CHAPTER ONE

INTRODUCTION

The liver is the largest glandular organ of the body enclosed within the right lower rib cage beneath the diaphragm and it is the chief site for metabolism and excretion. Its other functions are the metabolism of carbohydrate, protein, fats toxins and other xenobiotics. It also performs the function of detoxification, secretion of bile and storage of vitamins. It plays a good role inthe maintenance of homeostasis of the internal milieu (Rajib *et al*., 2009). The liver is involved in the biochemical pathways to growth, nutrient supply, energy provision, reproduction and fight against diseases, (Ward and Daly, 1999). Thus, maintaining a healthy liver is a crucial factor for overall health and wellbeing (Rajib *et al*., 2009). The liver is continuously exposed to various environmental toxins, pathogens and microorganism and is constantly abused by poor drughabit, (over the counter and prescribed drugs) and alcohol which can eventually lead to various liver disorders such as hepatitis, cirrhosis and alcoholic liver damage (Subramonium and Pushpangadan, 1999; Sharma *et al*., 1991). Liver diseases remain one of the serious health problems and inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective drug is available.

Plant remedies are known to play a vital role in the management of liver diseases but the availability of these remedies is actually scarce (Subramonium and Pushpangadan, 1999). Vegetablesserve as indispensable constituents of the human diet, supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy, (Aja, *et al.*, 2010).Leafy vegetables have been found to boost the concentration of Red Blood cell and significantly increase the serum activity of AST in experimental animals, (Ezekwe *et al*., 2013). Focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants for treatment purposes or for the production of drugs, (Dahanukar *et al*., 2001; Olamide and Mathew, 2013;

Udochukwu, *et al.,* 2015). Their use in ethnomedicine for the managementof ailments stem from the presence of phytochemicals, (Aja, *et al.,* 2010).It has been suggested that methanol extracts (Saud Asif Ahmed, *et al,* 2013) and ethanol extracts (Ladipo, *et al.,* 2010) of vegetables could be used in treating diseases caused by microorganisms.Polyphenols and flavonoids in plant extracts had been implicated in their antioxidant potentials byimproving the functionality of the antioxidant system of the test rats, (Edeoga, *et al.,* 2005; Sudha Medapa *et al.,* 2011; Imaga and Bamigbetan, 2013; Alamgir *et al.,* 2016).Such scientific studies have led to isolation of chemical substances with therapeutic properties and many of these isolates are useful as drugs while others have served as substrates for drugs synthesis. Therefore, many remedies from plant origin have been evaluated for their potential antioxidant and hepatoprotective effect in experimental animal model.

Acetaminophen (APAP) is an analgesic and antipyretic substance used in the production of the drug paracetamol. Although safe at therapeutic doses, APAP had been found to cause severe liver injury (Erica and Emily. 2014). Mitchell, *et al*. (1973), reported that Acetaminophen (APAP) overdose is the predominant cause of acute liver failure in the United States and that toxicity begins with a reactive metabolite that binds to proteins. These findings indicated that acetaminophen was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bonded to protein. It has also been shown by James, *et al.* (2009) that repletion of glutathione (GSH) prevented the toxicity. The mechanism of acetaminophen toxicity is by a complex sequence of events that include but not limited to CYP metabolism to a reactive metabolite which depletes glutathione and covalently binds to proteins, loss of glutathione with an increased formation of reactive oxygen and nitrogen species in hepatocytes undergoing necrotic changes, increased oxidative stress, associated with alterations in calcium homeostasis and initiation of signal transduction responses, causing mitochondrial permeability transition,

occurring with additional oxidative stress, loss of mitochondrial membrane potential, and loss of the ability of the mitochondria to synthesize ATP and loss of ATP which leads to necrosis,(Mitchell *et al*., 1973; Jack, *et al.,* 2009). The reactive metabolite was found to be *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is formed by a direct two-electron oxidation (Dahlin *et al*., 1984).It was also, showed that NAPQI is detoxified by glutathione (GSH) to form an acetaminophen-GSH conjugate. After a toxic dose of acetaminophen, total hepaticGSH is depleted by as much as 90%, and as a result, the metabolite covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts (Mitchell *et al*., 1973). Western blot assays for acetaminophen protein adducts have also been used (James, *et al.*, 2009) to study toxicity in human overdose patients. In these studies, adducts have been detected in the blood of human overdose patients with severe toxicity (hepatic transaminases above 5000 IU/l) (James *et al*., 2009).Depletion of GSH which is an intrinsic antioxidant is capable of introducing peroxidation of cell membrane lipids, regeneration of reactive oxygen free radicals and hepatocellular fatty regeneration with centriolobular necrosis of the liver.

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogeneous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals such as Fe^{2+} and Cu^{2+} (Halliwell and Guteridge, 1999). Overproduction of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates and enzymes resulting in several diseases. Living systems have specific pathways to overcome the adverse effects of various damages. However, sometimes these repair mechanism fail to keep pace with such deleterious effects (Nilsson*et al*., 2004). In chronic liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver diseases, drug- and chemical-induced hepatic toxicity), the antioxidant medicines such as silymarin can have beneficial effect, (Feher and Lengyey, 2017). Sylimarin is the active ingridient in the branded drug, Sylibon 140 (a known hepatoprotective drug), manufactured

by Micro Laboratory Ltd, India.Silymarin has cytoprotective activity mediatedby its antioxidative and radical-scavenging properties, (Křena and Walterovab, 2005).As an antioxidant, silymarin scavenges for free radicals that can damage cells exposed to toxins. Silymarin has been said to be at least ten times more potent in antioxidant activity than vitamin E.

Statement of the Problem:

In Nigeria, herbal medicines are used to treat various diseases and ailments such as: asthma, tuberculosis, ulcers, diarrhea, dysentery, etc. Leafy vegetables contribute greatly to the nutritional and medicinal needs of sub-tropical local populations especially in Nigeria as staples, flavours, condiments, spices, drinks and beverages in most developing countries (Mbang*et al.,* 2008). It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention and cure of diseases because plants have long served as a useful and natural source of therapeutic agents (Chevellier 1996). Pharmacological studies have demonstrated hepatoprotection, antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and antiulcer activitiessupporting the traditional uses of some leafy vegetables, (Perez, 2016; Ahmed, 2016). These effects have been attributed to the antioxidant components of such plant phenolic compounds including flavonoids and phenylpropanoids among others (Rice-Evan *et al.,* 1996). The intentional and unintentional abuse of paracetamol and the invasion of the living system by other toxins and toxicants which may lead to oxidative stress and consequent liver diseases need to be stermed. Living systems have specific pathways to overcome the adverse effects of various damages, but sometimes, these repair mechanism fail to keep pace with such deleterious effects (Stegmark and Akesson, 2004; Nilsson *et al*, 2004). Therefore, there is need to seek an alternative to augment the effort of the internal protection and repair mechanisms.

Justification of the Study:

Huge quantities of these vegetables, *Ocimum canum*(curry leaf), *Telfeiria occidentalis* (pumpkin), *Amarranthus viridis* (Green amaranth), *Ptericarpus santalinardes* (Red sandal wood), *Vernonia amygdalina* (bitter leaf) and *Ocimum gratissimum*(scent leaf)), are consumed all over Nigeria for flavouring and spicing of various types of food. Available information on their hepatoprotective property is scanty.Therefore, it is expedient to establish the hepatoprotective potentials of aqueous and the ethanol leaf extracts of these plants

The Aim of the Study:

This study was aimed at establishing the protective effect of the leaf extracts of*Ocimum canum Linn*(curry leaf), *Telfeiria occidentalis Hook. F* (pumpkin), *Amarranthus viridis Linn,* (Green amaranth), *Pterocarpus santalinardes Hook F* (Red sandal wood), *Vernonia amygdalina Delile,* (bitter leaf) and *Ocimum gratissimum* Linn, (scent leaf)on acetaminophen – induced acute liver toxicity in Albino rats.

Objectives of the Study:

The objectives of the study were:

1. To determine the proximate compositions of the leaf.
2. To determine the phytochemical compositions of the leaf extracts of the plants.
3. To determine the *in vitro* antioxidant properties of the plant materials via free radical scavenging activity using DPPH
4. To determine the hepatoprotective potentials of the extracts by assaying for the activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatse (ALP), Bilirubin – Total and direct, Total Protein, Lipid Profile (TC, TG, LDL and HDL) and biomarkers of oxidative stress (GSH, Catalase, SOD, Glutathione Peroxidase)
5. To determine the histopathological effect of the pre-treatment with the extractson the Livers of the Albino Rats

CHAPTER TWO

LITERATURE REVIEW

2.1 Leafy Vegetables and their medicinal uses

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases, (Ahmad, *et al*., 2009). The principle of this approach is that these plants contain certain biologically active compounds that can activate or inhibit the metabolic processes of humans.Some important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Soforowa, 1983). "*Let food be your medicine and medicine be your food*", is anincentive Hippocrates launched more than 2400 years ago (Hakim, 1988),gainingmore and more followers, as we become more aware of the benefits of a healthy living. Medicinal plants are affordable and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al*., 1999), but innocuous to man.

Many plants consumed as vegetables have various nutritional and medicinal values. The plants *Ocimum gratissimum, Vernonia amygdalina, Pterocarpus santaliniodes, Telfeiria occidentalis, Ocimum canum and Amaranthus Viridis*are some of those plants widely known and used for both medicinal and nutritional purposes.

Presently, there has been an increasing awareness on the need for including more vegetables in our diet in order to maintain a healthybody. To this date, several researchers have established numerous reasons why increasing the rate of producing vegetables and fruit should increase tremendously. (Okpala, 2015).The bioactivitiesof the leafy vegetables differ just as their effects on man differ.

2.1.1 *Ocimum gratissimum Linn* (Scent leaf)

The plant *Ocimum gratissimum* belongs to the family of plant known as *Lamiaceae*andis widely known and used for both medicinal and nutritional purposes. It is a perennialherbaceous, drought tolerant plant with lime-green pubescent leaves, a characteristically strong fragrance and a slight pungency that is widely distributed in the tropics of African and Asia.In Nigeria, it is found in the Savannah and coastal areas (Illoh *et al*., 2000).It is a fully developed flowering plant with root, stem and leaves systems (Iwu, 1993;Onajobi, 1986) and has an average height of 1-3m high. The leaves are broad and narrowly ovate, usually 5-13cm long and 3-9cm wide. The characteristic pleasant aroma is due to its volatile oil content (Dalziel, 1937).

Ocimum gratissimum is of versatile use in folk medicine and is commonly calledbush tea, fever plant or scent leaf. The common names of the plant are Basil fever plant or tea bush and vernacular names include Daidoya tagida (Hausa), Nchuanwu (Igbo), Tanmotswangiwawagi (Nupe) and Efinrin (Yourba) (Abdullahi *et al*., 2003). In Nigeria and several other countries, the plant plays important roles in traditional medicine preparations (Gill, 1992).*Ocimum gratissimum* leaf or the whole herbs are popular treatment, for diarrhoea, upper respiratory tract infection, headache, skin disease, pneumonia, fever, and conjuctivities (Dekkers *et al*., 1996; Onajobi, 1996).In folk medicine, *Ocimum gratissimum* is extensively used throughout West Africa as a febrifuge and anti- malarial. The crushed leaf juice is used in the treatment of stomach pain and catarrh. Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities,(Ezekwesili *et al*., 2004).In the coastal area of Nigeria, the plant is used in the treatment of epilepsy, (Osifo, 1992), high fever (Oliver 1980) and diarrhea (Oliver, 1980 and Sofowara, 1993). While in the savannah areas decoctions of the leaves are used to treat mental illness (Abdulrahman, 1992).*Ocimum gratissimum* is used by the Ibos of southern Nigeria in the management of the baby's cord. It is believed to keep the

baby's cord and wound surface sterile. It is used in the treatment of fungal infections, fever, cold and catarrh (Iwu, 1986).Clinical trials in creams formulated against dermatological disease have yielded favorable result (Edeoga and Eriata, 2001). Nutritional importance of this plant centers on it's usefulness as a seasoner because of its aromatic flavor (Ezekwesili *et al*., 2004).

Plate 1: The O*cimum gratissimum* plant as seen in a FADAMMA 111 vegetable garden in Keffi, Nasarawa state

Table 1: Scientific Classification of *Ocimum gratissimum*

Kingdom Plantae	plant
Subkingdom	Tracheobionta vascular plant
Super division	Spermatophyte - seed plant
Division	Magnoliophyta – flowering plant
Class	Magnoliopsida – Dicotyledon
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae – mint family
Genus	Ocimum L. – basil
Specie	*Ocimum gratissimum* L.-Africa

(Plant classification culled from: USDA 2008)

2.1.2 *Vernonia amygdalina Delile,* (bitter leaf)

Vernonia amygdalina, is a member of the Asteraceae family, is a small shrub that grows in tropical Africa. It typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Ijeh and Ejike, 2011). *Vernonia amygdalina* is commonly called bitter leaf in English because of its bitter taste. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa, (Egedigwe, 2010; Kokwaro, 2009). African common names include; Grawa (Amharic), Ewuro (Yoruba), Etidot (Ibibio), Onugbu (Igbo), Ityuna (Tiv), Oriwo (Edo), Etidot (Cross River State of Nigeria) Chusar-doki (Hausa), Mululuza (Luganda), Labwori (Acholi), Olusia (Luo), Andndoleh (Cameroon). (Egedigwe, *et al*, 2010; Kokwaro, *et al*, 2009)

The usage of *V. amygdalina*as medicinal herb started when zoopharmacologists found that sick chimpanzees with empty stomach sucked pith and juice from the unsavory*Vernonia*plant stalk (which was not their common diet) for self-deparasitization, enhanced body fitness,

increased strength or appetite and reduced constipation or diarrhoea especially during rainy season (Jisaka *et al*., 1993a). The bitter taste of *V. amygdalina*was suspected as a guide for them to choose for the appropriate plant, plant part and amount of intake. Other than animals, some of the citizens in Africa especially patients who were less educated with low or middle income also liked to use this plant, due to cultural and economic reasons.

Furthermore, the presence of the bitter taste also protects *V. amygdalina*from most of the animals, insect, sand microbes, where it only suffers from the attack by *Coleoptera curculionidae*, weevil *Lixus camerunus* and *Zonocerus variegates* (which utilized it as a source of protein). The processed *Vernonia amygdalina*was even exported to Europe and North America restaurants for preparation of African dishes. *Vernonia amygdalina*with just a little amount of processing can be classified as healthy food because it promotes the healthy development of the body.It contains not only the active drug molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity (Iwu, 2002). As a result, *V.amygdalina*serves well as a low cost and readily available source of important nutrients to humans (Ojiako and Nwanjo, 2009).Due to its bitterness, it also can be used as a bittering agent, a hop substitute and for the control of microbial contamination in beer brewing without affecting the quality of malt(Ojiako and Nwanjo, 2009),in Ethiopia, to make honey wine called Tei (Kasolo and Temu, 2008; Eleyinmi *et al*., 2004; Okoh *et al*., 1995).

Table 2: Ethnomedicinaluses of *Vernonia amygdalina*

Country	Preparation	Ailments
Ethiopia	Leaves (not root)	Stomach disorder, skin wound, diarrhea, scabies,hepatitis,ascarasis,tonsillitis, fevermastitis, tapeworm and worms infection.Stomach ache (worm expulsion)
DemocraticRepublic of Congo.	Leaves and root bark	Diarrhea, dysentery, gastroenteritis, malaria,hepatitis and worm infections.
Ghana	Leaves decoction	Malaria, fever, constipation, abortifacient,stomach sores,ulcer pain,upper respiratory tract infection anddermatitis.
Nigeria (Hausa)	Leaves, Rootand twig	Stomach ache, gastrointestinal troubles, oralhygiene,itches,parasitic infection, ringworm,typhoid, fever, Headaches constipation,diabetes, pile (haemorrhoids) and reduceaflatoxin contamination of storage cobs.
South Africa	Root	Schistosomiasis, infertility, amenorrhoea and diuretic.
Tanzania	Leaves and root	Snake bite (chew), fever, stomachache,as appetizer and for trematode
Uganda	leaves	Convulsions, cough, painful uterus, inducing uterine contraction,management of retainedplacenta postpartum bleeding, induced abortion, irregular painful menstruation, infertility, colic pains bacterial and fungalinfections.

