.17 (120.00) | 0.705 | 0.048* | 0.273 |
| DBP (mmHg) | 79.21 ± 9.90 (80.00) | 79.20 ± 6.92 (80.00) | 76.84 ± 7.49 (80.00) | 0.922 | 0.013* | 0.067 |
| **Elements** | | | | | | |
| Lead (µg/dL) | 48.09 ± 9.52 (54.00) | 47.43 ± 9.65 (52.20) | 45.38 ± 9.66 (48.76) | 0.277 | 0.007* | 0.022* |
| Cadmium (µg/dL) | 2.10 ± 0.50 (2.00) | 2.23 ± 0.48 (1.95) | 2.07 ± 0.48 (1.91) | 0.304 | 0.259 | 0.711 |
| Selenium (µg/dL) | 7.11 ± 1.57 (5.92) | 7.23 ± 1.51 (5.51) | 7.81 ± 1.46 (7.80) | 0.008* | 0.0001* | 0.001* |
| Zinc (µg/dL) | 100.14 ± 12.27 (95.60) | 100.68 ± 11.99 (93.88) | 103.12 ± 11.81 (97.80) | 0.227 | 0.0001* | 0.0001* |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.29 ± 0.48 (2.00) | 2.23 ± 0.48 (2.00) | 2.07 ± 0.48 (1.79) | 0.030* | 0.0001* | 0.001* |
| TAC (µmol/L) | 725.81 ± 122.18 (708.00) | 804.40 ± 99.40 (792.00) | 1016.51 ± 185.28 (986.00) | 0.0001* | 0.0001* | 0.0001* |

Test Statistics – Wilcoxon Test

## 4.26 Sex Hormones and Lipid Profile levels in Eugonadal Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.26, there were no significant differences in the mean levels of testosterone, follicle stimulating hormone and luteinizing hormone in the eugonadal automobile workers at different stages of vitamin C supplementation ($p>0.05$). The mean levels of total cholesterol, low density lipoprotein choesterol, very low density lipoprotein choesterol and triglyceride decreased progressively while the mean level of high density lipoprotein choesterol increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean levels of total cholesterol and low density lipoprotein choesterol were significant at one month and two months of vitamin C intake compared with the baseline values as well as at two months compared with one month ($p<0.05$) whereas the decrease in the mean levels of very low density lipoprotein choesterol and triglyceride as well as the increase in the mean high density lipoprotein choesterol level were significant at two months of vitamin C intake compared to their values at baseline and one month ($p<0.05$).

**Table 4.26: Sex Hormones and Lipid Profile levels in Eugonadal Automobile Workers at different stages of Vitamin C Supplementation.**

Data expressed as Mean ± SD (Median)

| Parameters | A<br>Baseline<br>(n = 19) | B<br>One month<br>(n = 19) | C<br>Two months<br>(n = 19) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 7.14 ± 0.83 (6.56) | 6.87 ± 0.76 (6.34) | 7.39 ± 0.89 (6.57) | 0.398 | 0.133 | 0.433 |
| FSH (IU/L) | 10.85 ± 1.05 (11.69) | 10.96 ± 0.86 (11.70) | 11.11 ± 0.87 (11.67) | 0.520 | 0.365 | 0.421 |
| LH (IU/L) | 5.92 ± 0.50 (5.94) | 6.10 ± 0.56 (6.10) | 6.12 ± 0.72 (6.01) | 0.376 | 0.329 | 0.276 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 4.95 ± 0.95 (5.04) | 4.81 ± 0.90 (4.99) | 4.51 ± 0.82 (4.40) | 0.0001* | 0.0001* | 0.0001* |
| HDLC (mmol/L) | 1.20 ± 0.07 (1.22) | 1.21 ± 0.06 (1.23) | 1.22 ± 0.06 (1.24) | 0.109 | 0.044* | 0.047* |
| LDLC (mmol/L) | 3.31 ± 0.80 (3.40) | 3.18 ± 0.78 (3.30) | 2.91 ± 0.71 (2.94) | 0.009* | 0.0001* | 0.0001* |
| VLDLC (mmol/L) | 0.44 ± 0.09 (0.39) | 0.42 ± 0.07 (0.40) | 0.38 ± 0.06 (0.35) | 0.336 | 0.001* | 0.012* |
| TG (mmol/L) | 0.97 ± 0.19 (0.86) | 0.92 ± 0.14 (0.87) | 0.84 ± 0.13 (0.76) | 0.275 | 0.004* | 0.012* |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG – Triglyceride. Test Statistics – Wilcoxon Test

## 4.27 BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Compensated Hypogonadic Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.27, there were no significant differences in the values of body mass index and cadmium levels in the automobile workers with compensated hypogonadism at different stages of vitamin C supplementation ($p>0.05$).

The mean values of systolic and diastolic blood pressures as well as lead and malondialdehyde levels decreased progressively while the mean levels of selenium, zinc and total antioxidant capacity increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean systolic blood pressure and lead level as well as the increase in the mean level of zinc were significant at two months of vitamin C intake compared with their values at baseline.

The decrease in the mean diastolic blood pressure and malondialdehyde level as well as the increase in the mean selenium level were significant at two months of vitamin C intake compared with their values at baseline and one month, whereas the increase in the mean level of total antioxidant capacity was significant at one month and two months of vitamin C intake compared with the baseline value as well as at two months compared with one month ($p<0.05$).

**Table 4.27: BMI, Blood Pressure, and Levels of Elements and Oxidative Stress Markers in Compensated Hypogonadic Automobile Workers at different stages of Vitamin C Supplementation.** Data expressed as Mean ± SD (Median)

| | A | B | C | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline (n =8)** | **One month (n = 8)** | **Two months (n = 8)** | **B/A** | **C/A** | **C/B** |
| BMI ($kg/m^2$) | 23.77 ± 2.75 (23.69) | 23.33 ± 2.20 (23.84) | 23.27 ± 2.02 (23.71) | 0.612 | 0.575 | 0.932 |
| **Blood Pressure** | | | | | | |
| SBP (mmHg) | 129.50 ± 10.66 (132.50) | 128.00 ± 8.56 (130.00) | 126.00 ± 8.76 (127.50) | 0.257 | 0.046* | 0,053 |
| DBP (mmHg) | 86.50 ± 8.18 (85.00) | 83.50 ± 6.67 (85.00) | 80.50 ± 7.62 (80.00) | 0.058 | 0.006* | 0.034* |
| **Elements** | | | | | | |
| Lead (µg/dL) | 55.60 ± 9.94 (60.04) | 55.33 ± 10.25 (61.67) | 52.90 ± 9.67 (59.18) | 0.878 | 0.044* | 0.059 |
| Cadmium (µg/dL) | 2.68 ± 0.58 (2.30) | 2.60 ± 0.58 (2.09) | 2.56 ± 0.56 (2.01) | 0.068 | 0.052 | 0.058 |
| Selenium (µg/dL) | 6.25 ± 1.44 (5.46) | 6.35 ± 1.45 (5.42) | 7.40 ± 1.67 (6.80) | 0.333 | 0.009* | 0.009* |
| Zinc (µg/dL) | 90.72 ± 17.27 (87.70) | 91.42 ± 17.30 (89.23) | 94.28 ± 18.01 (90.75) | 0.508 | 0.017* | 0.074 |
| **Oxidative Stress Markers** | | | | | | |
| MDA (µmol/L) | 2.57 ± 0.73 (1.97) | 2.49 ± 0.69 (1.97) | 2.30 ± 0.71 (1.76) | 0.114 | 0.007* | 0.007* |
| TAC (µmol/L) | 705.11 ± 134.95 (669.50) | 818.30 ± 118.88 (790.50) | 963.61 ± 158.30 (958.50) | 0.006* | 0.005* | 0.005* |

* - Significant. BMI - Body Mass Index. SBP - Systolic Blood Pressure. DBP – Diastolic Blood Pressure. MDA - Malondialdehyde. TAC - Total Antioxidant Capacity.
Test Statistics – Wilcoxon Test

### 4.28 Sex Hormones and Lipid Profile Levels in Compensated Hypogonadic Automobile Workers at different stages of Vitamin C Supplementation.

In table 4.28, there were no significant differences in the mean levels of testosterone, follicle stimulating hormone and luteinizing hormone in compensated hypogonadic automobile workers at different stages of vitamin C supplementation ($p>0.05$).

The mean levels of total cholesterol, low density lipoprotein, very low density lipoprotein cholesterol and triglyceride decreased progressively while the mean level of high density lipoprotein cholesterol increased progressively from baseline to two months of vitamin C intake. However, the decrease in the mean levels of total cholesterol and low density lipoprotein cholesterol were significant at one month and two months of vitamin C intake compared with the baseline values as well as at two months compared with one month ($p<0.05$) whereas the decrease in the mean levels of very low density lipoprotein cholesterol and triglyceride, and the increase in the mean high density lipoprotein cholesterol level were only significant at two months of vitamin C intake compared to their values at baseline and one month ($p<0.05$).

**Table 4.28: Sex Hormones and Lipid Profile levels in Compensated Hypogonadic Automobile Workers at different stages of Vitamin C Supplementation.** Data expressed as Mean ± SD (Median)

| Parameters | A<br>Baseline<br>(n = 8) | B<br>One month<br>(n = 8) | C<br>Two months<br>(n = 8) | B/A | C/A | C/B |
|---|---|---|---|---|---|---|
| **Sex Hormones** | | | | | | |
| Testo (ng/mL) | 5.30 ± 1.02 (4.66) | 5.81 ± 0.93 (5.77) | 5.99 ± 0.92 (5.61) | 0.079 | 0.093 | 0.071 |
| FSH (IU/L) | 17.72 ± 0.95 (17.99) | 17.02 ± 1.06 (17.25) | 15.89 ± 1.29 (15.87) | 0.093 | 0.090 | 0.082 |
| LH (IU/L) | 10.21 ± 0.66 (10.28) | 9.82 ± 0.83 (9.05) | 9.22 ± 0.92 (8.59) | 0.074 | 0.071 | 0.073 |
| **Lipid Profile** | | | | | | |
| TC (mmol/L) | 4.95 ± 0.69 (5.07) | 4.73 ± 0.72 (5.03) | 4.54 ± 0.67 (4.92) | 0.008* | 0.005* | 0.012* |
| HDLC (mmol/L) | 1.20 ± 0.07 (1.26) | 1.20 ± 0.07 (1.26) | 1.21 ± 0.07 (1.27) | 0.351 | 0.039* | 0.196 |
| LDLC (mmol/L) | 3.26 ± 0.55 (3.42) | 3.07 ± 0.60 (3.34) | 2.88 ± 0.53 (3.13) | 0.013* | 0.009* | 0.019* |
| VLDLC (mmol/L) | 0.46 ± 0.06 (0.42) | 0.44 ± 0.06 (0.40) | 0.40 ± 0.07 (0.40) | 0.171 | 0.046* | 0.137 |
| TG (mmol/L) | 1.00 ± 0.14 (0.92) | 0.94 ± 0.13 (0.88) | 0.90 ± 0.14 (0.87) | 0.173 | 0.045* | 0.146 |

* - Significant. Testo - Testosterone. FSH - Follicle Stimulating Hormone. LH - Luteinizing Hormone. TC - Total Cholesterol. HDL - High Density Lipoprotein. LDL - Low Density Lipoprotein. VLDL - Very Low Density Lipoprotein. TG – Triglyceride. Test Statistics – Wilcoxon Test

# CHAPTER FIVE

# DISCUSSION

The automobile workers due to the nature of their occupation which involves the use of metals and metal containing products like pigments, oil, grease, leaded gasoline as well as automobile battery repairs/recycling are exposed to toxic metals including lead and cadmium. Occupational exposure to these toxic metals may as well be enhanced by certain factors like poor occupational hygiene, lack of use of personal protective equipment as well as by certain unwholesome practices engaged in by these workers which include sucking of gasoline and washing of hands and feet with same.

Additionally, most of these artisans are oblivious of the hazards they may be exposed to, probably due to their low level of education which was observed in these workers, and as such pay little or no attention in protecting themselves from possible ingestion or inhalation of these toxic chemicals.

Blood lead and cadmium levels are used to assess recent exposures to these metals (Usuda *et al*., 2011), and the elevated blood levels of lead and cadmium in the automobile workers observed in this study indicates that these group of workers are more exposed to these metals than the general population because they come in contact with these metals in their daily activities at their various workstations. These findings correspond with those of Ahmad *et al*., (2014), Alli (2015) and Bal *et al*., (2015) who also reported similar increase in blood lead and cadmium levels in humans occupationally exposed to these toxic metals.

Notably, blood lead and cadmium levels did not differ significantly among the different professional groups of automobile workers (including the mechanics, spray painters/panel beaters, auto electricians and welders) which may be suggestive of equal exposure of these workers to the toxic metals since they all work in the same environment or workshop, and most times assist each other in their daily activities. This corroborates with the findings of Adejumo

*et al*., (2017) who also observed no differences in the blood levels of lead and cadmium among automotive workers in Benin City, Nigeria. Aloysius *et al*., (2013) had earlier reported that the various activities in the automobile workshop release heavy metals-containing wastes into the work environment, and these wastes may be generated from spent engine oil, gasoline, diesel and paints.

Blood lead and cadmium levels in this study were found to increase with respect to job duration, suggesting accumulation of lead with increase in years of work or job duration in the automobile workers. Al-Rudainy (2010) had earlier reported that the concentrations of these toxic metals in blood are determined by duration and dose of exposure among other factors.

Higher blood lead and cadmium levels were as well observed in automobile workers who smoke than the automobile workers who do not smoke, and this may be due to the high contents of these metals in tobacco smoke, suggesting that both occupational exposure and cigarette smoking contributes to elevated blood concentrations of these toxic metals. This is in line with the studies done by Sciskalska *et al*. (2014) who also reported elevated cadmium levels in blood of smelters who smoke. Lazarevic *et* al. (2012) had earlier reported that tobacco plants may take up metals including lead from the soil and gets concentrated in the leaves. The higher blood lead level observed in alcohol consuming automobile workers may be from contamination of lead during processing and canning. Izah *et al*., (2017) explained that elevated lead level in alcohol consumers may result from feedstocks and water used during production, moreover, the containers used in the packaging may be significant sources of metal contamination.