Table 3: Classification of *Vernonia amygdalina* (Egedigwe, 2010; Kokwaro, 2009)

Kingdom:	Plantae
Division:	Angiosperms
Class:	Eudicots
Sub-class:	Asterids
Order:	Asterales
Family:	Asteraceae
Genus:	Vernonia
Species:	amygdalina
Binomial name:	*Vernonia amygdalina* Delile

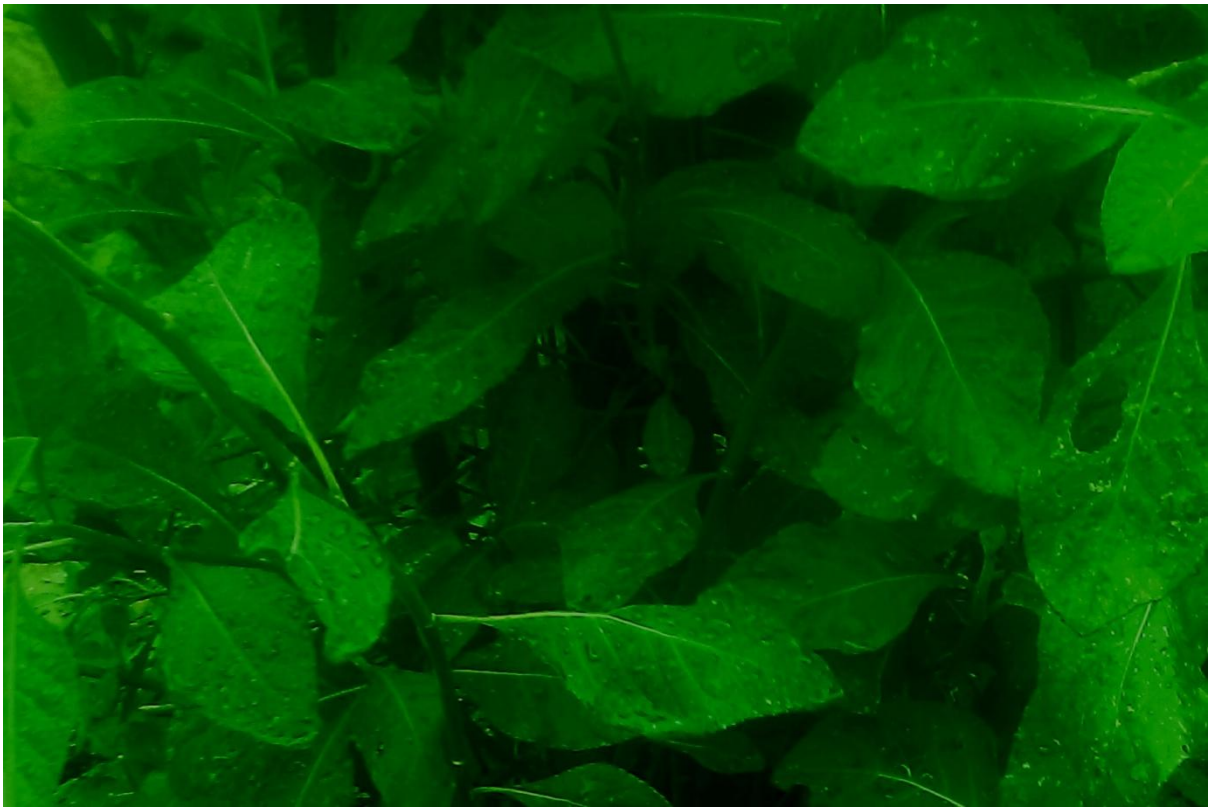

Plate 2: Bitter leaf (*Vernonia amygdalina*) plant as seen in a Mr. Daniel Adokwe's vegetable garden in Keffi, Nasarawa state

2.1.3 *Pterocarpus santalinardes Hook F* (Red sandal wood),

Pterocarpus santalinoides is a tree species in the legume family fabaceae. It is usually an evergreen tree with a dense crown of more or less drooping branches. (Tropical plants)It grows to 3-7 m tall with a trunk up to 1m in diameter with low strangling branches. Thin bark and flaking in small patches, slash yellowish white exuding drops of red gum, (Offor *et al*., 2015).The leaves are compound, about 5-9 leaflets ovate-elliptic, abruptly acuminate and rounded at the base or slightly luneate. They are glabrous, glossy and rather coriaceous with eight pairs of prominent main lateral nerves looping away from the margin. The leaf stalk is slender, glabrous stalk about 10-12 cm long and the leaflet stalk stout is 2-5 mm long. The flowers are orange-yellow and produced in panicles.The fruit is a pod 3.5-6 cm long with a wing extending three quarters around the margin. Bees commonly visit the flowers and

probably serve as pollinators. Fruits mature in 2-3 months after flowering in the rainy season, (Lemmens, 2008).

The common names of *Pterocarpus santalinoides* (Offor *et al*., 2015; Lemmons, 2008), are Red sandal wood (English), Ouokisse (French), Gunduru (Hausa), Nturukpa (Igbo), Gbengbe (Yoruba), Uturukpa (Igbede).

Decoctions are administered externally to wounds to promote healing, and to treat haemorrhoids and fever. They are taken internally to treat bronchial complaints, amoebic dysentery, stomach-ache and sleeping sickness, to prevent abortion and ease childbirth, and as a tonic (Lemmens, 2008). In Southern Nigeria the tender stem bark and leaves extract of *Pterocarpus santalinoides* usually called "Nturu Ukpa" in Igbo language is used in stopping stooling and vomiting in both children and adults.

The use of the leaves in treating skin diseases such as eczema, Candidiasis, and acnes and the use of concoctions made from its roots in treating asthmatic patients have been reported (Adesina, 1982). It is used in treating diarrhoea which is a major cause of death as it has a proven anti-enteropooling activity in traditional medicine, (Adesina, 1982). The anti-malaria activity has been reported also as well as use of the stem bark decoction in treating infertility in females (Alexis *et al*, 2000). The bark extracts is used in treatment of cough and diabetes. The leaves are used in Veterinary medicine to reduce abdominal pains in goats (Ama, 2010; Igoli *et al*, 2005)

Plate 3: The *Pterocarpus santaliniodes* Plant as seen in a bush behind the FNAS building of Nasarawa State University, Keffi.

Table 4: Classification of *Pterocarpus santalinoides*(Lemmens, 2008**)**

Kingdom	Plantae
Division:	Angiosperms
Class	Eudicots
Subclass	Rosids
Order	Fabales
Family	Fabaceae or Leguminosae
Subfamily	Faboideae
Tribe	Dalbergieae
Genus	Pterocarpus
Species	Santalinoides

2.1.4 *Telfeiria occidentalis Hook. F* (pumpkin)

Telfairia *occidentalis* is a tropical vine grown in West Africa as a leafy vegetable. Common names for the plant include fluted gourd, fluted pumpkin, and ugu. T. *occidentalis* is a member of the Cucurbitaceae family and is indigenous to Southern Nigeria (Akoroda and Adejero, 1990). The fluted pumpkin grows in many nations of West Africa most frequently in Benin, Nigeria indigenous to the west tropical rainforest area from Bendel (presently Edo and Delta) to Cross Rivers states of Nigeria (longitude 7°–8° E and latitude 5°–6° N) (Floyd, 1969) and Cameroon, used primarily in soups and herbal medicines (Oboh, 2005). The fluted pumpkin as an important cucurbitaceous leaf and seed vegetable, which was originally wild throughout its current range, but have been harvested to local extinction and are now replaced by cultivated forms (Akubue *et al.*, 1980). The Curcubitaceae are reported to have been associated with man since 12,000 BC (Esquinas-Alcazar and Gulick 1983). Common examples of plants in this family are cucumber, watermelon, squash and melon(Oboh, 2005).*Telfeiria occidentalis* is an important staple vegetable grown in Nigeria and produces luxuriant edible green leaves, which are rich in iron and vitamins. Stem of the plants have branching, long twisted tendrils and the leaves are divided into 3 to 5 leaflets with the terminal leaflets up to 15cm long while the male plant is grown principally for leaves and seeds, (*Mensah, et al., 2008*; Akorada *et al* 1990). The fluted pumpkin fruit is quite large; one study documented a range of 16–105 centimetres (6.3–41.3 in) in length, and an average of 9 cm in diameter, (Akoroda, 1990). The same study found the seed count in larger gourds to reach upwards of 196 per fruit, typically measuring between 3.4 and 4.9 cm in length (*Mensah, et al., 2008*). In both the pistillate and staminate varieties, *Telfeiria occidentalis* flowers grow in sets of five, with creamy-white and dark red petals, contrasting with the light green colour of the fruit when young, and yellow when ripe (Okoli, 1983). Dioecious flowering is most common in the fluted gourd, with very few documented cases of

monoecious flowering.The names for the plant include fluted gourd, fluted pumpkin (Akoroda and Adejero, 1990). Other traditional names include Ugu (Igbo), Iroko or Akoroko (Yoruba), Ubong (Efik), Umee (Urhobo) and Umeke (Edo), (Badifu and Ogunsina, 1991).

The plant is cultivated majorly for its edible leaves and seeds.The pharmacological importance of this family of plants (Cucurbitaceae) is ample. Considerable evidence from several epidemiological studies concerning the use of its bioactive substances in a number of animal models, cell culture studies and clinical trials validate its immense pharmacological activities (Oboh *et al*, 2006; Nwozo *et al*., 2004; Eseyin *et al*., 2000; Sofowora, 1983; Odoemena and Essien 1995; Gbile, 1986). *Telfairiaoccidentalis* is popularly used in ethnobotany as antidiabetic, antihypertensive, antitumouric, antioxidant, immunodulator, antibacterial, anti-hypercholesterolemic, intestinal anti-parasitic and anti-inflammatory agent (Nwozo *et al*, 2004). Due to its antioxidant, antimicrobial properties, minerals (especially Iron), vitamins (especially vitamin A and C) and high protein contents (Kayode and Kayode, 2011), consumption of the leaves assists to combat certain diseases. The aqueous extract of *Telfeiria occidentalis* has been shown to be hepato - protective against garlic-induced oxidative stress (Olorunfemi *et al*., 2005; Odoh, 2005) while both aqueous and ethanol extracts have demonstrated hypoglycaemic properties both in normoglycaemic and alloxan-induced diabetic rats (Zhang and Yao, 2002; Zhang, 2001). Studies have also shown the haematinic capacity of this plant hence the use of the concoction of fresh leaves as a high-value health tonic for impotent men and a cheap and fast remedy for acute anaemia (Kayode and Kayode, 2011; Ajibade *et al*., 2006).Based on taxonomy, Badifu and Ogunsina, (1991) also classified*Telfeiria occidentalis*as follows:

Table 5: The classification of *Telfeiria occidentalis*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitalis
Family	Cucurbitaceae
Genus	Telfairia
Species	T. occidentalis

Plate 4: Fresh *Telfeiria ocidentalis* leaves harvested from a vegetable garden in Keffi.

2.1.5 ` *Ocimum Canum Linn* (Curry plant)

Ocimum Canum belongs to the Lamiaceae family. This annual plant is native to the African continent and grows to a height of 2 feet. It is also known as the African basil with a distinct mint flavour and scented flowers. Two important relatives with similar properties are the Ocimum gratissimum (basil), and Ocimum sanctum (holy basil). All of these are used in medicinal preparations in various parts of the world. The ocimum canum was used specially for treating diabetes. This bushy herb is common in tropical Africa and other areas of the tropics and was later introduced into the Americas. The plant branches from the base and has an angled stems and oval pubescent leaves. Its leaves are tiny and fuzzy and and have beautiful violet or white flowers, having a sweet scent resembling that of the clove. The leaves of the ocimum canum are opposite and toothed in shape, with small flowers. It is irregular and occurs in crowded whorls.

Ocimum Canum is grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose, especially in type 2 diabetes levels. The herb can be used to treat colds, fevers, parasitic infestations on the body and inflammation of joints and headaches. The traditional medicine recognized its value in the treatment of fevers, dysentary and tooth problems. It was used as an insect repellent to counter the insect damages post harvest. It is used in various types of religious functions as part of rituals and as incense to protect the home. The leaves are used for flavoring purposes. The oil of the ocimum canum is composed of Linalool. The seeds may provide dietary fiber or reduce constipation.

According to catalogue of life China, 2013 annual Checklist (Biodiversity committee, Chinese Academy of Science), *Ocimum canum* is classified as follows:

Table 6: Scientific classification of curry Plant *(Ocimum canum*)

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridi plantae
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Asteranae
Order	Lamiales
Family	Lamiaceae – mints, menthes
Genus	Ocimum L. – basil
Species	Ocimum basilicum L. – sweet basil

(Plant classification culled from: USDA, 2008)

Plate 5: The *Ocimum canum* leaf, as seen in the vegetables garden of Fac. of Agric.of Nasarawa State University, Keffi.

2.1.6 *Amaranthus viridis Linn* (Amaranthus green)

Townsend (1985) typically described the plant, *Amaranthus viridie* as an annual herbaceous plant, stem erect or usually ascending 6-80 (sometimes up to 100) cm tall, glabrous to pubescent, pubescent especially upwards. Its petioles are long (up to 10cm), occasionally longer than the blade. Blade ovate to rhombic oblong, 2.7×1.5-5.5 cm. Base tapered to blunt, tip rounded, minutely mucronate, barely to clearly emarginated. Green flower unisexual, male and female intermixed in slender axillary to terminal particulate spikes 2-12cm long and 2-5 mm wide or in dense axillary cluster in the lower parts of the stem. Capsule nearly globose 1.25-1.75mm long not rupturing or rupturing irregularly, surface rough. Seed 1-1.25mm round, slightly compressed dark brown to black with a paler thick boarder. Green amaranths thrive in hot weather, tolerate drought, respond to high level of available nutrients and are adapted to avoid shading through rapid stem elongation. They compete aggressively against warm season crops and reproduce by prolific seed production (Mark 2014). The seed lose viability over time and loss in viability is faster in higher temperature (Purwanto and Poerba 1990). *Amaranthus viridis* has been found to grow best in intermediate light intensities (Simbolon and Sutamo, 1986).it can co-exist with the equally common *Amaranthus spinosus,* because the two have different nutritional requirement (Ramakrishnam, 1976), with basic chromosome number of 10 (Sammour *et al*., 1993). *Amaranthus viridis* is cosmopolitan in all warm region of the world. It is one of the most common weeds in the tropics, subtropics and warm temperate regions. It is listed in virtually all the warm temperate anf tropical floras of the world. *A.viridis* has been recorded from former USSR (Vasil'chenko, 1936). *A.viridis* grows in heavy organic to very sandy soil, including muck soil after the water has gone down for the season. *Amaranthus viridis* is grown and utilized in many areas of the world as both a wild and cultivated pot herb (Uphof, 1968)**.** The plant is rich in calcium and iron and it is a good source of vitamin B and C

(Morton, 1981). The seed can survive in the digestive tracts of chicken (Rodriguez *et al*., 1983). It is a good cattle fodder and it is used medicinally and for soap making (Dalziel, 1937) but it is poisonous to pigs (Salles *et al*., 1991). *A.viridis* is quiet common and can be a serious weed in virtually any crop.*Amaranthus viridis*' common names include: green amaranth, slender amaranth, African spinach, wild amaranth, (English), inine (Igbo), tete (Yoruba) is similar to other species of amaranthus having distinct leaf venation and long petioles.

Table 7: Scientific classification of *Amaranthus viridis*

Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta (Vascular Plants)
Superdivision	Spermatophyte (Seed Plants)
Division	Magnoliophyta (Flowering Plants)
Class	Magnoliopsida (Dicotyledons)
Sub Class	caryophyllidae
Order	caryophyllales
Family	Amaranthaceae (Amaranth Family)
Genus	*Amaranthus*
Specie	*Amaranthus viridis L*

(Classification of *Amaranthus viridis* culled from USDA, 2008)

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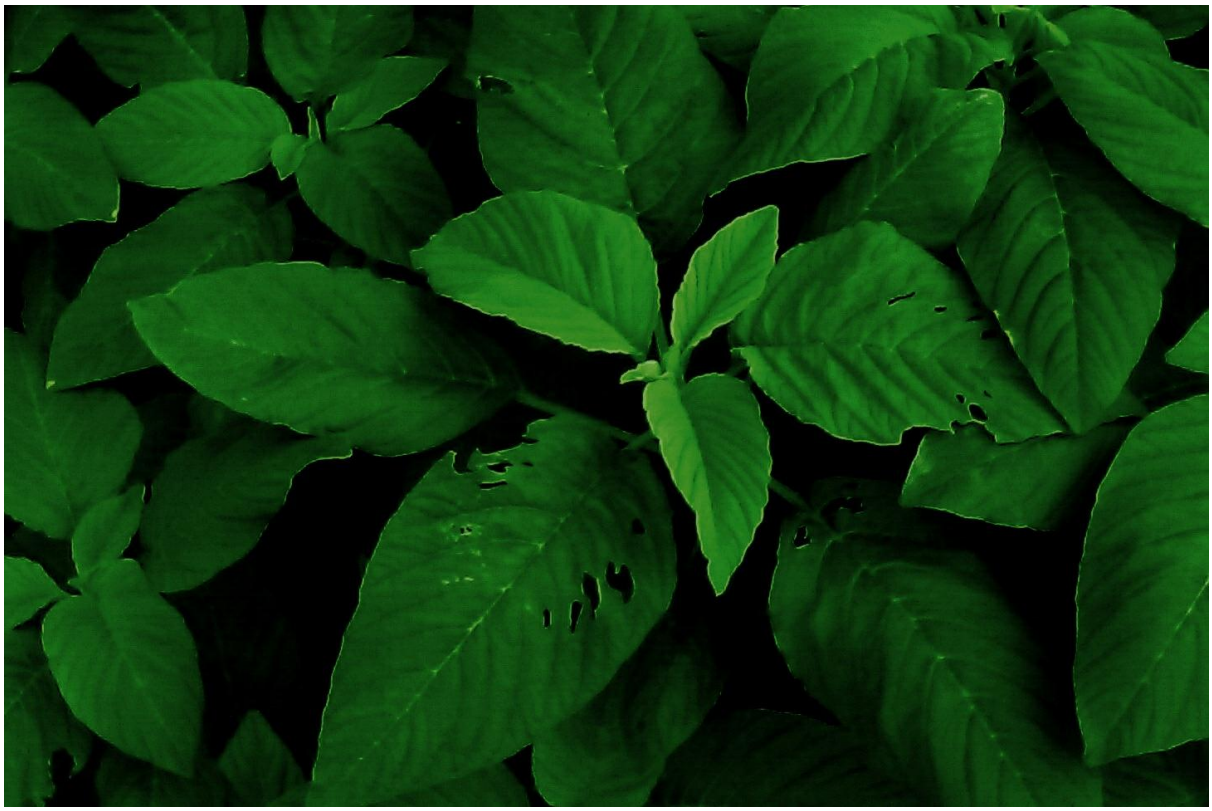


Plate 6: The *Amaranthus viridis* plant, as seen in the vegetables garden of Fac. of Agric.of Nasarawa State University, Keffi.

2.2 Oxidative stress:

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (Ananya, 2016). A free radical is an oxygen containing molecule that has one or more unpaired electrons, making it highly reactive with other molecules.Oxygen by-products are relatively unreactive but some of these can undergo metabolism within the biological system to give rise to these highly reactive oxidants. Not all reactive oxygen species are harmful to the body. Some of them are useful in killing invading pathogens or microbes.However, free radicals can chemically interact with cell components such as DNA, protein or lipid and steal their electrons in order to become stabilized. This, in turn,

destabilizes the cell component molecules which then seek and steal an electron from another molecule, therefore triggering a large chain of free radical reactions.