In addition to the increased blood levels of lead and cadmium observed in the occupationally exposed, the study also showed that the blood lead level in the occupationally unexposed was higher than the previously recommended WHO permissible range of <10µg/dL of lead in adults (WHO, 2007), which could be from other environmental contamination and exposures

(Ugwuja *et al*., 2014) including inhalation of exhaust fumes or contaminated dust particles or inadvertent ingestion of contaminated foods or drinks which is a possible indication of the extent of lead pollution in Nigeria. This is of serious concern as many Nigerians may be exposed to lead from the environment irrespective of their occupations. Similarly, Orisakwe (2009) and Galadima and Garba (2012) also reported high blood lead levels in occupationally unexposed persons. These findings support the fact that lead is a generally recognized occupational and environmental pollutant according to Pourmand *et al*., (2012).

Lead and cadmium were reported to adversely affect various body organs as well as altered biochemical indices (Orisakwe *et al*., 2007; Tchounwou, 2012) probably by disrupting various biochemical processes including alteration of antioxidant defense system via inhibition of various enzymes as well as displacement of essential trace elements resulting in oxidative stress which triggers the adverse health outcomes. Accordingly, the present study showed certain alterations in the measured biochemical profile of automobile workers possibly resulting from exposure to these metals.

One of the alterations in lead and cadmium accumulation in blood is their well known endocrine disrupting effect (Orisakwe, 2014; De Toni *et al*., 2017; Ujah *et al*., 2017) capable of interfering with testosterone synthesis as well as hypothalamus pituitary signaling, and consequently affecting testosterone concentration and release of sex hormones including GnRH, FSH and LH.

Among the automobile workers and control group studied, 74.2% and 96.8% respectively were eugonadic (normal levels of testosterone, FSH and LH) while 25.8% and 3.2% respectively had compensated hypogonadism (normal testosterone, elevated FSH and LH levels). Although the serum testosterone level was within the normal range, it was found to be lower in compensated hypogonadic workers compared with the eugonadic workers. On the other hand, FSH and LH were within normal range in eugonadic automobile but significantlty higher in

compensated hypogonadic workers. The differences in the reproductive hormones level with respect to gonadal status may reflect the lower mean lead and cadmium levels in the eugonadic automobile workers, it may therefore stand to reason that gonadal function and heavy metals exposure may be related in a concentration dependent manner. Moreover, Georgescu *et al*. (2011) had earlier reported that at higher level of exposure, lead adversely affects male sex hormone. FSH and LH are pituitary hormone which regulate testicular function, testosterone on the other hand regulate LH release by negative feedback mechanism. The altered hormone level in compensated hypogonadism may therefore indicate testicular dysfunction resulting to suppressed testosterone production and decline within the normal range. Hence, despite the normal testosterone levels, compensated hypogonadism may indicate impaired testicular function (Ventimiglia *et al*., 2017). Tajar *et al*., (2010) reported that the wide normal range of testosterone may possibly cause a decline in the hormone level from previously high normal to low normal in men with compensated hypogonadism. Additionally, in compensated hypogonadism when leydig cell function is compromised, normal or nearly normal testosterone levels may be sustained by augmented LH levels (Kristensen *et al*., 2018), FSH secretion may as well be stimulated, which may be the reason for the elevated FSH and LH in the present study. Elevated FSH levels in compensated hypogonadism may as well indicate impaired spermatogenesis (Barbotin *et al*., 2015).

The reduced testosterone level may be attributed to the disruption of testosterone production by these toxic metals (Rotter *et al*., 2016) possibly by interfering with enzymes like 3ß-Hydroxysteroid dehydrogenase (3ß-HSD) or 17ß-Hydroxysteroid dehydrogenase (17ß-HSD) involved in testosterone production (Tena-Sempere, 2010). This result agrees with that of Okoli *et al*., (2016) who also observed a reduction in testosterone level in automechanics exposed to mixed chemicals. Moreover, a recent animal study by Ujah *et al*., (2017) reported similar reductions in the levels of 3ß-HSD, 17ß-HSD and testosterone in cadmium chloride-exposed

rats. Although the present findings correspond with the reports of Vigeh *et al*., (2011) and El-Beltagy *et al*., (2015) who also observed low serum testosterone levels with high serum levels of LH and FSH following exposures to lead and cadmium, they however disagree with those of Telisman *et al*., (2000) who observed increased serum testosterone levels with no change in serum LH and FSH levels in male industrial workers exposed to lead and cadmium. Algafari *et al*., (2011) as well reported normal levels of serum testosterone and FSH in car customizers and welders whereas Chikezie *et al*., (2017) in their study on auto mechanics in Ibadan, Nigeria observed an increased serum testosterone and FSH levels with no change in serum LH level.

In this study, serum levels of these hormones were observed to be same among the different groups of automobile workers, however, reduced testosterone and elevated FSH were found with increased job duration which may indicate higher tendency of male reproductive dysfunction with increased duration of exposure to these toxic metals.

Further reductions in testosterone with no differences in FSH and LH levels were also observed in automobile workers who smoke and consume alcohol against the non-smokers and non-alcohol consuming automobile workers, therefore these habits may exacerbate the adverse effect of heavy metal exposure on the reproductive functions in these workers. The reduction in testosterone which may be from increased hepatic metabolism of this hormone in smokers (Dai *et al*., 2015), or from the action of nicotine (one of the toxic substances in cigarette smoke) which has been reported to impair androgen biosynthesis and leydig cell growth (Funabashi *et al*., 2005). The finding from this study is in line with the report of Bassey *et al*., (2018) who also observed lower testosterone in smokers but not in line with that of Blanco-Munoz *et al*., (2012) who reported elevated testosterone and LH levels in smokers. Additionally, excessive alcohol consumption may also damage leydig cells or impair the hypothalamus pituitary gonadal axis (Maneesh *et al*., 2006).

The endocrine disrupting function of lead and cadmium were also evident from the inverse association of lead and cadmium with testosterone, and the direct association of lead with FSH and LH, suggestive of reproductive function impairment. These findings corroborate with earlier studies on toxic metals associations with male sex hormones (Chen *et al*., 2016; Chikezie *et al*., 2017).

Another alteration attributable to lead and cadmium accumulation is the reduction in blood selenium and zinc levels in the automobile workers as observed in this study, and could be as a result of these toxic metals displacing or interfering with the metabolism of the trace elements (Matovic *et al*., 2011) at various levels including intestinal absorption, distribution in tissues and biological functions (Al-Fartosy *et al*., 2017). Other researchers also reported a reduction in blood zinc (Dioka *et al*., 2004; Bal *et al*., 2015) and selenium (Kasperczyk, 2012; Basu *et al*., 2015) levels in workers exposed to lead. However, Malekirad *et al*., (2010), reported elevated blood zinc levels in zinc-lead miners which may be associated with the zinc content of the mines. Adejumo *et al*., (2017) in their study on occupationally exposed automotive workers in Benin City also reported increased blood zinc levels which they attributed to inhalation of welding fumes containing zinc oxide during welding of galvanized materials or oral intake of contaminated foods in the auto repair workshops. Among the different groups of automobile workers, the levels of selenium and zinc were similar. However, they declined with increased duration on the job, which is an indication that increased duration of exposure to heavy metals may enhance the antagonistic effects of these toxic metals on the trace elements, selenium and zinc as observed in the present study.

Similarly, the eugonadic and compensated hypogonadic automobile workers had lower selenium and zinc levels than the eugonadal control, and this may be linked to the higher lead and cadmium levels which may possibly deplete the trace elements in these workers. Interestingly, selenium and zinc have been shown to play crucial roles in male reproductive

function, while selenium enhances testosterone production of leydig cell via enhancing expression of stAR and 3ß HSD genes (Shi *et al*., 2017) zinc is required by 17ß HSD for testosterone synthesis. Hence, the lower but normal testosterone level in the automobile workers may as well be linked to the lower selenium and zinc levels in these workers.

Lower selenium and zinc levels were also observed in automobile workers who smoke compared with their automobile workers who do not smoke, which corresponds with the findings of Isife and Dioka, (2010) who also observed lower selenium and zinc levels in smokers exposed to lead in Coal Camp, Enugu. Although the present study showed no significant differences in the levels of these trace elements in alcohol consuming and non-alcohol consuming workers, Ude *et al*., (2016) however observed higher levels of selenium and zinc in Nigerian alcohol beverage consumers, which may probably be linked to increased activity of antioxidant enzymes as protective mechanism in moderate drinkers (Kafai and Ganji, 2003).

Additionally, blood lead and cadmium levels showed an inverse association with both selenium and zinc levels in the automobile workers, and this further showed that lead and cadmium have antagonistic effect on essential elements. This is similar to the reports of Mogwasi *et al*., (2013) and Pawlas *et al*., (2016) who also observed an inverse association between lead and selenium and zinc. Selenium and zinc are components of the antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) respectively, and as such unwarranted reductions in these essential elements invariably results in the disruption of certain cellular activities including inhibition of antioxidant enzymes, and consequently excessive production of ROS which eventually results in oxidative stress and tissue damage (Ahamed and Siddiqui, 2007). Oxidative stress, which is an imbalance between ROS and antioxidants defence is regarded as the main culprit in heavy metal induced toxicity as the increased ROS produced are capable of

damaging lipid membranes, proteins and nucleic acids through oxidation of lipids, proteins and nucleic acids respectively (Bartosz, 2008).

The level of oxidative stress in biological samples can be measured using MDA and TAC which are known reliable markers (Jomova and Valko, 2011; Khajehnasiri *et al*., 2013; Christensen *et al*., 2015), and as such were used in this study to evaluate oxidative stress level in the automobile workers, which may result from accumulation of lead and cadmium. MDA which is the final product of lipid peroxidation is widely used as indicator of tissue damage (Dhir *et al*., 2004) whereas TAC estimates the cumulative activity of both endogenous and exogenous antioxidants (Krajcir *et al*., 2008) present in biological samples (Mohammadi *et al*., 2006), moreover, since antioxidant effects are additive, the measurement of TAC may be particularly useful in monitoring alterations in endogenous antioxidant levels (Erel, 2005). The elevated MDA level as well as reduced TAC level observed in the automobile workers is suggestive of oxidative stress possibly induced by lead and cadmium (Thuppil and Tannir, 2013). Similarly, the elevated MDA level may indicate lipid peroxidation from enhanced generation of ROS which impairs the antioxidant system (Flora *et al*., 2012) as evidenced by the reduction in TAC level observed in these workers. Other human and animal studies also reported similar elevation in MDA levels (Mahmood *et al*., 2015; Ogunrinola *et* a*l*., 2015; Adekola *et al*., 2016) and reduced TAC levels (AL-Fartosy *et al*., 2014; Okoli *et al*., 2015; Adekola *et al*., 2016; Chikezie *et al*., 2017) in lead and cadmium exposures, which were attributed to increased generation of reactive oxygen species induced by these toxic metals.

The oxidative stress markers remained same among the different automobile workers groups, however, MDA level increased while TAC level decreased with increased duration on the job, which may be suggestive of increased lipid peroxidation which increasingly suppresses the antioxidant defence with increased duration of exposure to heavy metals. MDA levels were as

well higher while TAC levels were lower in eugonadic and compensated hypogonadic automobile workers than in the control.

Additionally, the automobile workers who smoke also had higher MDA and lower TAC levels than the non-smokers, and this may be an indication of greater tendency of oxidative stress induction from additional toxic substances in cigarette smoke. Hence, smoking may exacerbate the oxidative stress-induced adverse health effects of heavy metal exposure in these workers. Obi *et al*., (2017) also reported higher MDA with lower TAC levels in smokers in Nnewi, Metropolis, Nigeria.

In addition to the above, MDA was directly associated with lead and cadmium whereas TAC was inversely associated with these metals, and these support the reports of Singh *et al*., (2013) and AL-Fartosy *et al*., (2014; 2017) who also observed similar associations in workers occupationally exposed to heavy metals.

The present study as well showed significant elevations in the serum levels of total cholesterol, LDLC, VLDLC and TG with no significant change in HDLC level in the automobile workers, and this may be attributed to the adverse effects of lead and cadmium on lipid metabolism (Zhou *et al*., 2016) possibly by altering normal lipid and lipoprotein fractions via lipid peroxidation (Olisekodiaka *et al*., 2012). It may as well be attributed to the up-regulation of plasma cholesterol and triglyceride concentrations by these toxic metals (Ugbaja *et al*., 2013), and these alterations in cholesterol metabolism increases the risk of CVD in persons exposed to these heavy metals (Ademuyiwa *et al*., 2005). These toxic metals probably enhance the activity of 3-hydoxy 3-methylglutaryl coenzyme A (HMG CoA) reductase which is the rate limiting enzyme in cholesterol biosynthesis thereby enhancing cholesterol synthesis. Other researchers had earlier reported cases of dyslipidemia (deviation from the normal lipid profile levels of a subject (Allouche *et al*., 2011)) in both humans and animals exposed to lead (Ademuyiwa *et al*., 2005; Ugbaja *et al*., 2013) and cadmium (Murugavel and Pari, 2007;

Samarghandian *et al*., 2015). Studies on exposure to these toxic metals have demonstrated similar increase in total cholesterol, LDL-C and triglyceride levels as well as decreased HDL-C level (Olisekodiaka *et al*., 2012; Samarghandian *et al*., 2015; Adejumo *et al*., 2016). Human studies as well reported similar increase in total cholesterol, LDL-C and VLDL-C levels (Sharma *et al*., 2012), elevated triglyceride level (Ajani *et al*., 2011) and normal levels of HDL-C (Ademuyiwa *et al*., 2005). Among the different automobile workers group however, the serum levels of total cholesterol, LDL-C, VLDL-C and triglyceride remained same, but increased with increased duration on the job. This suggests that increased duration of exposure to toxic metals may increase the risk of developing cardiovascular related diseases in these workers.

Similarly, the higher levels of total cholesterol and LDL-C observed in the eugonadal and compensated hypogonadic automobile workers compared with the control may still be suggestive of higher risk of cardiovascular diseases in these workers irrespective of the gonadal status. In the automobile workers who smoke, total cholesterol, VLDL-C, LDL-C and triglyceride levels were higher compared with the non-smoking workers, indicating that smoking may adversely affect lipid metabolism which further aggravates cardiovascular disease risk and hypertension in this group of workers. This may be linked to nicotine, a toxin present in tobacco smoke which stimulates hypothalamus pituitary adrenal axis, thus increasing catecholamine and cortisol secretion (Tweed *et al*., 2012), altering lipid metabolism and consequently dyslipidemia by enhancing lipolysis and elevating free fatty acids. Altered lipid profile levels in healthy smokers has also been reported by Garish and Harish (2018).