Oxidative stress is another mechanism that has been postulated to be important in the development of acetaminophen toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions by Fenton-type mechanisms, (James, *et al*., 2009). A significant amount of evidence has pointed to the potential involvement of oxidative stress in acetaminophen toxicity. Nakae *et al*. (1990) reported that administration of encapsulated superoxide dismutase decreased the toxicity of acetaminophen in the rat. Moreover, the iron chelator, deferoxamine, has been shown to decrease toxicity in rats (Sakaida, *et al*., 1995). Several laboratories have studied the role of macrophage activation (Laskin *et al*., 1995) in acetaminophen toxicity. Kupffer cells are the phagocytic macrophages of the liver. When activated, Kupffer cells release numerous signaling molecules, including hydrolytic enzymes, eicosanoids, nitric oxide, and superoxide. Kupffer cells may also release a number of inflammatory cytokines, including IL-1, IL-6, and TNF-α (Laskin *et al*., 1995), and multiple cytokines are released in acetaminophen toxicity (Blazka *et al.,* 1995); Hogaboam *et al*.,1990a, 2000; Bourdi *et al*.,2002a,b). There is also superoxide formation in acetaminophen toxicity. This may be done through a number of mechanisms, including formation from cytochrome P_{450} (Puntarulo and Cederbaum, 1996) and other enzymes. In the evaluation of the importance of activation of Kupffer cells, macrophages, or neutrophils (the so-called respiratory burst) in acetaminophen toxicity by Iames *et al* (2009), the sudden excess utilization of oxygen by activated phagocytes was found to be as a result of increased activity of the enzyme, NADPH-oxidase. The result is release of superoxide anion at the outer surface of the plasma membrane (Baggiolini and Wymann, 1990). The figure below shows the pathway for mitochondrial toxicity of Acetaminophen as depicted in James, *et al.,* (2009).

Mitochondrial Permiability Transition (MPT) in Acetaminophen Toxicity

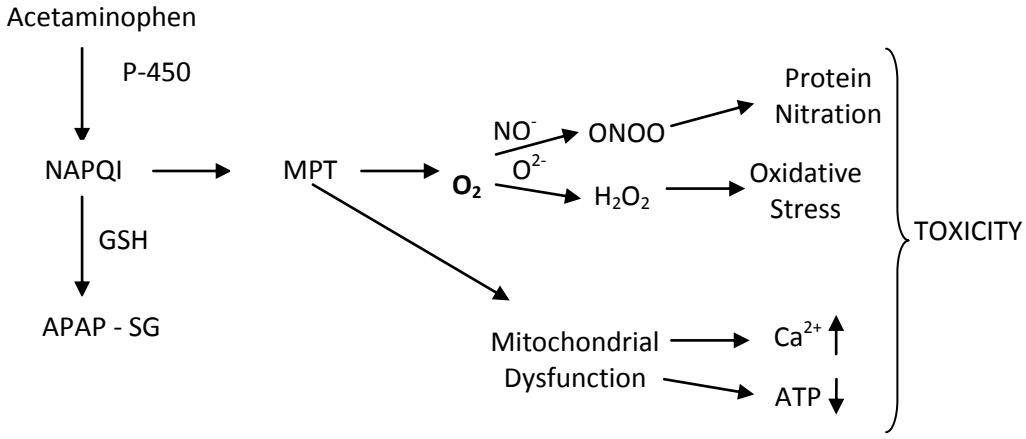


Figure 1: Schematic representation of the role of mitochondrial permeability transition in acetaminophen toxicity

2.2.1 Damage caused by oxidative stress

Oxidative stress leads to many pathophysiological conditions in the body. Some of these include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, gene mutations and cancers, chronic fatigue syndrome, fragile X syndrome, heart and blood vessel disorders, atherosclerosis, heart failure, heart attack and inflammatory diseases.

Table 2.8: Oxidants and their effects on biochemical reactions

Oxidants	Description
$^{\bullet}O_2^-$, superoxide anion	One-electron reduction state of O2, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe^{2}+ from iron-sulfur proteins and ferritin. Undergoes dismutation to form H_2O_2spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed •OH formation.
H_2O_2, hydrogen peroxide	Two-electron reduction state, formed by dismutation of $\bullet O^{2}$- or by direct reduction of O_2. Lipid soluble and thus able to diffuse across membranes.
$^{\bullet}$OH, hydroxyl radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components
ROOH, organic hydroperoxide	Formed by radical reactions with cellular components such as lipids and nucleobases.
RO$^{\bullet}$, alkoxy and ROO$^{\bullet}$, peroxy radicals	Oxygen centred organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.
HOCl, hypochlorous acid	Formed from $H_2O_2$by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.
$ONOO^-$, peroxynitrite	Formed in a rapid reaction between $\bullet O^{2}$- and NO•. Lipid soluble and similar in reactivity to hypochlorous acid. Protonation forms peroxynitrous acid, which can undergo homolytic cleavage to form hydroxyl radical and nitrogen dioxide.

James, *et al., (2009)*

2.2.2 Antioxidants

Every cell that utilizes enzymes and oxygen to perform functions is exposed to oxygen free radical reactions that have the potential to cause serious damage to the cell. Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves. An imbalance between oxidants and antioxidants is the underlying basis of oxidative stress.Antioxidants have been known to play protective role in human body against deleterious effect of reactive free radicals and it has been defined as any substance that when present at low concentrations compared to those of an oxidized subtract, significantly delays or prevents oxidation of that substrate (Halliwell, 1990.) They are chemical compounds that can prevent, stop, or reduce reactive effect of radicals.

2.2.3 The use of plant materials for Hepatoprotection and in the management of oxidative stress

The record of the use of plant materials as medicine is as old as the history of human existence (Prerna *et al.,* 2015). Prerna *et al.,* (2015) reported the use of *Ocinum sanctum, Bryophylum calycinum, Asparagus racemosus, Coleus aromaticus, Cynodon dactylon, Hibiscus rosa-sinensis, Jasminum sambac, Lawsonia inermis, Nelumbo nucifera, Adhatoda vascular* and *Cymbopogon citrates* in either hepatoprotection and in the management of oxidative stress or in both situations. These claims however, were not accompanied with corresponding doses.

Other researchers have shown the use of specific concentrations of plant materials in oxidative stress management and in hepatoprotection. Temburne and Sakartar (2015), reported effective doses of 300mg/kg b.w and 500mg/kg b.w of ethanol extract of *Murraya koenigii.* Ogbunugafor, *et al*., (2010), Ekor, *et al., (*2006), Anyasor *et al.,* (2010) and Olamide and Mathew, (2012), have all reported the use of plant materials in the management of

oxidative stress and by extension in hepatoprotection. While Ogbunugafor, *et al*., (2010),used 200mg/kgbw of aqueous and methanol extracts of *Hymenocardia acida* Tul, Ekor, *et al.*, *(*2006), used 400mg/kgbw of methanol extract of *Persea americana* and Olamide and Mathew (2012), employed 100mg and 200mg of hexane, chloroform, ethyl acetate and methanol extracts of stem bark of *Enantia chlorantha* to achieve effective *in vivo* antioxidant and/or hepatoprotection in rats. Anyasor *et al.,* (2010), reported the *in vitro* antioxidant properties of aqueous and methanol extracts of *Vernonia amygdalina* and *Talinum triangulare.*

2.2.4 Antioxidant Enzymes

Superoxide dismutase (SOD) (EC 1.15.1.1): This is the antioxidant enzyme that catalysed the dismutation of the highly reactive superoxide anion to O^2 and to the less reactive species H_2O_2.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

2.2.4.1 Catalase (CAT) (EC 1.11.1.6):

It is an enzyme responsible for the degradation of hydrogen peroxide. It is a protective enzyme present in nearly all animal cells. CAT is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferri-proto-porphyrin group per subunit, and has a molecular mass of about 240 kDa (Buschfort, *et al.,* 1997). CAT reacts very efficiently with H^2O^2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

$$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

2.2.4.2 Glutathione peroxidase (GPx) (EC 1.11.1.19):

Glutathione peroxidaseis an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The GPx contains a single selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity (Speranza, *et al.*, 1993). GPX, a 80 kDa enzyme, catalyses the reduction of hydro peroxides using GSH, thereby protecting mammalian cells against oxidative damages. In fact, glutathione metabolism is one of the most essential antioxidative defence mechanisms.

$$ROOH + 2GSH \longrightarrow ROH + GSSG + H_2O$$

2.3 Silymarin

Silymarin was discovered in 1968, by a group of German scientists as an active flavonoid complex, which provides milk thistle's medicinal benefits.It is a unique flavonoid complex, containing silybin, silydianin, and silychrisin, which are derived from the milk thistle plant. After hundreds of studies have been done on silymarin, it was approved in the German Commission E Monographs (the most accurate information available on the safety and efficacy of herbs) as a supportive treatment for inflammatory liver conditions such as cirrhosis, hepatitis, and fatty infiltration caused by alcohol and other toxins. In chronic liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver diseases, drug- and chemical-induced hepatic toxicity), the antioxidant medicines such as silymarin can have beneficial effect. Liver (Feher and Lengyey, 2017)

According toCatalina, *et al.*, 2003 andPresser,(2000), Silymarin has been used to:

1. Regenerate liver cells damaged by alcohol or drugs
2. Decongest the liver (A liver decongestant stimulates bile flow through the liver and gallbladder, thus reducing stagnation and preventing gallstone formation and bile-induced liver damage.)

3. Increase the survival rate of patients with cirrhosis
4. Complement the treatment of viral hepatitis
5. Protect against industrial poisons, such as carbon tetracholoride (a colorless gas that leaks into air, water and soil near manufacturing and waste sites)
6. Protect the liver against pharmaceuticals that stress the liver, such as acetaminophen and tetracycline
7. Antidote and prevent poisoning from the death cap mushroom, Amanita phalloides it It was also found to work as follows:
8. As an antioxidant, silymarin scavenges for free radicals that can damage cells exposed to toxins. Silymarin has been said to be at least ten times more potent in antioxidant activity than vitamin E.
9. It increases glutathione in the liver by more than 35% in healthy subjects and by more than 50% in rats.Glutathione is responsible for detoxifying a wide range of hormones, drugs, and chemicals. High levels of glutathione in the liver increases its capacity for detoxification.
10. Silymarin also increases the level of the important antioxidant enzyme superoxide dismutase in cell cultures.
11. It stimulates protein synthesis in the liver, which results in an increase in the production of new liver cells to replace the damaged ones.
12. Silymarin inhibits the synthesis of leukotrienes (mediators of inflammation, which can result in psoriasis, among other things)

2.4.0 Proximate Composition of Materials

Proximate composition means the 6 component of moisture, crude protein, crude fat, crude fibre, crude ash and nitrogen free extracts, which are expressed as the content (%) in the feed. Leafy vegetables have been found to contain varying percentages of carbohydrate, protein,

ash, fat, moisture and fibre. Same type of vegetable (leaf), have also been found to contain varying percentages of these crude materials though they are within certain ranges. Proximate composition, studied using amaranth, cowpea, peanut, pumpkin and sweet potato leaves by Mosha *et al* (1995), indicated that, crude protein, crude fat, carbohydrate and ash contents were in the range of 20.64--46.56 percent, 2.57--4.34 percent, 35.43--63.50 percent and 8.92--15.69 percent respectively.

A study carried out by Javid *et al*., (2009) also showed that *Amaranthus viridis*and *C. Album* contained a moisture of 6.46 and 12.21 percent respectively. While *Amaranthus viridis*leaf was found to have a moisture content of 10% by Asaolu *et al.,* (2012), in a study conducted on Nigerian Leafy Vegetables. Folade *et al.,* (2004) reported *Amaranthus viridis*leaf to contain moisture of 8.6%. The crude protein content of *Amaranthus viridis*leaf had been shown to be 26% and 16% byFolade *et al.,* (2004) and Javid *et al.*,(2009) respectively.

Carbohydrate constitutes a major class of naturally occurring organic compounds which are essential for the maintenance of life in plant and animals and also provide raw materials for many industries.Leafy vegetables have been reported to contain a percentage carbohydrate varying between 1.16% in *Telfeiria occidentalis*by Asaolu *et al.,* (2012) and 52.18% in *Amaranthus viridis*by Javid *et al*., (2009). These wide differences in these composition is not limited to the plant type but may be as a result of the locality where the research was carried out, the age of the plant, the season of the year and other environmental factors. Folade *et al.*, (2004) also reported the same *Amaranthus viridis*leaf to contain 52.0% carbohydrate.

Dietary fat increases the palatability of food by absorbing and retaining flavour,(Antia *et al.,* 2006*).*A diet providing 1.20% of its caloric of energy as fat is said to be deficient for human beings.Excess fat consumption is implicated in certain cardiovascular disorders (Antia *et al.,* 2006). Leafy vegetables have been found to contain varying percentages of crude fats. Fat values ranging from 1.72 – 14.02 (%), have been reported by different researchers like Mosha

et al (1995), Folade *et al.,* (2004) and Javid *et al.,*(2009), for leafy vegetables. *V.amygdalina*contains crude fat (2 to 15 g/100g DW with24.54% saturated and 65.45% polyunsaturated, (Oboh, 2006; Okoli *et al*., 2003a).

Plants are good sources of crude fibre and when adequately consumed, dietary fibre can lower the serum cholesterol level, heart disease, hypertension, constipation, diabetes and breast cancer (Ishida *et al.,* 2000). A fibre content of between 4.02% and12.08% had been reported in leafy vegetables, (Asaolu *et al.,* 2012;Javid *et al.,* 2009;Folade *et al.,* 2004 andMosha *et al.*,1995)Out of the dry matter, *V. amygdalina* contained 6.5 to 29.2% of crude fibre content (Alabi *et al*., 2005a; Antia *et al*., 2006; Ifon&Bassir, 1979; 1980; Oboh, 2006; Okoli *et al*., 2003a).

The ash content is a reflection of the amount of mineral elements present in a sample. This can be as low as 5.02% as found in Indian spinach by Asaolu *et al.,* (2012) and as high as 22.8% as reported for *Amaranthus viridis* by Javid *et al.,* (2009) in leafy vegtables. While Asaolu *et al.,* (2012), stated that the ash contents of *Vernonia amygdalina*was (9.56%), Indian spinach (5.02%), *occimum gratissimum* (13.01%), *Amaranthus hybridus* (15.55%), *telfeiria occidentalis* (8.56%), Javid *et al.,* (2009), reported *Amaranthus viridis*(22.84%) and *C. Album*(22.15%), and Folade *et al.,* (2004) reported that *Amaranthus viridis* contained 11.7% crude ash.

2.5.0 Phytochemicals

Phytochemicals (from the Greek word “phyto”, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999).

They protect plants from disease and damage and contribute to the plant’s colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards

such as pollution, stress, drought, ultra-violet (UV) exposure and pathogenic attack are called phytochemicals (Gibson *et al*., 1998; Mathai, 2000). Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases.

A quick analysis of literature reveals varied quantitative and qualitative levels of phytochemicals in different parts of plants. While some of the variations are due to methods of extraction, that is, type of solvent used, others are due to the phase, that is, liquid or solid extracts. There is a vast literature review on analyses of phytochemicals in plants, mostly qualitative.

Chemical composition of plant is not an intrinsic constant factor but varies over a wide range. Environmental factors, such as temperature, illumination, pH-value, mineral contents, CO_2 supply, or population density, growth phase and plant physiology, can greatly modified chemical composition (Hahn, 1998).

According to Hahn (1998), the exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In recent years, phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll and so on. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glycosides, which have no direct role in the growth reproduction and development of the plant but may act as protective agents to the plant. Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituents.

2.5.1 Alkaloids

These are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building

blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen (Sarker and Nahar, 2007). The solutions of alkaloids are intensely bitter. These nitrogenous compounds function in the defense of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities. In nature, the alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetable acids. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al*., 2010). More than 12,000 alkaloids are known to exist in about 20% of plant species and only few have been exploited for medicinal purposes. The name alkaloid ends with the suffix *–ine* and plant-derived alkaloids in clinical use include the analgesics morphine and codeine, the muscle relaxant (+)-tubocurarine, the antibiotics sanguinafine and berberine, the anticancer agent vinblastine, the antiarrythmic ajmaline, the pupil dilator atropine, and the sedative scopolamine.

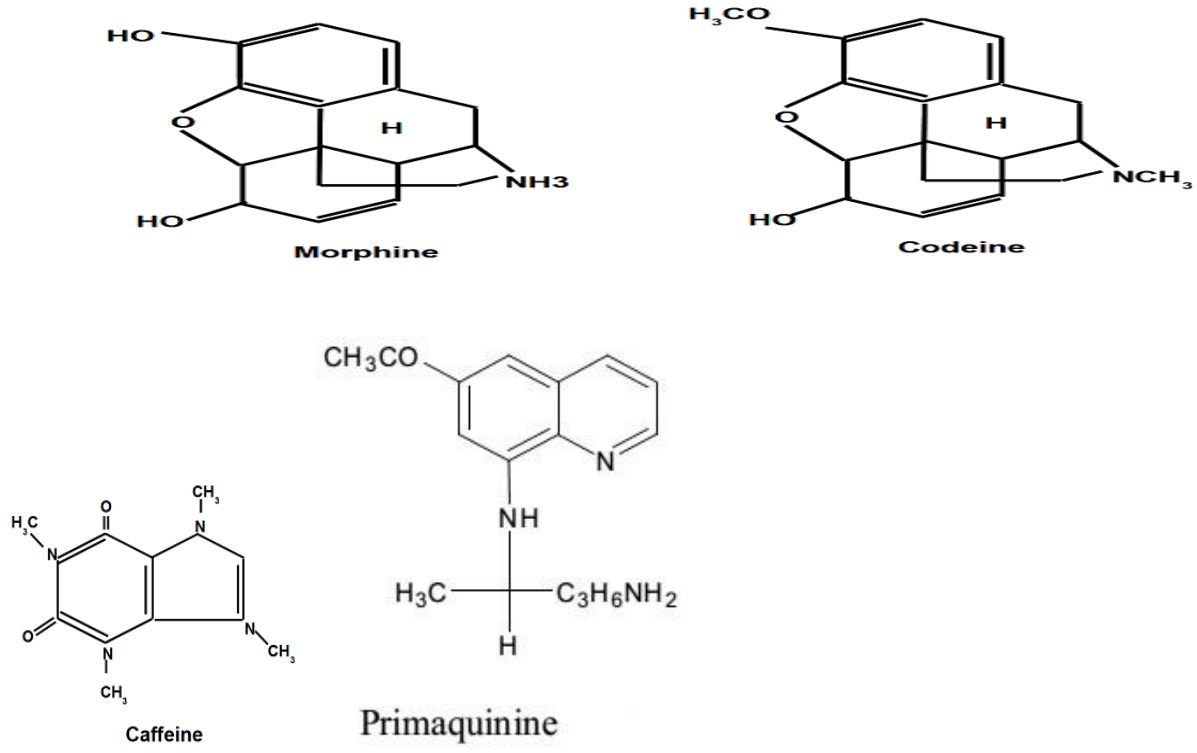
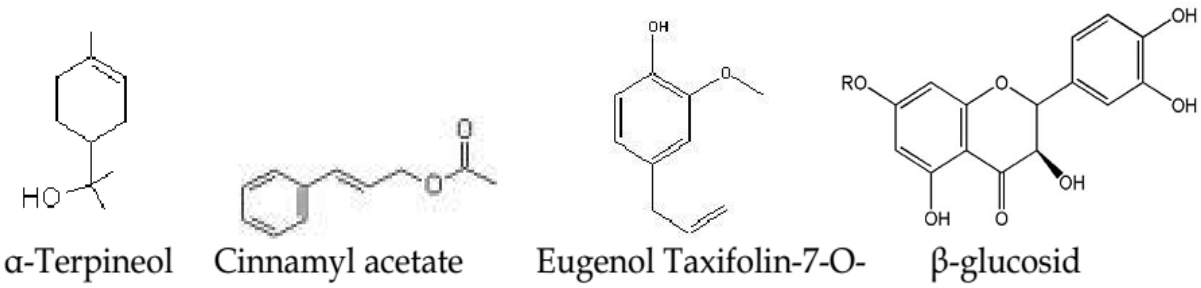


Figure 2: Structures of pharmacologically important plant derived alkaloids

2.5.2 Glycosides

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemiacetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colourless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulphur) water-soluble phytoconstituents, found in the cell sap. Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genin) (Kar, 2007; Firn, 2010).