Although the lipid profile in alcohol and non-alcohol consuming automobile workers were not significantly different in this study, a previous study however reported that alcohol may increase the risk of cardiovascular diseases and alter lipid profile depending on the frequency of consumption as well as quantity consumed, though moderate consumption may increase

HDL-C concentration, thereby reducing cardiovascular disease risk via enhancing liver metabolism of low density lipoprotein cholesterol (Toffolo *et al*., 2012).

The present study further showed a direct association of lead and cadmium with total cholesterol, LDL-C, VLDL and triglyceride, with no significant association with HDL-C in the automobile workers. These findings correspond with those of Ademuyiwa *et al*., (2005) and Sharma *et al*., (2012) who also observed a similar association between blood lead and total cholesterol and LDL-C.

In addition to the dyslipidemia observed in these workers, elevations in both systolic and diastolic blood pressures were also observed, which further increases the chances or risks of developing cardiovascular disorders in this group of workers. The elevated blood pressure may be attributed to the alteration in the renin-angiotensin system through the activation of angiotensin converting enzyme (ACE) (Simeos *et al*., 2011), or as a result of oxidative stress which enhances the production of ROS thereby limiting the availability of nitric oxide (NO) (Pierini *et al*., 2015), and consequently enhancing vascular contraction and endothelial dysfunction (Virdis *et al*., 2011). The findings from this study support earlier studies which reported that exposures to lead and cadmium are significant risk factor for the development of hypertension (Tsao *et al*, 2000; Varoni *et al*., 2003; Satarug *et al*., 2006).

Other studies as well reported similar elevations in both systolic and diastolic blood pressures associated with heavy metal exposures (Poreba *et al*., 2010; Dongre *et al*., 2011; Caciari *et al*., 2013). Although both the systolic and diastolic blood pressures were similar among the automobile workers group, they however increased with increased job duration.

The adverse cardiovascular effect of tobacco smoke constituents including nicotine and carbon monoxide may as well be shown in the elevated systolic and diastolic blood pressures of smoking automobile workers, which is consistent with the reported blood pressure elevation in smokers according to Jena and Purohit (2017). Elevated diastolic blood pressure was equally

observed in automobile workers who consume alcohol, therefore excessive alcohol intake may therefore elevate the arterial pressure promoting hypertension.

The present study further showed a direct association of lead and cadmium with blood pressure (systolic and diastolic), and these findings correspond with the reports of Kasperczyk *et al*., (2009), Alghasham *et al*., (2011) and Lee *et al*., (2016) who also reported a direct relationship between blood lead levels and blood pressures. Although, An *et al*., (2017) in their study on workers of smelting industry reported a positive relationship between blood cadmium and blood pressure, they however found no association between blood lead and blood pressure which could be as a result of the low blood lead levels (<7µg/dL) in these workers.

**Interventional Study**

Since automobile workers are at higher risk of exposure to lead and cadmium which is detrimental to their health, and because the symptoms of lead and cadmium toxicity are not very specific and most times go undiagnosed, a control measure targeted at reducing the levels of these metals and subsequently mitigating the accompanying adverse health effects must have to be in place in order to curb the lethal effects. Although synthetic chelators have been used as regimen for heavy metal toxicity, they are however not without their shortfalls. These shortfalls have led to the quest for cheaper and easily available alternative with little or no side effects for the reduction of the levels of these toxic metals as well as alleviating their adverse health outcomes.

In light of the above, an intervention study using green tea and vitamin C was undertaken in order to explore the possible ameliorating potentials of these supplements on blood lead and cadmium as well as the altered biochemical profile observed in the automobile workers. These supplements were selected based on the understanding that some dietary supplements including dietary phytochemicals (Nwokocha *et al*., 2012) and vitamins (Ugbaja *et al*., 2013) possess

protective effects against heavy metal toxicity which act by alleviating or preventing the adverse health effects induced by these metals (Monachese *et al*., 2012; Zhai *et al*., 2015).

In this study, green tea supplementation resulted in the reduction of the elevated blood lead and cadmium levels in the automobile workers, however, the reduction in blood cadmium level was significant after two months of green tea intake. Similar reductions were as well observed in both eugonadal and compensated hypogonadic automobile workers at different stages of green tea supplementation. The reduction in the blood lead and cadmium levels may be attributed to the abundant catechins present in green tea which may inhibit the absorption of the metal ions or enhance their excretion (Paul, 2008). These flavonoids present in green tea tend to bind with these metal ions to form an insoluble complex-ionic salt, and consequently enhancing their removal (Idowu *et al*., 2017) in addition to reducing gastrointestinal absorption (Abdel-Moneim *et al*., 2014). Few animal studies as well reported significant reductions in blood lead following administration of green tea extracts (Meki *et al*., 2011; Yosef *et al*., 2012; Abdel-Moneim *et al*., 2014).

Green tea supplementation for one month in the automobile workers was found to improve serum testosterone level whereas the serum FSH and LH levels improved after two months. Considering the gonadal status of these workers, this supplement also improved the levels of these hormones in compensated hypogonadic automobile workers with no significant effect in the eugonadic workers. The reason for the observed differences in green tea effect on the male reproductive hormones with respect to gonadal status may possibly be due to enhanced testicular function following improved testosterone level after green tea intake in compensated hypogonadic workers. The improved testosterone level may have resulted in subsequent reductions in LH and FSH compared with the baseline values, and this may be suggestive of a more profound effect of green tea on impaired gonadal function. The improvement in testosterone level may possibly be associated with the ability of green tea to inhibit the activity

of aromatase (Satoh *et al*., 2002; Monteiro *et al*., 2006; Balunas *et al*., 2008) which is the enzyme that converts androgens like testosterone to estrogen. The catechins, especially EGCG in green tea may as well inhibit the enzyme 5-α reductase (Hipakka *et al*., 2002; Grant and Ramasamy, 2012) which converts testosterone to dihydrotestosterone (DHT).

It was observed in few animal studies that green tea administration in lead and/or cadmium administered rats increased serum testosterone levels (Thuppil and Tannir, 2013; El-Beltagy *et al*., 2015; Sha'bani *et al*., 2015) and reduced serum LH levels (Jassem *et al*., 2008; Gawish *et al*., 2010). However, some other studies observed that green tea administration in experimental models reduced serum testosterone and increased serum LH (Chandra *et al*., 2011; Das and Karmakar, 2015) as well as serum FSH levels (Das and Karmakar, 2015), which were attributed to the decreased activity of steroidogenic enzymes by green tea catechin. It has earlier been proposed that the beneficial or detrimental effect of green tea on testosterone level is dependent on the ratio of EC + EGC to ECG + EGCG, whereby a higher amount of EC + EGC would favor testosterone production, and a higher ECG + EGCG would inhibit its production and vice versa (Seeram *et al*., 2006). Similarly, in the present study, EC + EGC and ECG + EGCG were 90.6mg/g and 71.6mg/g respectively. Additionally, most of the studies on green tea supplementation with respect to heavy metal exposure were performed on experimental models, and as such, may not be well extrapolated in human cases. Therefore, the positive or negative effect of green tea may be dose-dependent (Murakami, 2014), and 90-300mg EGCG has been recommended by EFSA (2018) for optimal health benefits. In other words, at moderate concentrations, green tea catechins exhibit positive effects on reproductive function parameters while eliciting an opposite action at relatively higher concentrations, green tea may therefore exert some degree of protection on reproductive health especially in persons exposed to gonadotoxin(s) (Roychoudhury *et al*., 2017).

The lowered blood selenium and zinc levels in the automobile workers including the eugonadal and compensated hypogonadic were significantly raised following green tea supplementation, however, blood zinc level was significantly elevated after two months of supplementing with green tea. The improved blood levels of these essential elements may be due to the reduction in blood lead level following green tea intake since lead is known to compete with as well as displace these minerals/elements. These findings are consistent with those of Hamdaoui *et al*., (2005) who also reported improved serum levels of selenium and zinc after supplementing with green tea in experimental models. Similar elevation in serum zinc level was also observed in obese individuals after supplementing with green tea (Sulibursk*a et al*., 2012).

In addition to the improved blood levels of the essential trace elements in these workers after taking the green tea supplement, the present study also observed an improvement in the oxidative stress markers which was marked by a significant decrease in serum MDA level as well as a significant increase in serum TAC level following supplementation with green tea. This may be suggestive of the anti-oxidative property of green tea which may possibly act through scavenging or suppressing ROS and lipid peroxidation, thereby enhancing antioxidant defence (Suresh *et al*., 2010). The high concentrations of catechins in green tea, especially EGCG with strong antioxidant properties (Ashbeans *et al*., 2011, Ly *et al*., 2014) which is much higher than vitamin C, a known antioxidant is capable of reducing free radical species and blood metals, and subsequently reducing oxidative stress levels (Hijazi *et al*., 2015).

The lead and cadmium reducing as well as selenium and zinc increasing property of green tea may also play a role in their antioxidant activity, thereby preventing the production of heavy metal induced ROS and their subsequent interactions with membrane proteins and lipids as well as nucleic acids, thus, preventing oxidative tissue damage (Suresh *et al*., 2010). These observations are in line with previous human and animal studies which also reported an improvement in oxidative stress level depicted in the reduction in serum MDA levels and

elevation of serum TAC levels after green tea supplementation (Yosef *et al*., 2012; Haidari *et al*., 2013; El-Beltagy *et al*., 2015; Arab *et al*., 2016; Zadkhosh *et al*., 2016). However, Mozaffari-Khosravi *et al*., (2014) in their study on type 2 diabetes patients reported that green tea supplementation did not affect the serum levels of MDA, and this may be as a result of the lower steeping temperature of the green tea bags which was between 60-70$^{o}$C against 100 $^{o}$C.

Green tea supplementation was as well found to improve blood lipids and lipoproteins in the automobile workers irrespective of the gonadal status by reducing the elevated serum levels of total cholesterol, LDLC, VLDLC and triglycerides as well as increasing the serum HDLC level. Green tea supplementation for one month lowered serum total cholesterol, LDLC, VLDLC and triglyceride levels, and the reductions in these parameters as well as elevated HDLC levels were equally observed after two months of green tea supplementation. This improvement in lipids and lipoprotein fractions suggests that green tea has a positive effect on cardiovascular health which may be linked to the influence of green tea catechins on lowering intestinal lipid absorption (Frejnagel and Wroblewska, 2010) as well as the enhancing fecal excretion (Koo and Noh, 2007). An in vitro study had reported that green tea can inhibit HMG-CoA reductase (Singh *et al*., 2009) which is the rate limiting enzyme of the mevalonate pathway thereby reducing cholesterol synthesis. Additionally, green tea polyphenols have also been shown to simultaneously inhibit three enzymes (mevalonate kinase, mevalonate diphosphate decarboxylase and farnesyl pyrophosphate synthase) in the mevalonate pathway (MVP) of cholesterol biosynthesis (Ge *et al*., 2014). Other researchers also observed lowered serum levels of total cholesterol and LDLC (Sulibursk*a et al*., 2012; Belcaro *et al*., 2013; Onakpoya *et al*., 2014; Zhou *et al*., 2016), decreased serum levels of triglyceride (Sulibursk*a et al*., 2012; Belcaro *et al*., 2013; Zhou *et al*., 2016) and increased serum levels of HDLC (Basu *et al*., 2010; Sulibursk*a et al*., 2012; Mozaffari-Khosravi *et al*., 2014; Zhou *et al*., 2016) after green tea supplementation in both human and animal studies. On the other hand, Senger *et al*., (2012)

observed no improvement in lipid profile parameters after green tea supplementation, however, this study was on elderly subjects with metabolic syndrome.

Additionally, moderate concentrations of EGCG (90-300mg/day) has been shown to have beneficial effects on cardiovascular and metabolic functions in normal physiology as well as pathophysiology of other oxidative stress-induced disease conditions, and may elicit hepatotoxicity at concentrations equal or greater than 800mg/day (Kim *et al*., 2014; EFSA, 2018). The positive effect of green tea on lipid metabolism was also evident in the reduction of BMI after two months of supplementation as observed in the present study, and may explain the reason behind the use of green tea as a natural agent of weight loss (Basu *et al*., 2010; Wang *et al*., 2010). Some researchers suggested that the anti-obesogenic effect of green tea polyphenols on fat homeostasis may follow diverse mechanisms, like enhancement of thermogenesis, reduction of fat absorption and modification of appetite (Rains *et al*., 2011; Thavanesan, 2011).

Similarly, the elevated systolic and diastolic blood pressures in the automobile workers were reduced after two months of green tea supplementation, and this could be attributed to the ability of green tea polyphenols to improve endothelial dilatory function (Antonello *et al*., 2007) or increase bioavailability of endothelium-derived nitric oxide (Amiot *et al*., 2016) by regulating endothelial nitric oxide synthase (eNOs) activation as well as regulating ROS production (Babu and Liu, 2008). It may as well be due to the ability of green tea polyphenols to maintain vascular tone by balancing vasoconstrictory substances (angiotensin-II (Ang-II)), endothelin-1(ET-1)) and vasodilatory substances (prostaglandins, various endothelium-derived hyperpolarizing factors (EDHFs)) (Bhardwaj and Khanna, 2013). Moreover, green tea may likey inhibit ACE as a possible mechanism for blood pressure reduction (Actis-Goretta *et al*., 2006; Persson *et al*., 2012).

Similar reductions in systolic and diastolic blood pressure were reported by Khalesi *et al*., (2014) and Peng *et al*., (2014), Onakpoya *et al*., (2014) observed a reduction in systolic blood pressure following green tea consumption whereas Kafeshani *et al*., (2017) in their study on healthy adult men observed that green tea supplementation did not affect both the systolic and diastolic blood pressures. The disparity may be due to the six weeks of green tea supplementation compared with two months supplementation in the present study.

Vitamin C supplementation for two months also reduced the blood lead levels in the automobile workers, and this reduction may be attributed to increased urinary elimination of lead which subsequently reduces blood and tissue levels. This is consistent with other studies which also reported a reduction in blood lead levels following vitamin C supplementation in human subjects and animals exposed to lead (Tandon *et al*., 2001; Gulani *et al*., 2013; Ugbaja *et al*., 2013). Blood cadmium levels were however not altered by vitamin C supplementation, and this may probably be due to the inability of the given dose (500mg) to effectively reduce the metal ions, or that vitamin C may not be an appropriate agent for the reduction of blood cadmium in the exposed persons.