α-Terpineol Cinnamyl acetate Eugenol Taxifolin-7-O- β-glucosid

Figure 3: Structures of some pharmacologically important plant-derived glycosides

2.5.3 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C_{15} aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers (Kar, 2007). The compounds are derived from parent compounds known as flavans. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other group of flavonoids include flavones, dihydroflavons, flavans,

flavonols, anthocyanidins, proanthocyanidins, calchones and catechin and leucoanthocyanidins.

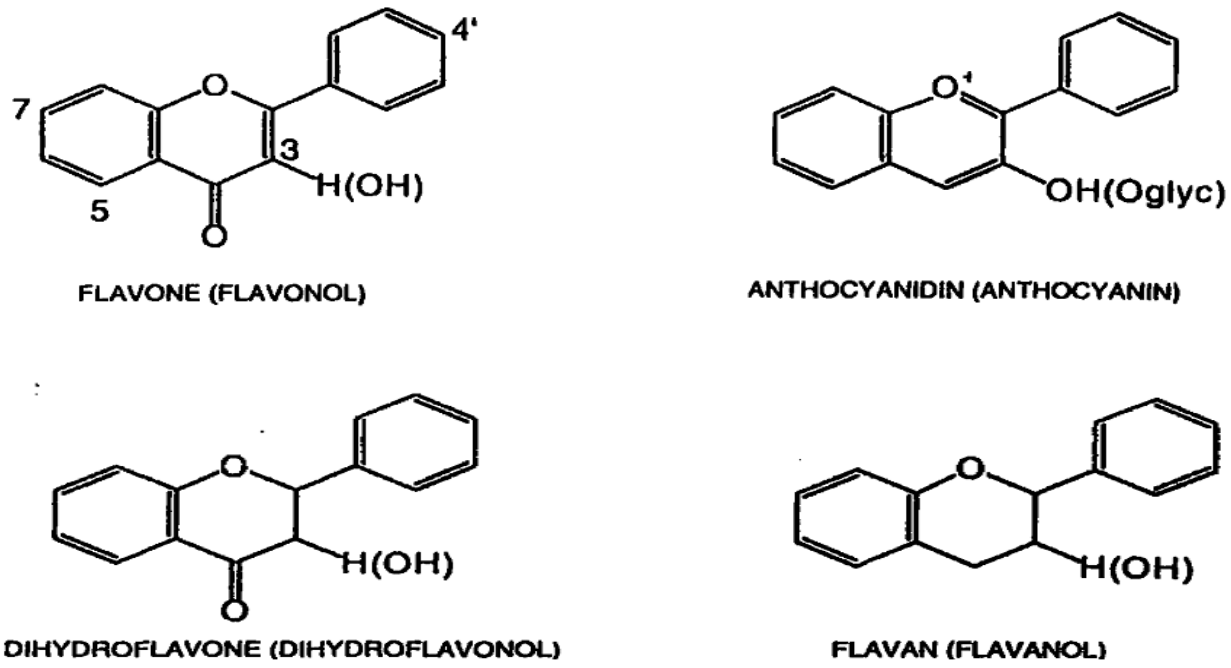


Figure 4: Structures of some pharmacologically important plant-derived flavonoids

2.5.4 Phenolics

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolics are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants (Walton *et al*., 2001). They are plant secondary metabolites, and they have an important role as defense compounds. Phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. Flavonoids are the largest group of plant phenols and the most studied (Dai and Mumper, 2010).

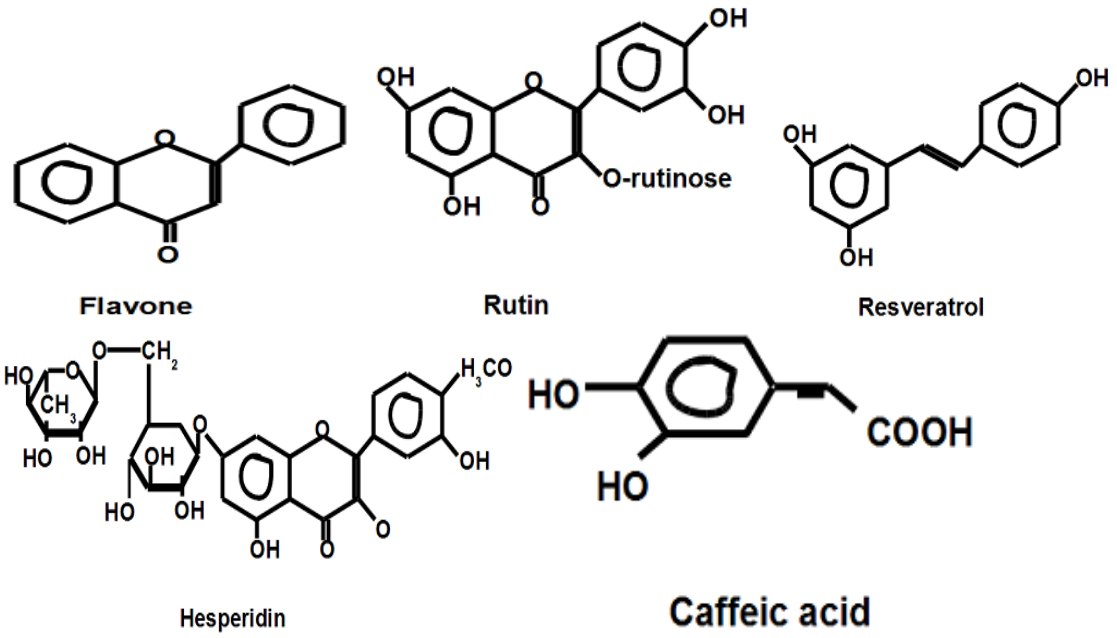


Figure 5: Structures of some pharmacologically important plant derived phenolics

2.5.5 Saponins

The term saponin is derived from *Saponaria vaccaria* (*Quillaja saponaria*), a plant, which abounds in saponins and was once used as soap. Saponins therefore possess 'soaplike' behaviour in water, that is, they produce foam. Onhydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. Usually, the sugar is attached at C-3 in saponins, because in most sapogenins there is a hydroxyl group at C-3. Saponins are regarded as high molecular weight compounds in which, a sugar molecule is combined with triterpene orsteroid aglycone. There are two major groups of saponins and these include: steroid saponins and triterpene saponins. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they give aglycones. Saponins are extremely poisonous, as they cause heamolysis of blood and are known to cause cattle poisoning (Kar, 2007).

2.5.6 Tannins

These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group (Kar, 2007). Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein.

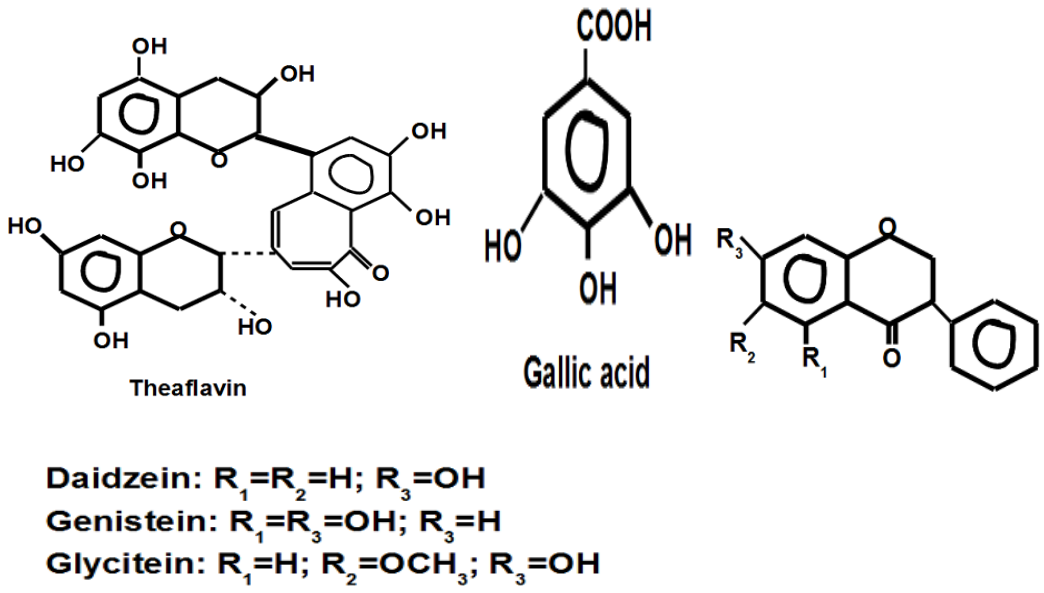


Figure 6: Structures of some pharmacologically important plant derived tannins

2.5.7 Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Firn, 2010). Terpenoids includes hydrocarbons of plant origin of general formula $(C_5H_8)_n$ and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Examples of commonly important monterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C_{20}) are classically considered to be resins and taxol, the anticancer agent, is the common example.

The triterpenes (C_{30}) include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes: amyrins, ursolic acid and oleanic acid sesquiterpene (C_{15}) like monoterpenes, are major components of many essential oils (Martinez *et al.*, 2008).

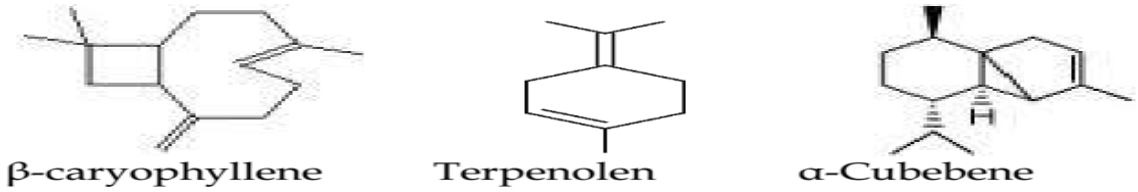


Figure 7: Structures of some pharmacologically important plant derived terpenes

2.5.8 Essential Oils

Essential oils are the odorous and volatile products of various plant and animal species. Essential oils have a tendency evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils. They mostly contribute to the odoriferous constituents or 'essences' of the aromatic plants that are used abundantly in enhancing the aroma of some spices (Martinez *et al.*, 2008). Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odour (Firn, 2010).

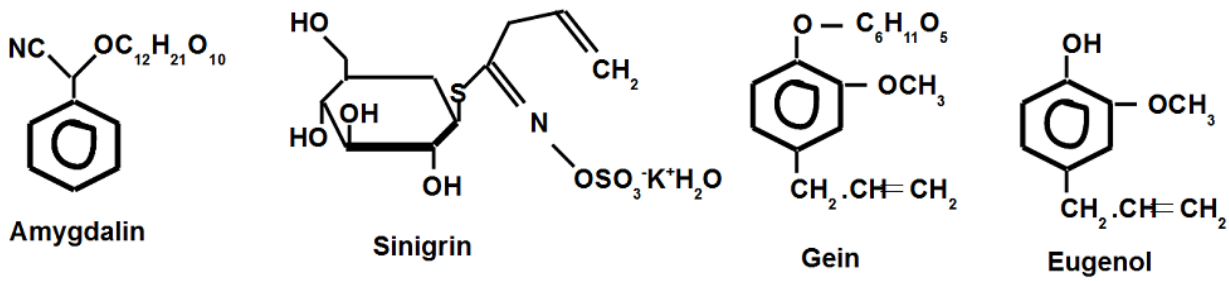


Figure 8: Structures of some pharmacologically important plant derived essential oils

2.5.9 Steroids

Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010). The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness (Maurya *et al*., 2008; Madziga *et al*., 2010)

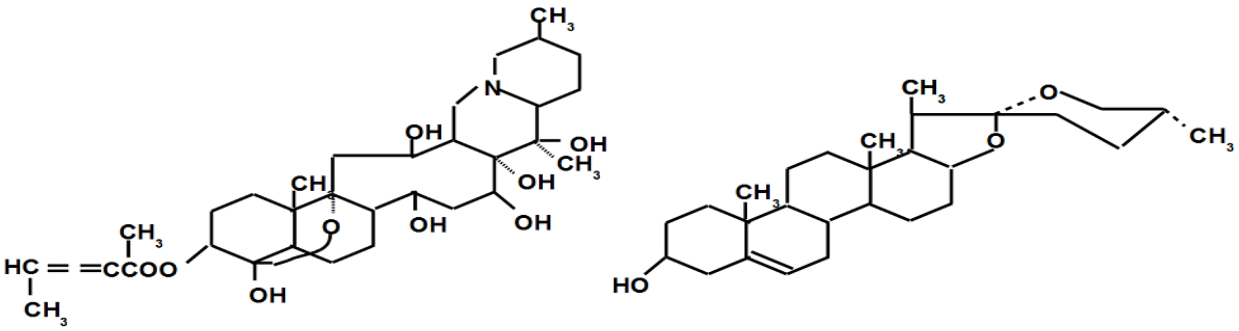


Figure 9: Structures of some pharmacologically important plant derived steroids

2.6.0 Extraction of Phytochemicals

Tiwari *et al*. (2011), defined extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Remington, 2007).Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from

the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al*., 2008).

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al*., 2008).

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) may be employed.

The basic parameters influencing the quality of an extract are plant part used as starting material, solvent used for extraction and extraction procedure. While the effect of extracted plant phytochemicals depends on the nature of the plant material, its origin, degree of processing, moisture content and particle size (Ncube *et al*., 2008). The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and polarity (Ncube *et al*., 2008).

2.6.1 Plant Materials

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, and so on. That is, any part of the plant may contain active components. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction (Parekh *et al*., 2006).

2.6.2 Choice of Solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Eloff, 1998). The solvent of choice should be non-toxic and should not interfere with the bioassay.

The various solvents that are used in the extraction procedures are:

1. **Water:** Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to

water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound (Das *et al*., 2010).

2. **Acetone:** Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. Both acetone and methanol have been found to extract saponins which have antimicrobial activity ((Eloff, 1998; Ncube *et al*., 2008).
3. **Alcohol:** The higher activity of the ethanol extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr, 2010). Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results.
4. **Chloroform:** Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).
5. **Ether:** Ether is commonly used selectively for the extraction of coumarins and fatty acids (Cowan, 1999).

6. **Dichloromethanol:** It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only terpenoids (Cowan, 1999).

Table 9: Solvents used for active component extraction (Cowan, 1999)

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

2.6.3 Extraction Procedures

a. Plant tissue Homogenization:

Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 minutes or left for 24 hours after which the extract is filtered. The filtrate then may be dried under reduced pressure and redissolved in the solvent to determine the concentration.

b. Serial Exhaustive Extraction:

It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted.

c. Soxhlet Extraction:

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al*., 2010).

d. Maceration:

In maceration (for fluid extract), whole or coarsely powdered plant drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs (Ncube *et al*., 2008).

e. Decoction:

This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Remington, 2007).

f. Digestion:

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby (Remington, 2007).

g. Percolation:

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.

h. Sonication:

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfi a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Handa *et al*., 2008).

2.7.0 The Liver

The liver is the largest organ of the body, constituting 2-5% of the adult body weight. It receives blood supply from two major blood vessels. The hepatic artery supplies oxygenated blood, whereas the portal vein, which provides80% of the total blood supply, supplies nutrient-rich deoxygenated blood. The liver thus acts as a guard between the digestive tract and the rest of the body, transforming, detoxifying, and accumulating metabolites. The liver also produces different types of plasma proteins, such as albumin, which are delivered into the blood, as well as metabolites that are constituents of the bile (Arias *et al.*, 1997; Sasse*et al*. (1992).

2.7.1 The Liver Enzymes

Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT) are enzymes found mainly in the liver, they can also be found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys. AST and ALT formerly are called serum Glutamic Oxaloacetic Transaminase (GOT) and Serum Glutamic Pyruvic Transaminase (GPT), respectively. AST or ALT levels are a valuable aid primarily in the diagnosis of liver disease. The normal concentrations in the blood are from 5 to 40µl for AST and from 5 to 35µl for ALT. While that of ALP is 30 to 120 Iu/l. However,

when body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise.

Therefore, the amount of ALT, AST and ALP in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise 10 to 20 times and greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal range (250–1,400µl)) (Huang *et al*., 2006). . On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged (Hafkenscheid, 1979).Liver enzyme abnormalities are useful surrogates of the burden of liver disease in the population (Ioannou *et al*., 2006),

In various forms of liver disease, serum levels of numerous cytosolic, mitochondria, and membrane associated enzymes are increased. The degree of elevation varies with the type of disease. Alanine and Aspartate Aminotransferases and alkaline Phosphatase are the enzymes that are most often measured for the evaluation of liver disease (Tietz *et al.,* 1983). The knowledge of the intracellular location of enzymes can therefore assist in the determination of the nature and severity of the pathological process if suitable enzymes are assayed in the blood.

Liver function tests (LFT), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver (Lee*et al*., 2001). The parameters measured include prothrombin time (PT), albumin, bilirubin (direct and indirect) and others. Liver transaminases (AST (SGOT) and ALT (SGPT)) are useful biomarkers of liver injury in a patient with some degree of intact liver function (McClatchey*et al.*, 2011). Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance.

2.7.3 Liver Diseases

There are more than a hundred kinds of liver disease; these are some of the most common:

1. **Fascioliasis**, a parasitic infection of liver caused by a Liver fluke of the *Fasciola* genus, mostly the *Fasciola hepatica* (Suchy*et al.,* 2014).
2. **Hepatitis, inflammation** of the liver, is caused by various viruses (viral hepatitis) also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions (Benoval*et al.,* 2014; DeClercq*et al.,* 2010).
3. **Alcoholic liver** disease is a hepatic manifestation of over consumption of alcohol, including fatty liver disease, alcoholic hepatitis, and cirrhosis. Analogous terms such as "drug-induced" or "toxic" liver disease are also used to refer to disorders caused by various drugs (Williams *et al.,* 2014; Suk *et al.,* 2014).
4. **Fatty liver disease** (hepatic steatosis) is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells (Reddy and Rao, 2006).Non-alcoholic fatty liver disease is a spectrum of disease associated with obesity and metabolic syndrome (Angulo, 2002).
5. **Hereditary diseases** that cause damage to the liver include hemochromatosis, involving accumulation of iron in the body, and Wilson's disease. Liver damage is also a clinical feature of alpha 1-antitrypsin deficiency and glycogen storage disease type II (Suchy *et al.,* 2014).
6. In **transthyretin-related hereditary amyloidosis**, the liver produces a mutated transthyretin protein which has severe neurodegenerative and/or cardiopathic effects. Liver transplantation can give a curative treatment option (Suchy*et al.,* 2014).
7. **Gilbert's syndrome**, a genetic disorder of bilirubin metabolism found in a small percent of the population, can cause mild jaundice (Suchy *et al.,* 2014).

8. **Cirrhosis** is the formation of fibrous tissue (fibrosis) in the place of liver cells that have died due to a variety of causes, including viral hepatitis, alcohol overconsumption, and other forms of liver toxicity. Cirrhosis causes chronic liver failure (Benoval *et al.*, 2014; DeClercq *et al.*, 2010).
9. **Primary liver cancer** most commonly manifests as hepatocellular carcinoma and/or cholangiocarcinoma; rarer forms include angiosarcoma and hemangiosarcoma of the liver. Many liver malignancies are secondary lesions that have metastasized from primary cancers in the gastrointestinal tract and other organs, such as the kidneys, lungs (Benoval *et al.*, 2014; DeClercq *et al.*, 2010).
10. **Primary biliary cirrhosis** is a serious autoimmune disease of the bile capillaries (Suchy *et al.*, 2014).
11. **Primary sclerosing cholangitis** is a serious chronic inflammatory disease of the bile duct, which is believed to be autoimmune in origin (Suchy *et al.*, 2014).
12. **Budd–Chiari syndrome** is the clinical picture caused by occlusion of the hepatic vein (Komatsu, 2014).

These numerous liver diseases are directly or indirectly caused by the involvement of the liver the metabolism of the toxins and toxicants that find their ways into the system. Therefore, the health of the organism is dependent on the functionality of the liver.

2.8.0 Acetaminophen and Acetaminophen Induced Hepatotoxicity

Acetaminophen, is N-acetyl-para-aminophenol, (APAP or paracetamol), which is the most widely used as drug prescribed for pains, and it is popular the world over (Daly *et al.*, 2008). While safe at therapeutic doses of up to four grams per day for adults, acetaminophen overdoses, either accidental or intentional, are the leading cause of acute liver failure in the United States,

accounting for some 56,000 emergency room visits, 2,600 hospitalizations and nearly 500 deaths annually (Lee, 2008; Nourjah*et al*2006).

Acetaminophen is metabolized by conjugation with sulfate and glucoronidate, which are inert and are excreted in the urine. Depending on dose, a fraction of APAP is converted into a highly reactive toxic intermediate, N-acetyl-p-benzoquinone imine (NAPQI) by several P450 cytochromes (Prescott, 1980). Substantial amounts of NAPQI are effectively eliminated by conjugation with glutathione (GSH). However, after a large dose of APAP, the sulfonation reaction becomes saturated and the build-up of NAPQI depletes GSH in the liver, causing further accumulation of NAPQI. Unconjugated NAPQI binds to proteins and subcellular structures and induces rapid cell death and necrosis that can lead to liver failure. N-acetylcysteine (NAC) can be an effective antidote for APAP poisoning. NAC limits hepatotoxicity by increasing GSH synthesis in the liver (Schilling *et al.*, 2010). Current protocols recommend treating patients with an initial dose of 150 mg/kg NAC, infused over a period of an hour, upon hospitalization, followed by decreasing amounts of NAC infused over the next 20 hours (Daly *et al.*, 2008). Fatal liver damage can be prevented if the initial dose of NAC is administered within 8-12 hours of an APAP overdose. This antidote dosage regime has been developed empirically over a period of many years based on outcomes from clinical cases. It is not known whether the current NAC treatment protocol is optimal.

The metabolism of APAP has been well-studied and the distributions of its metabolites in the plasma and urine of humans are well-documented (Prescott, 1980; Mitchell *et al.*, 2010; Davis *et al.*, 1976), as are the hepatic values in mice and rats (Moldeus, 1978).What has been lacking is an integrated and quantitative understanding of the kinetics of APAP metabolism, of how APAP dosage affects NAPQI synthesis and GSH concentrations in the liver, or how NAC stimulates the synthesis of GSH, and/or how the dosage and timing of NAC affect detoxification of NAPQI.

Remien *et al*. (2012) developed a mathematical model to estimate over dosage of APAP based on indicators of liver damage (blood levels of Aspartate Aminotransferase, Alanine Aminotransferase and the international normalized ratio of prothrombin time)that are measured upon admission to hospital emergency Departments. In a retrospective study, this model was able to accurately predict whether the overdose would lead to fatal liver damage.

2.8.1 Biochemical Mechanisms of Acetaminophen Toxicity

Events that produce hepatocellular death following the formation of acetaminophen protein adducts are poorly understood. One possible mechanism of cell death is that covalent binding to critical cellular proteins results in subsequent loss of activity or function and eventual cell death and lysis. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well as proteins involved in cellular ion control (Nelson, 1990). Tirmenstein and Nelson (1989) and Tsokos-Kuhn *et al*. (1988) reported alterations of plasma membrane ATPase activity following toxic doses of acetaminophen.

A number of proteins bound to acetaminophen have been isolated and identified. A review of the individual proteins that were isolated and identified by individual analysis has been published (Cohen and Khairallah, 1997; Pumford*et al*., 1997). Subsequently, Qui *et al*. (1998) used matrix-assisted laser desorption ionization mass spectrometry to identify 20 additional proteins containing covalently bound acetaminophen. The proteins identified by Pumford *et al*.,(1997) included a 100-kDa cytosolic protein determined by sequence analysis to be the enzyme, N-10-formyltetrahydrofolate dehydrogenase. This enzyme is involved in 1-carbon metabolism and oxidizes formaldehyde to carbon dioxide. Studies in mice reported that the activity of this enzyme was decreased by 20% at 2 h after toxic doses of acetaminophen (Pumford*et al*., 1997). A second protein, a 50-kDa mitochondrial protein, was isolated and determined to be glutamate dehydrogenase (Halmes*et al*., 1996). This enzyme reversibly metabolizes glutamate to α-ketoglutarate and ammonia. The activity of this enzyme was also

decreased approximately 25% at 2 hour. Based on the enzyme inhibition data for these two enzymes, it appeared that covalent binding with acetaminophen resulted in only partial inhibition of enzyme activities under toxic conditions in mice. Although it is plausible that partial inhibition of a large number of enzymes may contribute to cell death, the data generated questions relative to the validity of the hypothesis that covalent binding to critical proteins is the only mechanism of acetaminophen toxicity.

2.8.2 Acetaminophen overdose:

Acetaminophen is one of the most frequently used drugs in both intentional and unintentional overdoses. Acetaminophen overdose can result from acetaminophen-only preparations or from combinations of acetaminophen with other drugs. These acetaminophen-containing preparations can be prescription medication or over-the-counter preparations, (Gunnel *et al*., 2000).

There are an increasing number of case reports relating to iatrogenic acetaminophen poisoning relating to the use of intravenous acetaminophen in both the adult and pediatric population,(Berling, *et al.,* 2012; Beringer, *et al., 2011*). Acetaminophen overdose may occur after an acute single ingestion of a large amount of acetaminophen or acetaminophen-containing medication, or repeated ingestion of an amount exceeding recommended dosage. Patients are often asymptomatic or have only mild gastrointestinal symptoms at initial presentation. Untreated acetaminophen poisoning may cause varying degrees of liver injury over the 2 to 4 days following ingestion, including fulminant hepatic failure (Alastair and Allan, 2014).

An acute acetaminophen overdose in adults, in terms of FDA-labeled therapeutic dosing, is minimally defined as a cumulative dose of acetaminophen >4 grams and is ingested over 8 hours or less. (Some authors use a period of 4 hours.) In adults, hepatic toxicity may occur following ingestion of >7.5 grams (i.e., 24 regular-strength 325 mg or 15 extra-strength 500

mg caplets or tablets). Children are at risk for hepatotoxicity with acute acetaminophen ingestions >150 mg/kg.

Hepatotoxicity is extremely rare in patients treated with acetylcysteine within 8 hours of an acute acetaminophen overdose. The efficacy of acetylcysteine decreases subsequent to the first 8 hours following an acute acetaminophen overdose, with a corresponding stepwise increase in hepatotoxicity with increasing treatment delays between 8 and 16 hours, (Alastair Newton and Allan, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant Materials

The plant materials are; *Ocimum gratissimum, Vernonia amygdalina, Pterocarpus santaliniodes, Telfeiria occidentalis, Ocimum canum* and*Amaranthus viridis.* They were were collected in and around Keffi, in Nasarawa state, North Central Zone of Nigeria.The leaves were identified by a Taxonomist at the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi.

3.1.2 Chemicals and Reagents

The drug, Acetaminophen was a research support from Emzor Pharmaceutical Ltd, Lagos while silymarin is a branded drug (Sylibon 140) from Micro Laboratory Ltd, India. All other chemicals and reagents used were of analytical grade and are as shown in the appendix.

3.1.3 Equipments

The major equipments used for the study were:

Sanyo Harrier 18/80 Refrigrated Centrifuge

Becckman Coulter DU 800 UV/Visible Spectrophotometer

AmScope® Microscope Digital Camera (Model MU500)

Digital Photo Colorimeter (EI (312 Model), Japan)

HSC (1000-4000rpm) Bench Centrifuge

Searchtech Renounce 52-2 Rotary Evaporator

Searchtech XH-C, Vortex Mixer

Sherwood Electronic Balance

Blessed 321 Homogenizer, Mumbai

Search PS-3C p^H Meter

HH-6 Water Distiller, Ghangzou Jian Lingerie Med. Device Ltd, China

Search Tech Electronic Incubator, Model DNP.

Search Tech DHG-9101-1SA Dry Oven

HH-W420 Water Bath

3.1.4 Experimental Animals

Ninety (90) male Wister albino rats weighing between 120-140g were used for the study. These rats were purchased from the animal house of the National Veterinary Research Institute (NVRI), Vom, in Plateau state. They were housed in clean, well ventilated metal cages in the animal house of the Department of Biological Sciences (Zoology unit), Nasarawa State University Keffi. The animals were kept under 24 hours light/dark cycling. They were allowed assess to unlimited food and water supply and allowed to acclimatize for three (3) weeks before the commencement of the study.

3.2 Methods

3.2.1 Sample collection and preparation

The leaves of the the plants under study were rinsed in water to remove dust and sand particles, and then dried under room temperature for fourteen (14) days. The dried leaves were then ground into powder using electric blender.Part of the ground leaf materials were used to determine the proximate composition while the other was used for preparation of aqueous and ethanol extracts.

3.2.2 Proximate Analysis

Standard methods of the Association of AnalyticalChemist (AOAC), 2006was used to determine the moisture content, crude protein, crude fat, total ash, crude fiber and carbohydrate content of the materials.

3.2.2.1 Determination of Moisture Content

This is a measure of the percentage moisture lost due to drying at a temperature of 105ºC. Two (2) grams of the ground samples were weighed (W_1) into pre-weighed crucible (W_0) and placed into a hot drying oven at 105ºC for 3hours. The crucible were removed, cooled in desiccators and weighed. The process of drying, cooling and weighing were repeated after 30minutes at least until a constant weight (W_2) was obtained. The weight loss due to moisture was obtained by the equation:

Moisture (%) = $\frac{w1-w2}{w1-wo}$ x 100

Where: W_0= weight of the empty crucible (g)W_1= weight of the powder + sample + empty crucible (g) W_2= weight of dried sample + empty crucible (g)

3.2.2.2 Determination of Ash Content

This is a measure of the residue remaining after combustion of the dried samples in a furnace at a temperature of 550^{o}c for five (5) hours. Two(2) grams of the dried samples were weighed (w_1) into re-weighed empty crucible (W_0) and placed into a lent on muffle furnace at 550^{o}c for 5hours. The ash was cooled in desiccators and weighed (W_2). The weighed ash was determined by the difference between the ground samples, re-weighed and the ash in the crucible. Percentage ash was obtained by equation.

Ash (%)= $\frac{w1-w2}{w1-wo}$ x 100

Where: W_0= weight of the empty crucible (g)W_1= weight of crucible + powdered sample(g) W_2= weight of crucible + ash sample(g)

3.2.2.3 Determination of Crude fat

The crude fat content in the samples was extracted usingsoxhlet extraction procedure. The ground samples (2g), were weighed (W_0) into a thimble and covered with a clean white cotton wool. Petroleum ether (200ml) was poured into a 250ml extraction flask, which was

previously dried in an oven at 105^0C and weighed (W_2). The porous thimble was placed into the soxhlet and the rest of the apparatus was assembled. Extraction was done for 5hours. Thimble was removed and the extraction flask placed in a water bath so as to evaporate the petroleum ether and then dried in the oven at a temperature of 105^0C to completely remove all solvent and moisture. It was then cooled in a desiccator and re-weighed (W_1). The percentage crude fat was calculated using the equation below;

Crude lipid (%) = $\frac{w1-w2}{w1-wo}$ x 100

Where: Wo= weight of sample(g) W_1= weight of flask + oil(g) W_2=weight of flask(g).

3.2.2.4 Determination of Crude Fibre

Two (2) grams of ground sampleswere weighed (W_0) into a $1dm^3$ conical flask. One hundred (100) ml and twenty mililiters (20ml) of twentypercent (20%) H_2SO_4 were added and boiled for thirty minutes (30mins). The content was filtered through What Man No.1 filter paper. The residue was scrapped back into the flask with a spatula. One hundred mililiters (100ml) and twenty mililiters (20ml) of 10%NaOH was added and allowed to boil gently for 30minutes. The content was filtrered and residue was washed thoroughly with hot distilled water. It was rinsed once with ten percent (10%) hydrochloric acid(HCl), twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into the crucible and dried overnight at 105^oC in an oven. It was then removed and cooled in a desicator. The sample was weighed (W_1) and ashed at 550^oC for 90minutes in a lenton muffle furnance. It was finally cooled in a desiccator and weighed again (W_2). The percentage crude fibre was calculated using equation:

Crudefibre (%) =$\frac{w1-w2}{wo}$ x 100

Where:

W_0= weight of sample (g) W_1 =weight of dried sample (g) W_2 =weight of ash sample (g).

3.2.2.5 Determination of Crude Protein

The crude protein of the samples was determined using the micro-kjeddahl method. This is based on the transformation of protein and that of the other nitrogen containing organic compounds, other than nitrates into ammonium sulphate by acid digestion:

$$\text{Sample nitrogen} + H_2SO_{4\,(aq)} \longrightarrow (NH_4)_2\,SO_{4\,(aq)}$$

$$(NH_4)_2\,SO_{4\,(aq)} + 2NaOH_{\,(aq)} \longrightarrow 2NH_{3\,(aq)} + 2H_2O + Na_2SO_{4\,(aq)}$$

$$NH_{3\,(aq)} + H_3\,BO_{3\,(aq)} \longrightarrow NH_4^{+}{}_{(aq)} + H_2\,BO_{3\,(aq)}$$

$$H^{+}{}_{(aq)} + H_2\,BO_{3\,(aq)} \longrightarrow H_3\,BO_{3\,(aq)}$$

The sample (2g) was weighed along with 20mlof distilled water into a micro-kjedahl digestion flask. It was shaken and allowed to stand for thirty minutes (30mins). Five grams (5g) of selenium of catalyst was added followed by the addition of twenty mililiters (20ml)concentratedH_2SO_4. The flask was heated on the digestion block at 100^0C for 4hours until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 50mlvolumetric flask and diluted to the mark with water. An aliquot of the digest (10ml) was transferred into another micro-kjedahl flask along with 20mlof distilled water and placed in the distilling outlet of the micro-kjedahl distillation unit. A conical flask containing 20mlof boric and indicator was placed under the condenser outlet NaOH solution (20ml, 40%) was added to the content in the kjedahl flask by opening the funnel stopcock. The distillation start and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in 20mlof boric acid, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H_2SO_4; the end point was obtained when the colour of the distillate changed from green to pink. Crude protein is a measure of nitrogen content of the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.60. This is based on the assumption that, protein

contain about 16%N which includes both true protein and non-protein N and does not make a distinction between available or unavailable protein. The crude protein was calculated using

Crude protein (%) = %N 6.60

The nitrogen content of the sample is given by the formula below:

$$N\ (\%) = \frac{Tv\ 0.014\ V_1}{G\ V_2} \times 100$$

Where:

Tv= titre value of acid (ml)

Na =concentration or normality of acid

V_1= volume of distilled water used for distilling the digest (50ml)

V2 = volume of aliquot used for distillation (10ml)

G = original weight of sample used (g).

3.2.2.6 Determination of Carbohydrate

Total proportion of carbohydrate in the samples was obtained by Colorimetric Method.The Anthrone method is a colorimetric method of determining the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The sample was mixed with sulfuric acid (H_2SO_4) and the anthrone reagent and then boiled until the reaction was completed. The solution was then allowed to cool and its absorbance read at 620 nm. There is a linear relationship between the absorbance and the amount of sugar that was present in the original sample. This method determined both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. A calibration curve was prepared using a series of standards of known carbohydrate concentrations of 0.5g, 1g, 1.5g, 2g, 2.5g, 3g, 3.5g, 4g, 4.5g and 5g.

3.2.3 Preparation of Extracts

Ground plant material were divided into two; one was extracted with ethanol by soaking 100g of the ground samples in 500ml of 98% ethanol (i.e. ratio 1:5; weight to volume) for the 48hrs. The extracts were filtered using muslin cloth and then concentrated by heating in a water bath and stored in airtight containers.