Despite the previously reported role of vitamin C in male reproduction which includes its involvement in cholesterol synthesis (Sonmez et al., 2005), a requirement for steroid hormone synthesis and secretion of anterior pituitary hormones including FSH and LH (Okon and Utuk, 2016), the current study showed no significant effect of vitamin C (500mg) supplementation on serum testosterone, LH and FSH levels in automobile workers irrespective of the gonadal status. This may possibly be that the dosage or duration of intake was not sufficient to cause any significant change in the levels of these hormones or that this vitamin does not affect these hormones contrary to earlier reports. Bughdadi, (2014) had earlier reported that vitamin C supplementation maintains the pituitary-gonadal axis by keeping the hormonal pattern within normal ranges. Increased serum testosterone and FSH levels following vitamin C

supplementation were reported in few animal studies (Fernandes *et al*., 2011; Vijayprassad *et al*., 2014; Okon and Utuk, 2016).

Vitamin C supplementation was also found to increase the blood selenium and zinc levels in these workers irrespective of the gonadal status after two months of intake, and this may be related to the action of this antioxidant vitamin in reducing the blood lead level in the automobile workers, and subsequently increasing the levels of these trace elements. Other researchers likewise reported that vitamin C enhances the expression of antioxidant enzymes activity (Khassaf *et al*., 2003; Kathore *et al*., 2014; Assaikkutti *et al*., 2016), which possibly increased zinc and selenium levels.

Similar to green tea supplementation, vitamin C supplementation equally improved oxidative stress markers in both eugonadic and compensated hypogonadic automobile workers as depicted in the reduction in serum MDA level with a corresponding increase in serum TAC level as observed in this study. This may be due to the antioxidant nature of this vitamin which acts by forming less reactive compounds or quenching free radical reaction (Popovic *et al*., 2015), reducing oxidative stress and lipid peroxidation, and consequently improving antioxidant status (Ghanwat *et al*., 2016). Additionally, El-Tohamy and El-Nattat (2010) had earlier reported the protective effect of vitamin C against heavy metal induced oxidative stress. The findings from this study are in agreement with the reports of other human and animal studies which observed similar reduction in MDA levels (Abdollahazad *et al*., 2009; Alkhamees, 2013; Shittu *et al*., 2013; Popovic *et al*., 2015) and elevation in TAC levels (Ozdem *et al*., 2011; Kamodyova *et al*., 2013; Poulab *et al*., 2015; Ahmadi, 2016) levels in both humans and experimental models. Bakhtiari *et al*., (2012) however reported that a daily supplementation with 500mg vitamin C for three weeks did not affect salivary TAC levels, which may be due to shorter period of intervention.

The present study also recorded significant reductions in the elevated serum total cholesterol and LDLC levels after one month of vitamin C supplementation, serum VLDLC and triglyceride levels were however reduced after two months in addition to further reductions in serum levels of total cholesterol LDLC in the automobile workers irrespective of the gonadal status. The serum level of HDLC was also increased after two months of supplementation in these workers. These results indicate that vitamin C may be involved in the maintenance of normal lipid and lipoprotein levels which could be attributed to its ability to activate 7α-hydroxylase, the enzyme that enhances the conversion of plasma cholesterol to bile acid, and subsequently reducing cholesterol levels (Eteng *et al*., 2006). The decreased serum levels of TG may be from enhanced uptake and removal of VLDLC from plasma which is facilitated by this vitamin (Hasegawa *et al*., 2002).

Other human and animal studies also reported reduced serum cholesterol and LDLC levels (Gaur and Dixit, 2012; El Mashad *et al*., 2016), reduced VLDLC levels (Eteng *et al*., 2006), reduced triglyceride levels (McRae, 2008) and increased serum HDLC levels (El Mashad *et al*., 2016) following vitamin C supplementation. Additionally, Afkhami-Ardekani and Shojaddiny-Ardekani (2007) observed that daily intake of 1000mg vitamin C for six weeks reduced serum LDL-C and triglyceride levels whereas no significant effect was observed on lipid profile following a daily intake of 500mg vitamin C for six weeks in type 2 diabetes patients, the difference possibly being dose dependent.

Vitamin C supplementation for two months also reduced the elevated systolic and diastolic blood pressures in these workers irrespective of the gonadal status, and this may be attributed to the inhibition of the oxidation of tetrahydrobiopterin to dihydrobiopterin by this vitamin, which consequently increases the intracellular concentration of tetrahydrobiopterin, a co-factor of endothelial nitric oxide synthase (eNOS) thereby enhancing NO production (Huang *et al*., 2000). Vitamin C is therefore believed to improve the bioavailability and activity of NO which

is a potent vasodilator (Suematsu *et al*., 2010; Mortensen-Lykkesfeldt, 2014). Vitamin C has also been reported to restore hypertension associated with endothelial dysfunction resulting from impaired NO/L-arginine pathway and impaired vasodilation (Taddei *et al*., 1998).

Similar reduction in blood pressures were also reported by different studies using various doses and routes of administration of vitamin C (Brody *et al*., 2002; Mullan *et al*., 2002; Fernandes *et al*., 2011; Juraschek *et al*., 2012; Karin *et al*., 2016; Shateri *et al*., 2016).

## 5.1 Conclusion

The results obtained from this study showed that blood lead and cadmium levels were markedly elevated in the automobile workers, and may have led to altered biochemical indices including reproductive hormones, trace elements, oxidative stress markers and lipid profile levels. It is likely that the altered sex hormone levels in the automobile workers may impair gonadal function, and consequently affect reproductive health.

It was also observed that increased duration of exposure, smoking as well as alcohol consumption may enhance accumulation of lead and cadmium, alterations in biochemical indices, and consequently exacerbating the adverse health effects in these workers.

Lead was directly associated with FSH, LH, MDA, total cholesterol, LDL,VLDL and triglyceride, inversely associated with testosterone, Se, Zn and TAC. Cadmium also showed similar associations, except for FSH and LH which showed no significant associations with cadmium in the automobile workers. This is an indication that lead and cadmium accumulation in blood significantly alter the normal biochemical parameters.

Green tea supplementation showed ability to reduce blood lead and cadmium levels as well as improve the altered levels of reproductive hormones, trace elements, oxidative stress markers and lipid profile in these workers. Similarly, vitamin C supplementation reduced blood lead level, and improve trace elements, oxidative stress markers and lipid profile levels in these workers.

Green tea or vitamin C may represent potential and promising dietary supplement with ameliorating potentials for reducing certain aspects of lead and cadmium induced toxicity in the affected persons.

## 5.2 Recommendations

From the findings of this study, it is therefore recommended that;

I. Blood lead and cadmium tests be included in diagnostic tests especially when there is clear evidence or suspected case of toxic metal exposure.

II. As a result of the health benefits, availability and cost effectiveness of green tea and vitamin C supplements, they may be useful in the management of certain aspects of lead and cadmium toxicity as well as prevention of certain pathological conditions.