The other part was extracted by soaking 100g of the powdered samples in 500ml of distilled water (i.e. ratio 1:5; weight to volume) for 24hrs. The extracts were filtered using muslin cloth and then concentrated by heating to evaporate the water and stored in airtight containers.

3.2.4 Qualitative Phytochemical screening of the leaf extracts:

Portions of the concentrated extractswere used for phytochemical screening using standard procedures of the Association of AnalyticalChemist to identify the constituents as described byOdebiyi and Sofowora(1990), Fadeyi (1983), Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

3.2.4.1 Test for Alkaloids

The plant extracts (0.5g) were dissolved in 2ml of dilute Hydrochloric acid and filtered; the filtrate was treated with a few drops of Hager's reagent (saturated picric acid solution). Presence of yellow colour precipitate indicated the presence of alkaloids.

3.2.4.2 Test for Sterols

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. Presence of reddish brown colour at the interface indicated the presence of a steroid ring.

3.2.4.3 Test for Triterpenoids

Crude extractswere dissolved in chloroform and filtered. The filtrates were treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. Presence of a yellow colour at the layer indicated the presence of triterpenoids

3.2.4.4 Test for Flavonoids

Few drops of sodium hydroxide solution was added to (0.5g) of the extract. Persistent of intense yellow colour on addition of dilute acid indicated the absence of flavonoid.

3.2.4.5 Test for Saponins

Half (0.5) gram of the plant extract was shaken vigorously with 2ml of water in a test tube. Persistence of the foam formed indicated the presence of saponins.

3.2.4.6 Test of Tannins

In a test tube, 1 ml of ethanol solution was added to 2 ml of water and 2-3 drops of diluted solution of $FeCl_3$ and observed for a green. Presence of a blue black or a blue - green coloration showed the presence of tannins.

3.2.4.7 Test for Resins

Two mililiters (2ml) of sample extract was added to equal volume of acetic anhydride solution and then a drop of concentrated H_2SO_4 was added. Formation of violet colour indicated the presence of resin.

3.2.4.8 Test for Balsams

Three (3) drops of alcoholic $FeCl_3$ was added to 4ml of sample extract and warmed. Persistence of adark green colour indicated the resence of balsam.

3.2.4.9 Test for Terpenoids

Acetic anhydride (0.5ml) was added to the sample extract and few drops of H_2SO_4 were added. Presence of bluish green precipitate indicated the presence of terpenoid.

3.2.4.10 Test for Phenols

To 0.5g of extract 1% $FeCl_3$ in ethanol was added. Formation of a dirty green precipitate indicated the presence of phenol.

3.2.4.11 Test for Glycosides

Two hundred milligrams (200mg) of crude extract was mixed vigorously with 2ml of diluted sulphuric acid and 5% aqueous ferric chloride solution boiled for 5 minutes was added. Oxidation to anthroquinones indicated the presence of glycosides.

3.2.4.12 Test for Anthraquinones

The extract (0.2g) was added to 10ml benzer, shaken and filtered. A volume of 0.5ml 0f 1% NH_3 was added. Persistence of pink, red or violet colour at the lower phase indicated that anthraquinones were present..

3.2.4.13 Test for Phlobatannins

One percent (1%) HCl was added to 1ml of sample extract and boiled. Presence of a reddish colour indicated the presence of phlobatannin.

3.2.4.14 Test for Cardiac Glycosides

A quantity (0.5g) of sample extract was dissolved in 2ml of glacial acetic acid containing a drop of ferric chloride. 2ml of concentrated Sulphuric acid was added. Formation of a brown ring at the interface indicated the presence cardiac glycoside.

3.2.4.15 Test for Carbohydrate

A quantity (0.5 g) of the extract was treated with 2 drops of α-naphthol solution in a test tube. Absence of a violet ring at the junction indicated the presence of carbohydrate.

3.2.4.16 Test for Diterpenes

About 0.5g of extract was dissolved in water and treated with 3 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

3.2.5 Quantitative phytochemical determination of the leaf extracts:

The quantitative determination of the phytochemical composition of the leaf extracts were carried out using the methods of the Association of Analytical Chemists, AOAC, (2006). The respective processes for different phytochemicals are explained below:

3.2.5.1 Alkaloid determination

Five gram (5g) of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4minutes, this was filtered and extract was concentrated on a water bath to one quarter of the original volume (125ml). Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed

$$\% \text{ alkaloid} = \frac{W_3 - W_2}{W_1} \times 100\%$$

Where: W_1 =initial weight of sample, W_2 =weight of the extract, W_3 = final weight of the residue

3.2.5.2 Determination of saponins

Half gram (0.5g) of sample was added to 20ml of 1MHCl and was boiled for 4hours. After cooling, it was filtered with a filter paper. Fifty mililiters (50ml) of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. Then 5ml of acetone-ethanol was added to the residue. Four microliters 4µl of each was taken into 3 different test tubes and six mililiters (6ml) of ferric sulphate (Fe_2SO_4) reagent was added into then followed by 2ml of concentrated H_2SO_4. It was thoroughly mixed and after ten minutes (10ml) and the absorbance was taken at 490nm.

$$\text{Saponin} = \frac{\text{Absorbance of sample x Absorbance of standard}}{\text{Absorbance of atandard}}$$

3.2.5.3 Determination of tannins

Two milligram (0.2g) of the finely ground sample was weighed into a 50ml sample bottle. Ten millilitres (10ml) of 70% aqueous acetone was added and tightly covered. The bottle was suspended in an ice bath shaker and shaken for 2hours at 300^{o}C. The solution was then centrifuged and the supernatant stored in ice. A volume of 0.2ml of the solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5mg/ml of the stock and the solution made up to 1.0ml with distilled water, Folinciocateau reagent (0.5ml) was added to the sample and standard followed by addition of 2.5ml of 20% Na_2CO_3, the solution was then vortexed. This was allowed to incubate for 40minutes at room temperature and the absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared.

3.2.5.4 Determination of total phenolic content:

The total phenolic content of the leaveswas determined using the Folin-Ciocalteu reagent. Each evaporated thick and viscous extract (0.8±0.01 to 0.9±0.01mg) was diluted with 5ml methanol. The sample of each plant extract solution (200µl) was transferred into a test tube and then mixed thoroughly with 1.0ml of Folin-Ciocalteu reagent. After mixing for 3 minutes, 0.8ml of 7.5% (w/v) sodium carbonate was added. The mixture was agitated with vortex mixer, then allowed to stand for a further 30 minutes in the dark and then centrifuged at 3300g for 5 minutes. The absorbance of the sample and that of the blank were read at 765nm using a spectrophotometer. The concentration of total phenolic compounds in all plant is expressed as milligrammes of gallic acid equivalent, (mg gallic acid equivalent).

3.2.6 Determination of free radical- scavenging using DPPH:

The radical scavenging activity of the plant extracts against DPPH radical were determined by UV-Visible Spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by Afolayan *et al.*, (2014). The following

concentrations of the extracts were prepared, 0.03125, 0.0625, 0.125, 0.25 and 0.5mg/ml in methanol. Vitamin C was used as the antioxidant standard at concentrations of 0.03125, 0.0625, 0.125, 0.25 and 0.5mg/ml.One mililiter (1.0ml) of the extract was placed in a test tube and 3.0 ml of methanol was added, followed by 0.5 ml of 1 mM of DPPH in methanol. Thereafter, the decrease in absorption was measured on a UV-Visible Spectrophotometer after the incubation for 10 minutes. A control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against that of the control and the percentage inhibition was calculated. All test and analysis were run in duplicates and the results obtained. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation below:

% inhibition = {[Ab-Aa]/Ab} x 100

Where Ab is the absorption of the blank sample (without the extract) and Aa is the absorption of the extract.

3.2.7 Experimental Design

The animals were divided into fifteen (15) groups of six animals each. These include three (3), control groups; Normal Control (Water and Feeds only), Negative Control (Water, Feed and 2g/kgbw of Acetaminophen) and Positive Control (Water, Feed, Sylimarin and 2g/kgbw of Acetaminophen). The other groups were six groups administered with the aqueous leaf extracts of the plants and another six (6) groups administered with ethanol leaf extracts of the plants. This is further explained in table 11.

Table 11: Experimental Design

AQUEOUS EXTRACT			ETHANOL EXTRACT		
GROUP	PLANT	CONC.	GROUP	PLANT	CONC.
1A	*Ocimum canum*	400mg/kgbw	1B	*Ocimum canum*	400mg/kgbw
2A	*Telfeiria occidentalis*	400mg/kgbw	2B	*Telfeiria occidentalis*	400mg/kgbw
3A	*Amarranthus viridis*	400mg/kgbw	3B	*Amarranthus viridis*	400mg/kgbw
4A	*Ptericarpus santalinardes*	400mg/kgbw	4B	*Ptericarpus santalinardes*	400mg/kgbw
5A	*Vernonia amygdalina*	400mg/kgbw	5B	*Vernonia amygdalina*	400mg/kgbw
6A	*Ocimum gratissimum*	400mg/kgbw	6B	*Ocimum gratissimum*	400mg/kgbw

The animals were first fed with the chow (feeds) and intubated with the plant materials (aqueous and ethanol leaf extracts – 400mg/kgbw) for seven days (a week). They were fasted for up to seven hours, followed by intoxicated by intubation with Acetaminophen (2g/kgbw).

3.2.8 Preparation of Biological Samples

3.2.8.1 Collection of Blood Samples

At the end of the experimental period, (after 9 hours of intoxication), the animals were made unconscious by exposure to chloroform in an enclosed container, according to the method described by Ekor, *et al.* (2006) Incisions were quickly made into the animals' cervical region with the aid of sterile blades. Blood samples were collected by cervical decapitation into plain tubes. Serum was collected by centrifuging the clotted blood in a HSC (1000-4000rpm) bench centrifuge at 3000 rpm for 10 minutes.

3.2.8.2 Preparation of Liver Homogenate:

After bleeding, the livers were carefully removed, trimmed of extraneous tissues and rinsed in ice-cold 1.15% KCl. The livers were then blotted dry, two grams (g) was weighed and homogenized in 8 mililiters (8ml) of ice-cold phosphate buffer (100 mM, pH 7.4). The homogenate were then centrifuged first at 6,000 rpm for six minutes (6 mins) to remove nuclear debris after which the obtained supernatant were centrifuged at 10,000 rpm for twenty minutes (20 mins) to obtain the post-mitochondrial supernatant (PMS),using a refrigerated centrifuge. This was used for the assay of the antioxidant enzymes (Super Oxide Dismutase, Catalase and Glutathione Peroxidase).

3.2.9 Determination of the Liver Function

The prepared serum was analyzed for various biochemical parameters - Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), lipid profile (Triacyl glycerides, Total Cholesterol, High and low density lipoproteins), total protein, albumin and bilirubin using spectrophotometric procedures.

3.2.9.1 Estimation of Alkaline Phosphatase (ALP).

The serum Alkaline Phosphatase activities of the experimental animals were estimated using the method of King (1965b).

Principle

The determination of alkaline phosphate (ALP) was based upon the reactions below.

$$\text{P-Nitrophenyl phosphate} + H_2O \xrightarrow{\text{Alkaline phosphatise}} \text{p-Nitrophenyl} + \text{inorganic phosphate}$$

Procedure

One millilitre (1.0 ml) of serum (the ALP source) was pipette into 1.5 ml of phosphate buffer buffer (0.1M, pH 7.4) and made up to 3.0 ml with distilled water in the test tubes. The tubes were incubated at 37°C for 15 minutes. The reaction was arrested by the addition of 1.0 ml of Folin's phenol reagent. To the control tubes, the enzymes were added after arresting the reaction. The contents were centrifuged and to 1.0 ml of the supernatant, 1.0 ml of 15 % sodium carbonate, 1.0 ml of substrate and 0.1 ml of $MgCl_2$ were added and incubated for 10 minutes at 37°C. The colour developed was read at 640 nm using a spectrophotometer against a blank. The standard solutions of phenol of varying concentrations were also treated similarly. The enzyme activity in serum and tissues were expressed as moles of phenol liberated/minute/mg of protein.

3.2.9.2 Estimation of Aminotransferase Activities

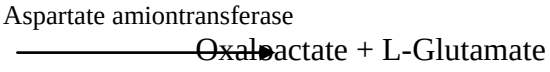
The determination of Aspartate aminotransferase and Alanine aminotransferase were carried out using the King (1965a) method.

Principle

The transaminase enzymes, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), catalyze the transfer ofthe amino group of glutamic acid to oxalo acetic acid and pyruvate in reversible reactions. The transaminase activity isproportional to the amount of oxaloacetate and pyruvate formedover a definite period of time and is measured by a reaction with2.4-dinitrophenylhydrazine (DNPH) in alkaline solution.

Estimation of Aspartate Aminotransferase (AST)

Principle



Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of enzyme source was added and incubated for one hour at 37°C. At the end of the incubation period, 0.07 ml of aniline-citrate reagent was added and incubated for another 20 minutes. Then, 1.0 ml of the dinitrophenyl hydrazine reagent was added and left for 20 minutes. At the end of 20 minutes, 10 ml of 0.4MNaOH was added and the color developed was read at 540 nm in a spectrophotometer after 10 minutes. The standards were also treated similarly.

Calculations

The enzyme activity in serum was expressed as units per liter (moles of pyruvate/hour/mg of protein).

Estimation of Alanine Amino Transferase (ALT),

Principle

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of enzyme source was added and incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 1.0 ml of dinitrophenyl hydrazine and left aside for20 minutes at room temperature. The colour developed by the addition of 10 ml of 0.4N sodium hydroxide was read at 540 nm in a spectrophotometer against the reagent blank.

Calculations

The enzyme activity in serum was expressed as moles of pyruvate/hour/mg of protein

3.2.9.3 Determination of other Liver Function Parameters

Estimation of Total Protein (TP):

The total protein was estimated using the colorimetric method of Lowry *et al*. (1951)

Principle

The peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a coloured complex. The absorbance which is proportional to the concentration of total protein in the specimens is measured at 550nm. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintained its solubility in alkaline solution.

Procedure

To 0.1 ml of serum, 0.9 ml of water and 4.5 ml of alkaline copper reagent were added and kept at room temperature for 10 min. To this, 0.5 ml of Folin's reagent (1:2) was added and the blue colour developed was read after 20 min at 640 nm. Protein content was expressed as g/dl of serum.

Calculations

$$\frac{\text{Abs (Assay) x concentration of standard = gm/dl}}{\text{Abs (Standard)}}$$

Determination of Albumin

To 0.01 ml of serum/tissue extract, 1.0ml of working solution was added and incubatedthe assay mixtu2```re for 1minute at 37o C. After completion of incubation period, the absorbancewas measured at 600 nm. The activity was calculated by using the formula:

Total albumin in g % = Absorbance of sample/Absorbance of standard x 5.

Total and Direct Bilirubin

The method of Malloy-Evelyn, (1937), was used.

Principle

Reaction between bilirubin and diazotized sulfanilic acid leads to a compound, the azobillirubin, coloured in very acidic or basic medium. In an aqueous solution, only direct bilirubin (DB) reacts. To enable one assay fortotal bilirubin (TB), it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethysulfoxide (DMSO).The absorbance of azobillirubin thus produced is proportional to the concentration of bilirubin and is measured at 546 nm.

Procedure

To 50 µl of serum samples, 1000 µl of total bilurubin reagent and direct bilirubin reagent and 20 µl of respective activator reagent were added. The reaction mixture was mixed well and incubated for 10 minutes at 37^0C. At the same time, blank and standard solution was prepared. The absorbance of sample against reagent blank was read at 546 nm.

Calculations

Bilirubin was calculated by using the formula:

Total bilurubin = O.D. of sample – O.D. of blank / O.D. of standard × 10

Direct bilirubin = O.D. of sample – O.D. of blank / O.D. of standard × 7.7

3.2.10 Determination of lipid profile

3.2.10.1 Determination of Triacyglycerol (TG)

Principle

The estimation of the serum triacylglycerol was based on the reactions below:

$$\text{Triacyglycerol} + 3H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + 3\text{R-COOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{Dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} 2H2O + HCl + \text{dye}$$

(Dye = 4-(p-benzoquinone-monoimino)-phenazone)

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of 4-Chlorophenol and (4-AAP) 4-aminophenazone in the presence of Peroxidase (POD) and hydrogen peroxide produces a rose colored dye which was measured at 550 nm. The intensity of the colour formed is directly proportional to the triglycerides concentration in the sample.

Procedure

Three test tubes were labaled blank, standard and sample and ten microlitres (10µ) of distilled water, standard triglyceride and the serum were pipetted into them respectively. One thousand microliters (1000µl), of the working reagent was then added to each of the test tubes, mixed and incubated at 37^{o}C for 5 minutes. The absorbance of the reaction mixture was read at 550nm and the triglyceride concentration was calculated as shown below:

Calculations

Concentration of triglyceride (mg/dl) =

$$\frac{\text{Abs of test} \quad \text{x} \quad \text{conc. of standard (mg/dl)}}{\text{Abs of standard}}$$

11.43mg/dl = 1mmol/L

3.2.10.2 Determination of Total Cholesterol;

The total cholesterol content of the serum was determined using the method of Parekh and Jung (1970).

Principle

Cholesterol esters are converted into their fatty acid and cholesterol components by cholesterol esterase. The cholesterol produced is converted to cholest-4-en-3-one and

hydrogen peroxide by cholesterol oxidase in the presence of oxygen. The peroxide reacts with hydroxybenzoic acid (HBA) and 4-aminoantipyrine by the action of peroxidase to form the red colour-producing quinone imine. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read at 500 nm.

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + free fatty acid}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten - 4, 3-one + } H_2O_2$$

$$2\ H_2O_2 + \text{Phenol + 4-amino-antipyrine} \xrightarrow{\text{Peroxidase}} \underset{\text{(Pink)}}{\text{Quinoneimine + 4 } H_2O}$$

Procedure

A quantity (0.1 ml) of the aliquot of the total lipid extract from the serum was evaporated to dryness and 3 ml with ferric chloride–Uranyl acetate reagent was added. Standard (20–60 µg) were made to 3 ml with ferric chloride–Uranyl acetate reagent. Blank consisted of 3 ml of the reagent. To all these tubes, 2 ml of sulphuric acid–ferrous sulphate reagent was added to all the tubes and the contents were mixed well and kept in ice bath. After 20 min the colour developed was read at 540 nm using a UV spectrophotometer.

Calculations

$$\frac{\text{Abs (Assay) x Concentration of standard}}{\text{Abs (Standard)}} = \text{mmol/L}$$

3.2.11 Determination of the activities of the antioxidant enzymes in the liver

3.2.11.1 Determination of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by the method described by Sun and Zigma (1978).

Principle

Superoxide Dismutase has the ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm.

Procedure

A reaction mixture (3 ml) containing 2.95 ml of 0.05 M sodium carbonate buffer pH 10.2; 0.02 ml of liver homogenate and 0.03 ml of 0.3mM epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water.

Calculation

Enzyme activity was calculated by measuring the change in absorbance at 480 nm after 5 mins. $\sum = 4020M^{-1}\ cm^{-1}$

3.2.11.2 Determination of Catalase activity

The catalase activity was determined according to the method of Beers and Sizer as described by Usoh *et al.,* (2005)

Principle

Catalase is assayed by measuring the decrease in absorbance at 240nm due to the decomposition of H_2O_2, using the UV spectrophotometer.

Procedure

The sample was prepared by homogenizing the liver in the phosphate buffer (1:4,), weigtht (in gram of liver) to volume (in millilitre of buffer). The reaction mixture (3 ml) contained 0.1 ml of homogenate inphosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0.

Calculations

An extinction coefficient for H_2O_2 at 240 nm of 40.0 $M^{-1}cm^{-1}$ (Aebi 1984) was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per mg proteinper minute

3.2.11.3 Determination of glutathione peroxidase (GSH-Px) activity

The method of Lawrence and Burk (1976) was employed to measure the activity of GSH-Px.

Principle – It is based on the oxidation of NADPH by the Glutathione peroxidise which is measured in terms of nmoles of NADPH oxidized/min/mg protein

Procedure

A hundred microliter (100 µl) of supernatant was mixed with 700 µl of reaction mixture containing, 1 mM EDTA, 1 mM NaN , 0.2 mM NADPH and 1 mM GSH in a phosphate buffer saline, pH 7.2, and 100 µl of glutathione peroxidase (containing 10 units of GPx). The tubes were vortexed and incubated for 5 min at room temperature. After incubation, 100 µl of 0.2 mM H_2O_2 was added to initiate the reaction and absorbance recorded at 340 nm and after every 30 sec over a period of 3 min, using a spectrophotometer.

Calculations

Changes in the rate of absorbance were converted into nmoles of NADPH oxidized/min/mg protein, using an extinction coefficient of 6.22 × 10 L mol cm

3.2.12 Histopathological study on the liver tissues

This was carried out at Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State, using the method described by Bancroft *et al*., (2013). The liver tissue, after washing in physiologic saline solution, were preserved in 10% neutral buffered formal saline immediately after removal from the animal and allowed to fix for about 72 hours. The

tissues were dehydrated in ascending grades of isopropyl alcohol by immersing in 50%, 70%, 80%, 90%, 95%, and 100% for 1 hour each. The dehydrated tissues were cleared in twice in xylene, with each clearing done in one hour (1hr). The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with rotary microtome at 5 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides that had been previously smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C for 5 minutes and the sections were allowed to cool.

The sections were deparaffinised by immersing in two changes of xylene for 10 mins each in horizontal staining jar. The deparaffinised sections were hydrated in descending grades of isopropyl alcohol for 2 mins each and taken to water, after which it was stained in Ehrlich's hematoxylin for 10 min in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (1% HCl in 70% alcohol). The sections were then placed in running tap water for 10 min for blueing (slow alkalization). The sections were counter stained in 1% aqueous eosin (1 gm in 100 ml tapwater) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was ensured by placing the sections in the incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mountant having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip). The tissue morphology was observed with low power objective under light microscope. The cell injury and other aspects were observed under high power dry objective. The report was written and signed by ECHEONWU, Bobmanuel Chimaroke (*BMLS (Histopath), AMLSCN, MFSc.).*

3.2.13 Statistical Analysis

Statistical analysis was conducted, for the enzyme assay and lipid profile values using the one-way ANOVA and data were expressed as mean ± standard deviation (SD) of six values. The differences between the mean values of the test groups were analysed for statistical significance using one-way ANOVA. The differences between groups were considered to be significant if $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 The proximate Compositions of the leaves

The leaves of the plants;*Vernonia amygdalina, Ocimum gratissimum, Telfeiria ocidentalis, Ocimum canum* and *Amaranthus viridis* were found to be rich in protein, having percentage compositions of between 13.26 ± 0.01% in *Ocimum canum* to 25.09 ± 0.07% in *Amaranthus viridi* while *Pterocarpus santalinoides*has alow protein and fat content of 1.14±0.00% and 1.00±0.00%, respectively. All the leaves also have high ash content of above 11%, (see Table 4.2). Three of the leaves (*Vernonia amygdalina, Telfeiria occidentalis* and *Amaranthus viridis*) are rich in protein content, having above 20%.

Table 12: The proximate Compositions of the leaf of the plants

Sample	Moisture (%)	Ash (%)	Fats (%)	Fibre (%)	Protein (%)	Carbohydrates (%)
Ocimumgratissimum	10.73±0.05	13.36±0.10	14.15±0.03	6.40±.0.08	14.69±0.02	1.65±0.11
Amaranthusviridis	9.80±0.06	17.77±0.07	11.97±0.05	9.54±0.10	25.09±0.07	4.88±0.10
Terifeiria occidentalis	8.34±0.04	15.83±0.08	13.28±0.02	9.41±0.01	23.26±0.08	1.25±0.00
Vernonia amygdalina	9.83±0.01	10.84±0.02	12.99±0.02	3.47±0.00	22.85±0.05	8.85±.-07
Ocimum canum	10.76±0.02	14.60±0.04	14.54±0.01	2.15±0.00	13.26±0.01	2.69±0.11
Pterocarpus santalinoides	14.19±0.03	11.12±0.01	1.00±0.00	9.46±0.03	10.14±0.01	09.09±0.15

Values are mean ± SD of three (3) results

4.2 The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of the plants

The results from the phytochemical analysis of the aqueous and ethanol leaf extracts of the plants showed the distribution of the phytochemicals among the samples. Only tannin was found in all the leaf extracts, while other phytochemicals were differently distributed in the aqueous and ethanol leaf extracts of the plants. Sterol was present only in the aqueous leaf extracts of *Amaranthus viridis* and *Pterocarpus santalinoides* and the ethanol leaf extracts of P*terocarpus santalinoides* and *Amaranthus viridis* as shown on Table 12.

Table 13: The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of some leafy vegetables

Parameters	*Ocimum gratissimum*		*Amaranthus Viridis*		*Telfairia occidentalis*		*Vernonia amygdalina*		*Ocimum canum*		*Pterocarpus santalinoides*	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Alkaloid	-	-	-	+	-	+	-	+	-	-	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	-	+	+	+	-	+	+	+
Cardic glycosides	-	+	+	+	-	+	+	-	-	+	-	+
Triterpenoid	-	+	-	+	+	+	+	+	-	+	-	+
Sterol	-	-	-	+	-	-	+	-	-	-	+	+
Terpenoid	-	+	+	+	-	+	-	+	+	+	-	-
Saponin	+	-	+	-	+	-	+	+	+	-	+	-
Balsam	-	-	+	+	-	+	-	+	-	+	-	+
Cardenolides	-	-	+	-	+	-	-	-	-	-	-	-
Resin	-	-	+	-	-	-	-	-	-	-	-	+
Phlobatanni	+	+	-	-	+	-	+	-	+	+	-	-
Glycoside	+	+	+	-	-	+	+	+	+	+	-	-

+ … indicates presence; - … indicates absence

4.3 The percentage quantitative phytochemical compositions of aqueous extracts of some leafy vegetables

The percentage quantitative tannin, alkaloid, saponin and phenol compositions of aqueous extracts of *Vernonia amygdalina, Ocimum gratissimum, Telfeiria ocidentalis, Pterocarpus santalinoides, Ocimum canum* and *Amaranthus viridis* leaf, showed that *Telfeiria ocidentalis* has the highest percentage content of tannin. *Vernonia amygdalina* has the lowest tannin content while *Amarantus viridis* has no tannin and alkaloid as shown in Figure10. Saponin is present in all the samples. Alkaloid and phenol were not found in *Telfeiria ocidentalis* and *Ocimum canum*.

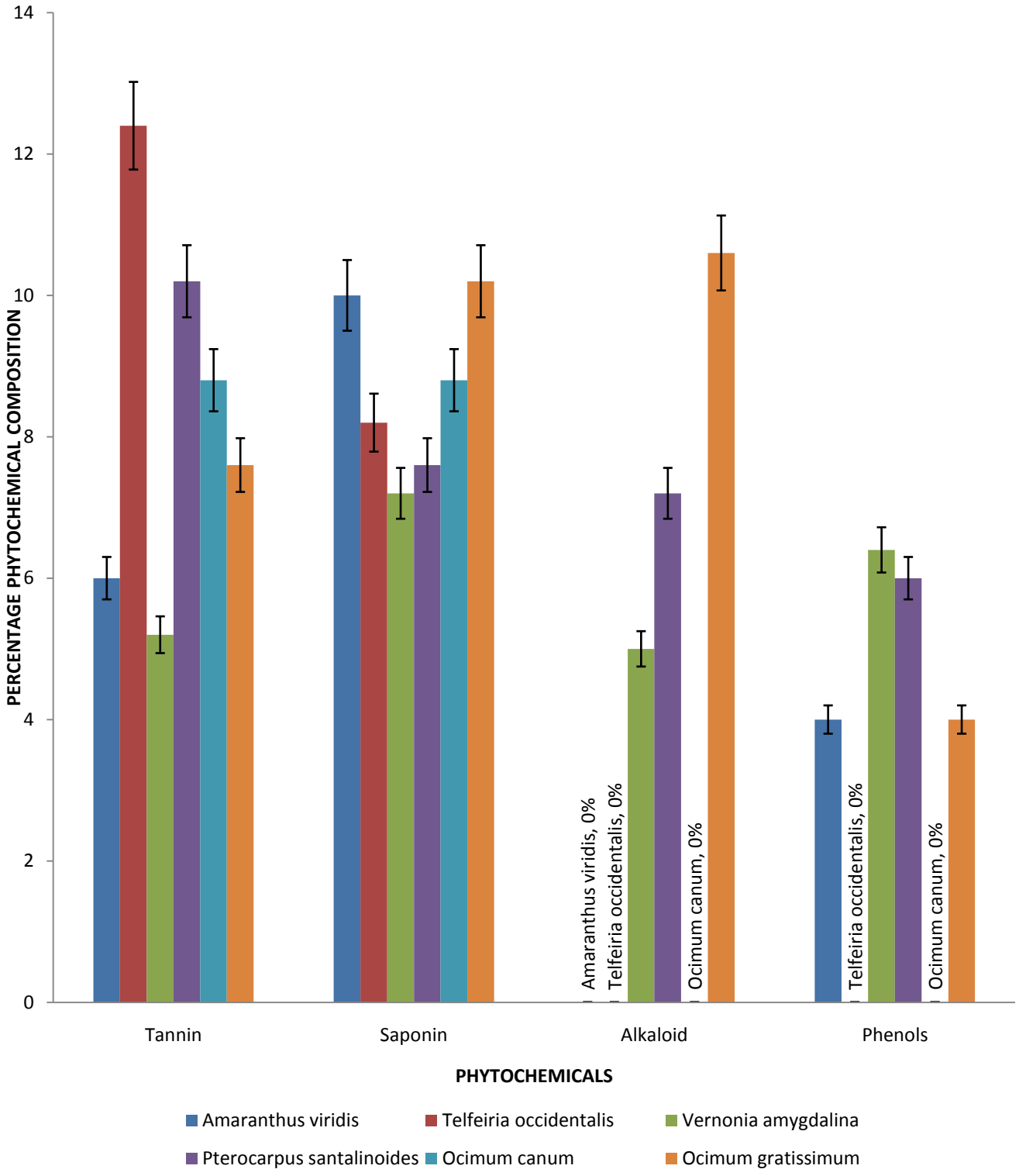


Figure 10: The percentage quantitative phytochemical compositions of aqueous extracts of some leafy vegetables

4.4 The percentage quantitative phytochemical compositions of ethanol extracts of some leafy vegetables

The ethanol extracted a sizable quantity (5.6% - 8.4%) of tannin from all the plants. Phenol was also extracted from all the plants, although it was as low as 2.8% and 4.8% in *Vernonia amygdalina* and *Ocimum gratissimum*, respectively, but as high as 13.4% in *Pterocarpus santalinoides* (Figure 11).

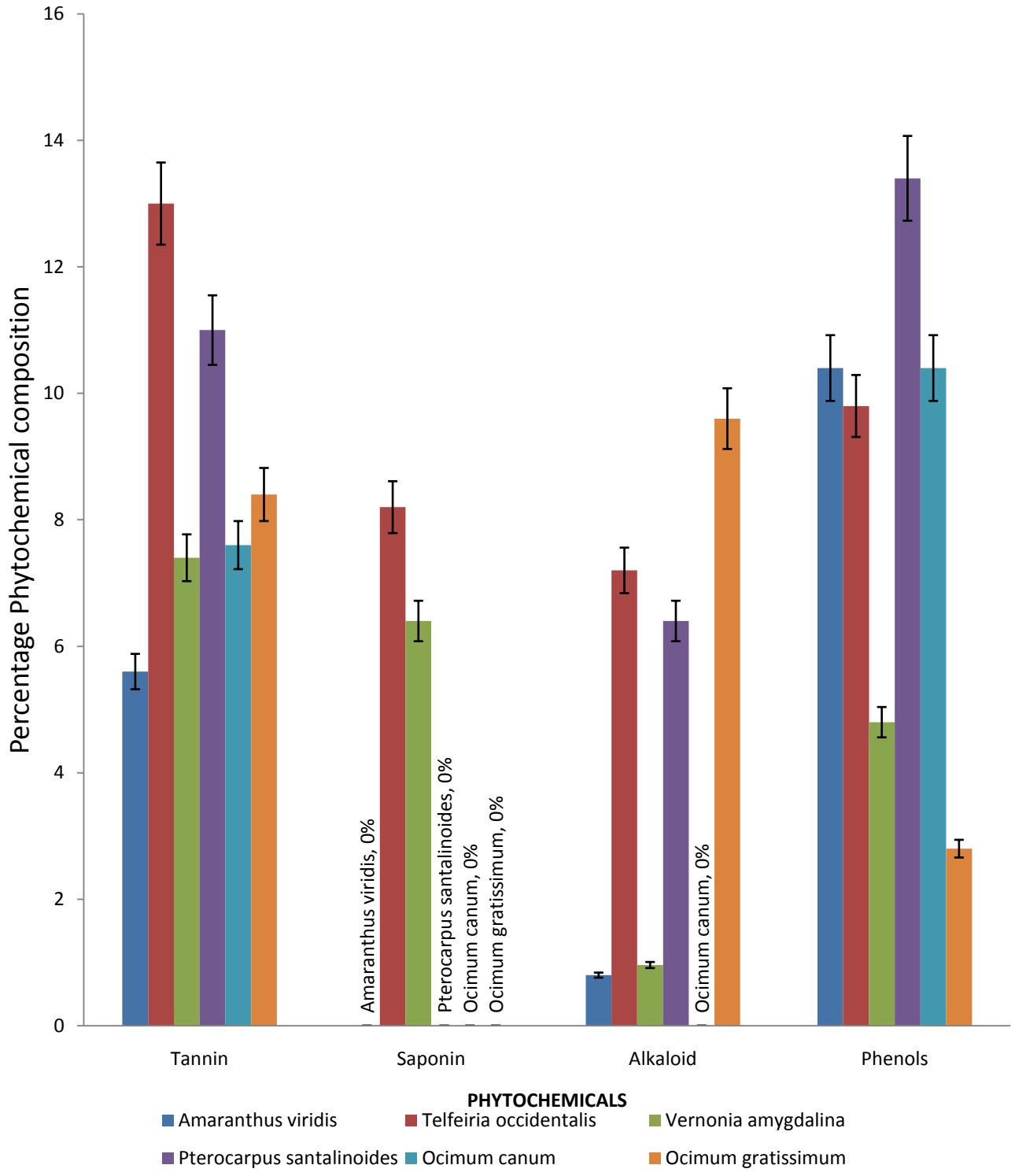


Figure 11: The percentage quantitative phytochemical compositions of ethanol extracts of some leay vegetables

4.5: Free Radical Scavenging Activity

A look at the (DPPH), scavenging activity of aqueous extracts of the leaves used in this study using Ascorbic acid as standard, showed that there was an increasing scavenging activity with increase in concentrations of the leaf extracts (Figure 12). All the extracts had antioxidant property comparable to that of Ascorbic acid at a concentration of 0.5mg/L.

The ethanol leaf extracts of the plants studied showed no defined parttern of increase or decrease in DPPH-scavenging activity in relation to increase in the concentrations of the extracts. The common decimal however, is the decrease in DPPH-scavenging activity at 0.25mg/L to 0.5mg/L and thatat all the concentrations considered in this study, the ethanol leaf extracts of these plants had very low DPPH-Scavenging potential compared with Ascorbic acid, (Figure 13).