## 5.3 Limitation of Study

Due to the unavailability of the recommended Trace Element Plus $K_2$ EDTA ($K_2$E) vacutainer tubes, $K_2$ EDTA were used for sample collection for blood metals and trace elements analyses.

## 5.4 Suggestion for Further Studies

It is recommended that further studies be carried out to evaluate the combined effects of green tea and vitamin C supplements in persons occupationally exposed to toxic metals in order to ascertain the possible ameliorating potentials of both supplements. Longer time of intervention is also recommended in order to ascertain the long term effects of green tea and vitamin C supplements.

## 5.5 Contribution to Knowledge

In the light of the elevated blood lead and cadmium levels as well as the possible alterations in the biochemical indices in automobile workers, this study suggests the importance of green tea and vitamin C in ameliorating the toxic effects of lead and cadmium in these workers.

Green tea and vitamin C are therefore regarded as important dietary supplements in the prevention and management of lead and cadmium-induced toxicities.

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## APPENDIX I

## INFORMED CONSENT

Dear Sir/Madam

I am Chikaodili Nwando Obi-Ezeani, a Ph.D student of the department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus intend to carry out a research on the topic: Effects of *Camellia sinensis* and Vitamin C Supplementation on Some Elements and other Biochemical Parameters of Automobile Workers in Enugu, Nigeria.

**Purpose of study**

The aim of this study is to examine the levels of some elements and other biochemical parameters of automobile workers, and the subsequent effects of green tea (*Camellia sinensis*) and vitamin c supplementation.

**Hazards of the Research**

This research is a non-hazardous interventional study for the subjects since the supplements (green tea and vitamin c) they will consume will not constitute any health hazard to them as none has been recorded so far based on the selected dosage.

**Benefits of the Research**

Since heavy metal toxicity is very prevalent in our society especially among the occupationally exposed persons, *Camellia sinensis* (green tea) and vitamin c may serve as safe and cheap substitutes for synthetic chelators which are associated with various adverse effects for reducing blood metal levels and the associated toxicities.

In other words, consumption of these products may prove to be beneficial in preventing or alleviating the adverse health effects of heavy metal poisoning, thereby improving the quality of life and reducing the overall burden in our healthcare system.

**Procedure**

The purpose of the study will be explained to each of the participants and informed consent sought before administering of questionnaire, supplementation and sample collection. Initial fasting sample of eight milliliters (8ml) will be collected from the subjects in all the groups and dispensed into $K_2$-EDTA and plain vacutainer tubes, and this will serve as baseline samples before commencement of the intervention study.

Green tea and vitamin C will be taken by the subjects for two months as outlined in the groups. Samples will be collected after one month and two months of supplementation. The samples will be used for analysis of the biochemical parameters.

**Voluntarism**

Participation in this research work is on voluntary basis and the human subjects will not be paid.

**Confidentiality**

All the information and data generated from the participants during the course of this research work will be handled with high level of confidentiality, they will only be available to the student and supervisors.

**Right to withdraw without repercussions**

The participant has the right to agree or decline from the study as well as the right to withdraw from participating in the study at any time without repercussion.
The information and data obtained will solely be used for the purpose of this research.

**Name of Researcher:** Chikaodili Nwando Obi-Ezeani

**Address of Researcher:** Department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus.

**Phone Number**: +2348038739762

**Signature/Thumbprint of participant**

## APPENDIX II

## QUESTIONNAIRE

I, Obi-Ezeani Chikaodili Nwando, a Ph.D. student of Chemical Pathology Department, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, is undertaking a research on "Effects of *Camellia sinensis* and Vitamin C Supplementation on Some Elements and other Biochemical Parameters of Automobile Workers in Enugu, Nigeria".

Kindly answer the questions rightly and with all sincerity. All information will be treated with confidentiality. Please tick and write appropriately.

### SECTION A (SOCIO-DEMOGRAPHIC & ANTHROPOMETRIC DATA)

1. Phone no.: _____
2. Age: _____
3. Sex: ____
4. Weight:_____
5. Height:_____
6. Body Mass Index: _____
7. Blood Pressure Measurement:_____
8. Marital Status: Tick(✓) appropriately
    a. Single [ ] c. Divorced [ ]
    b. Married [ ] d. Widowed [ ]
9. Educational Status
    a. Primary [ ] c. Tertiary [ ]
    b. Secondary [ ] d. No formal education [ ]

**SECTION B (SOCIAL HABITS/LIFE STYLE AND JOB DESCRIPTION DATA)**

Please read carefully and tick (✓) where appropriate.

10. Do you take alcohol? No [ ] Yes [ ]
11. Do you smoke? No [ ] Yes [ ]
12. History of chronic diseases
    a. Diabetes [ ]
    b. Hypertension [ ]
    c. Others (please specify): _____
13. Are you currently taking any vitamin/mineral supplement, herbal medication, lipid lowering or fertility drug?

    No [ ] Yes [ ]

    If Yes, please specify_____
14. What is the nature of your job?
    a. Vehicle repairs and services [ ]
    b. Panel beating/ Spray painting [ ]
    c. Welding [ ]
    d. Battery maintenance and services [ ]
    e. Civil service [ ]
    f. Business [ ]
    g. Student [ ]
    h. Others_____
15. Duration of job.
    a. 6-12 months [ ]
    b. 1-5 years [ ]
    c. 6-10 years [ ]
    d. 11-15 years [ ]
    e. 16-20 years [ ]
    f. 20 years and above [ ]

## APPENDIX III

# NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL

**P.M.B. 5025, NNEWI, ANAMBRA STATE, NIGERIA**


Chairman
Board of Management

Mrs. Chinyelu Ogoamaka Nwofor
B.Ed, M.Ed, MHP&M, AHA, FCAI
Director of Administration/
Secretary to the Board

Professor Anthony O. Igwegbe
MBBS, FWACS, FICS, FPCS
Chief Medical Director/
Chief Executive

Dr. E. A. E. Afiadigwe
B.Sc (Hons) Sig, MBBS (Lond), FWACS, FICS
Chairman
Medical Advisory Committee

E-mail: nauthmd@yahoo.co.uk
nauthnnewi@hotmail.com
Telegram: TEACHOS NNEWI


NAUTH/CS/66/VOL.10/20/2017/021

*Our Ref:*

*Your Ref:*

5th June, 2017

*Date:*

**Obi-Ezeani Chikaodili Nwando**
Department of Chemical Pathology,
Faculty of Health Sciences and Techology,
Nnamdi Azikiwe University,
Nnewi Campus

**ETHICS COMMITTEE APPROVAL**

**RE: EFFECTS OF CAMELLIA SINENSIS (GREEN TEA) AND VITAMIN C SUPPLEMENTATION ON MALE SEX HORMONES AND SOME BIOCHEMICAL PARAMETERS OF AUTOMOBILE WORKERS IN DESTINY LAYOUT IN ENUGU STATE, NIGERIA**

We write to inform you that after due consideration of your research proposal, approval is hereby conveyed for you to commence the study.

The principal investigator is required to send a progress report to the Ethics Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out her oversight function.

Please note that this approval is subject to revocation if you fail to obtain proper authorization from your study site/unit.

.....
**Dr. Joy Ebenebe**
Chairman, NAUTH Ethics Committee

.....
**Mrs. M.C Onwuka (JP)**
Sec., NAUTH Ethics Committee