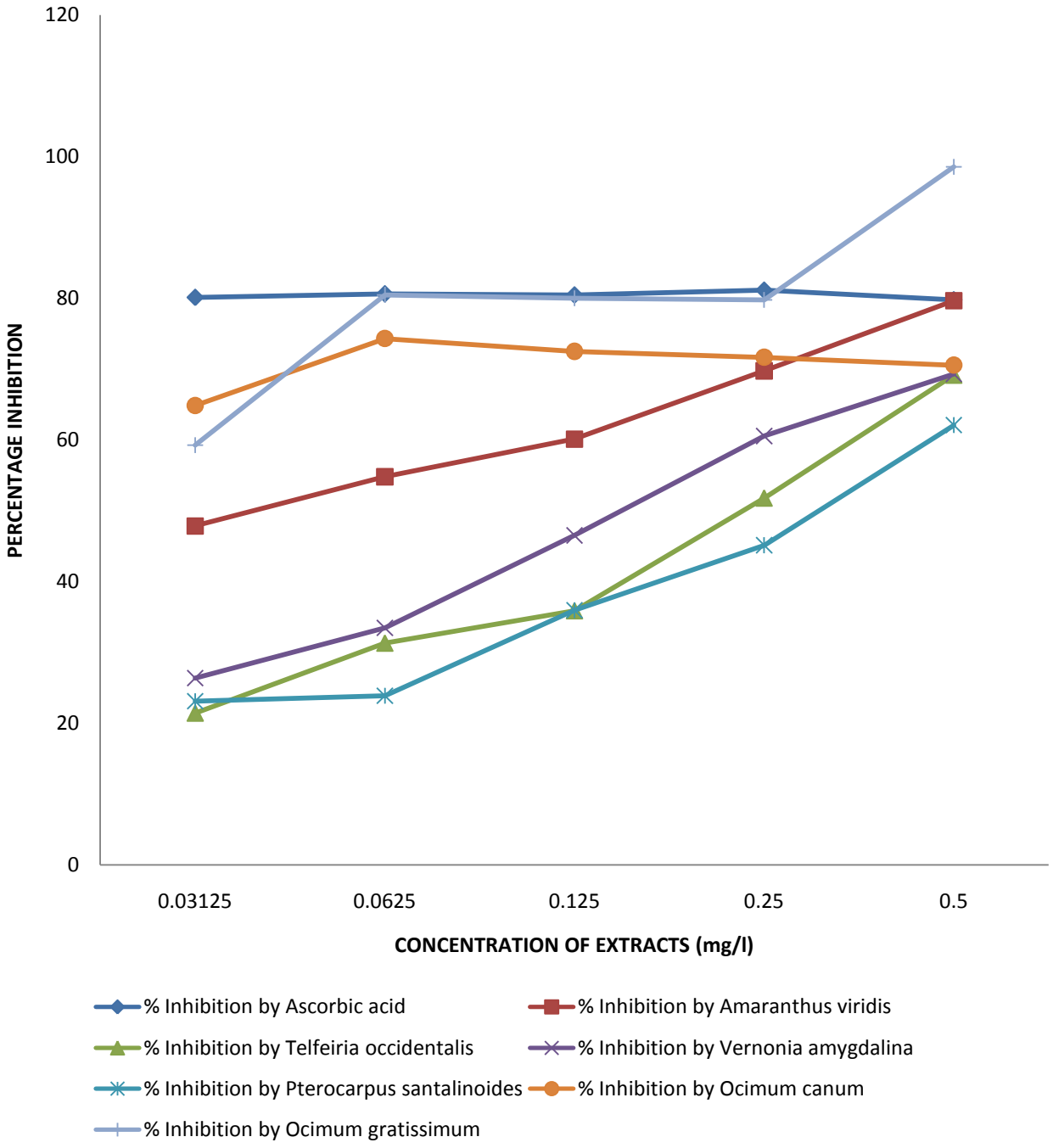


Figure 12: Comparing the free radical, (DPPH), scavenging activity of aqueous extracts of some leaves using Ascorbic acid as standard.

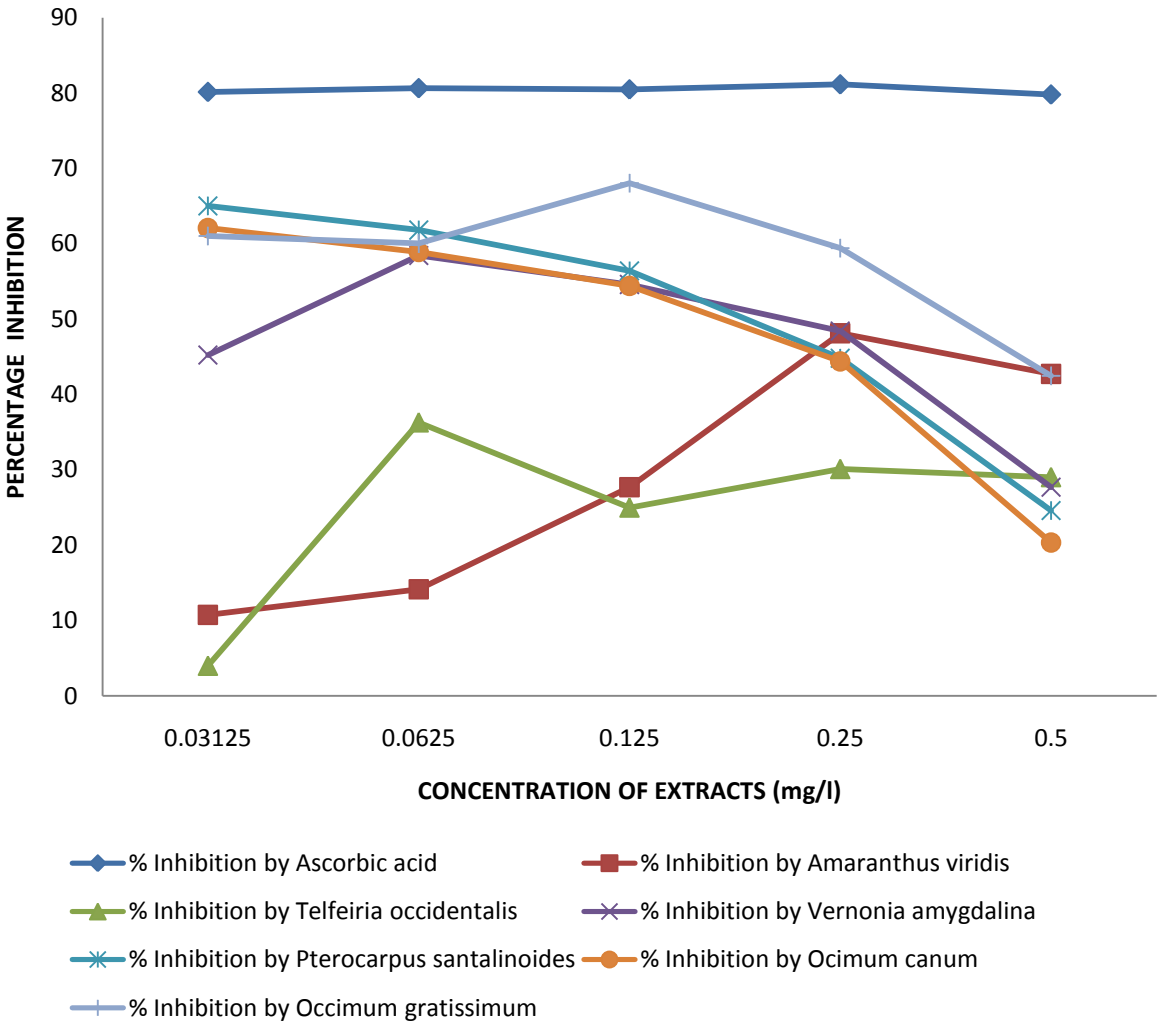


Figure 13: Comparing the free radical, (DPPH), scavenging activity of ethanol extracts of some leaves using Ascorbic acid as standard.

4.6: The effect of pre-treatment with 400gm/kgbw aqueous leaf extracts of some leafy vegetables on the serum activities of AST, ALT and ALP of rats intoxicated with 2g/kgbw single dose of acetaminophen.

The oral administration of a single 2g/kgbwdose of acetaminophen led to a marked increase in the serum activities of the ALT, AST and ALP of the animals in the negative control group compared to the normal control group (Tables 14 and 15). The groups pre-treated with 400mg/kgbw of aqueous leaf extracts of the plants showed significantly ($p<0.05$) decreased activity of ALT, insignificant change in the serum activity of ALP and significant increase in serum activity of AST of the animals, as shown on Table 14.

Table 14: The effect of pre-treatment with 400gm/kgbw aqueous leaf extracts of some leafy vegetables on the serum activities of AST, ALT and ALP of rats intoxicated with 2g/kgbw single dose of acetaminophen.

TREATMENT	AST (u/l)	ALT (u/l)	ALP (u/l)
Normal control	41.90±3.30	27.90±8.20	86.30±58.80
Standard control	42.80±10.80	17.90±3.10	167.30±107.70
Negative control	67.40±26.40^{++}	117.30±57.50^{++}	209.80±67.00^{++}
Amaranthus viridis	144±19.97*	45.53±18.53**	210.33±171.59
Telfairia occidentalis	111.33±11.15*	35.60±5.82**	185.33±26.35
Vernonia amygdalina	118.00±13.00*	9.05±3.76**	104.00±56.00**
Pterocarpus santalinoides	102.82±9.50*	51.75±9.60**	47.67±6.00**
Ocimum canum	147.00±4.48*	66.33±14.58**	201.00±51.88
Ocimum gratissimum	133.90±46.71*	41.00±5.79**	150.50±110.29**

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control. AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phoshatase

4.7: The effect of pre-treatment with 400gm/kgbw ethanol leaf extracts of some leafy vegetables on the serum activities of AST, ALT and ALP of rats intoxicated with 2g/kgbw single dose of acetaminophen.

The 400mg/kgbwethanol leaf extracts of the vegetables;*Vernonia amygdalina, Ocimum gratissimum, Telfeiria ocidentalis, Pterocarpus santalinoides, Ocimum canum* and *Amaranthus viridis*elicited significant ($p<0.05$) decrease in the activity of ALT, insignificant change in the serum activity of ALP and significant increase in serum activity of AST of the animals, as shown on Table 4.4.

Table 15: The effect of pre-treatment with 400gm/kgbwethanol leaf extracts of some leafy vegetables on the serum activities of AST, ALT and ALP of rats intoxicated with 2g/kgbw single dose of acetaminophen.

TREATMENT	AST (u/l)	ALT (u/l)	ALP (u/l)
Normal control	41.90±3.30	27.90±8.20	86.30±58.80
Standard control	42.80±10.80	17.90±3.10	167.30±107.70
Negative control	$67.40\pm26.40^{++}$	$117.30\pm57.50^{++}$	$209.80\pm67.00^{++}$
Amaranthus viridis	131.3±14.57*	73.4±14.03	33.5±19.09 **
Telfairia occidentalis	125.33±2.31*	55.93±6.76**	211.67±13.20
Vernonia amygdalina	74.73±11.97	29.27±13.59**	104.67±25.01**
Pterocarpus santalinoides	182.75±44.90*	20.03±13.50**	201.00±42.80
Ocimum canum	142.60±51.81*	57.70±25.08**	129.00±25.36
Ocimum gratissimum	135.62±14.57*	27.34±5.47**	115.20±61.38**

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control.

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

ALP = Alkaline phoshatase

4.8: **The effect of pre-treatment with 400gm/kgbw aqueous and ethanol leaf extracts of some leafy vegetables on the serum concentrations of total proteien, albumin, and bilirubin of rats intoxicated with 2g/kgbw single dose of acetaminophen.**

The oral administration of a single 2g/kgbw dose of acetaminophen to the rats elicited a significant ($p < 0.05$) decrease on the serum albumin concentration of the rats. However this intoxication caused insignificant ($p > 0.05$), change on the serum concentrations of total protein, total bilirubin, direct bilirubin and indirect bilirubin (Tables16 and 17).

The 7day pre-treatment of the animals with both aqueous and ethanol leaf extracts of the plants elicited significant ($p < 0.05$) increase on the serum concentrations of the albumin of some of the animals, see Tables 16 and 17.

Table 16: The effect of pre-treatment with 400gm/kgbw aqueous leaf extracts of some leafy vegetables on the serum concentrations of total proteien, albumin, and bilirubin of rats intoxicated with 2g/kgbw single dose of acetaminophen.

TREATMENT	Total Protein(g/dl)	Albumin (g/dL)	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/dl)	Indirect. Bilirubin(mg/dl)
Normal control	75.10±5.70	18.67±5.00	12.50±2.70	5.30±1.40	5.90±0.70
Standard control	76.80±10.50	4.70±2.30	9.00±5.20	6.50±2.90	4.00±3.30
Negative control	62.80±1.20	4.00±1.42^{--}	16.40±4.20	4.30±2.50	7.20±1.90
Amaranthus viridis	69.63±5.95	14.67±6.11*	15.83±21.74	11.97±16.92	3.87±4.90
Telfeiria Occidentalis	76.70±8.21	13.33±4.62*	15.40±9.91	18.33±16.20*	16.17±7.30
Vernonia amygdalina	61.16±11.94	6.00±2.00	11.80±2.00	2.65±0.75	9.15±2.75
Pterocarpus santalinoides	76.15±14.80	14.00±2.30*	3.97±1.80**	2.47±1.80**	1.50±1.10**
Ocimum canum	77.57±12.48	10.67±2.31*	8.87±1.96	1.20±0.35	4.67±0.83
Ocimum gratissimum	68.57±5.48	5.50±4.73	25.03±18.82	5.75±1.29	5.20±2.00

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control.

Table 17: The effect of pre-treatment with 400gm/kgbwethanol leaf extracts of some leafy vegetables on the serum concentrations of total proteien, albumin, and bilirubin of rats intoxicated with 2g/kgbw single dose of acetaminophen.

TREATMENT	Total Protein(g/dl)	Albumin (g/dL)	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/dl)	Indirect. Bilirubin(mg/dl)
Normal control	75.10±5.70	18.67±5.00	12.50±2.70	5.30±1.40	5.90±0.70
Standard control	76.80±10.50	4.70±2.30	9.00±5.20	6.50±2.90	4.00±3.30
Negative control	62.80±1.20	4.00±1.42^{--}	16.40±4.20	4.30±2.50	7.20±1.90
Amaranthus viridis	80.73±6.49	10.67±2.31*	7.47±3.32**	1.87±1.45**	3.47±1.08**
Telfeiria Occidentalis	81.93±1.10*	13.33±2.30*	3.03±0.70**	1.83±0.21**	1.30±0.36**
Vernonia amygdalina	78.48±11.29	12.00±6.93*	18.43±5.40	6.13±4.52	8.97±6.81
Pterocarpus santalinoides	77.15±10.40	16.00±5.70*	2.75±0.90**	1.9±1.10**	1.76±1.10**
Ocimum canum	75.00±2.65	13.33±2.31*	3.63±1.85**	2.33±1.60	0.83±0.58**
Ocimum gratissimum	67.02±4.54	7.80±5.40	10.36±4.81**	5.64±3.49	4.72±2.81**

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control.

4.9: The effect of pre-treatment with 400mg/kgbw of leaf extracts of some leafy vegetables on the serum lipid profile of rats intoxicated with a 2g/kgbw single dose of acetaminophen.

A single 2g/kgbw dose of acetaminophen orally administered to the rats did not cause a significant Change on the serum concentration of the lipids [Triacyglycerol (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Total Cholesterol, (T. chol)], of the rats.

The leaf extracts however, caused different degrees of effects on the serum lipid profile of the animals in the test groups, see Tables 18 and 19.

Table 18: **The effect of pre-treatment with 400mg/kgbw of aqueous leaf extracts of some leafy vegetables on the serum lipid profile of rats intoxicated with a 2g/kgbw single dose of acetaminophen.**

SAMPLES	T. Chol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	T.G (mmol/L)
Normal Control	1.30±0.30	0.40±0.01	0.70±0.30	1.00±0.40
Standard Control	1.70±1.00	0.30±0.01	1.40±0.70	1.00±0.20
Negative Control	1.50±0.30	0.50±0.20	1.30±0.50	1.00±0.30
Amaranthus viridis	1.46±0.65	0.45±0.04	0.31±0.08**	0.95±0.59
Telfairia occidentalis	2.13±0.57	0.29±0.05**	0.70±0.36	0.68±0.27
Vernonia amygdalina	1.32±0.34	0.26±0.00	0.74±0.39	0.72±0.11
Pterocarpus santalinoides	*2.53±1.80*	*0.35±0.09*	*1.62*±1.40	1.25±0.82
Ocimum canum	1.81±0.51	0.30±0.09	1.02±0.18	0.41±0.20**
Ocimum gratissimum	1.22±0.39	0.30±0.05**	0.58±0.40**	1.04±0.48

Values are mean ± SD of six (6) results, ** shows values with significant decrease ($p<0.05$) compared to the Negative control.

T.chol. = Total cholesterol, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, TG = Triacyl glycerol.

Table 19: **The effect of pre-treatment with 400mg/kgbw ofethanol leaf extracts of some leafy vegetables on the serum lipid profile of rats intoxicated with a 2g/kgbw single dose of acetaminophen.**

SAMPLES	T. Chol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	T.G (mmol/L)
Normal Control	1.30±0.30	0.40±0.01	0.70±0.30	1.00±0.40
Standard Control	1.70±1.00	0.30±0.01	1.40±0.70	1.00±0.20
Negative Control	1.50±0.30	0.50±0.20	1.30±0.50	1.00±0.30
Amaranthus viridis	1.19±0.12	0.35±0.15	0.28±0.04**	0.35±0.15**
Telfairia occidentalis	0.80±0.06**	0.26±0.00**	0.27±0.15	0.72±0.11
Vernonia amygdalina	2.75±1.98	0.38±0.13	2.07±1.84	0.66±0.21
Pterocarpus santalinoides	1.69±0.50	0.32±O.10	1.25±0.50	0.55±0.10**
Ocimum canum	2.99±0.40*	0.28±0.04**	2.03±0.17*	0.47±0.12**
Ocimum gratissimum	1.82±0.76	0.38±0.17**	1.19±0.60	0.89±0.12

Values are mean ± SD of six (6) results, ** shows values with significant decrease ($p<0.05$) compared to the Negative control.

T.chol. = Total cholesterol, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, TG = Triacyl glycerol.

4.10: The effect of pre-treatment with 400mg/kgbw of leaf extracts of some leafy vegetables on the activities of the liver antioxidant enzymes of rats intoxicated with a 2g/kgbw single dose of acetaminophen

Acetaminophen overdose (2g/kgbw), reduced significantly ($p<0.05$) the serum activities of the SOD and GPx in the negative control group. The serum activities of CAT of the was minimally affected by the intoxication with the stated dose of acetaminophen compared to the negative control group.

However, pre-treatment of the animals with 400mg/kgbw of the leaf extracts of the plants used in this study caused significant ($p< 0.05$) increase in activities of SOD, CAT and GPx of most of the test animals. Tables 20 and 21 are a depiction of the results of the effect of pre-treatment with 400mg/kgbw of aqueous and ethanolleaf extracts of these leafy vegetables on the activities of the liver antioxidant enzymes of rats intoxicated with a 2g/kgbw single dose of acetaminophen

Table 20: The effect of pre-treatment with 400mg/kgbw of aqueous leaf extracts of some leafy vegetables on the activities of the liver antioxidant enzymes of rats intoxicated with a 2g/kgbw single dose of acetaminophen

SAMPLES	SOD (U/mg)	CAT (U/mg)	GPx (μ/mg)
Normal Control	0.05±0.01	3.00±2.30	397.00±100.00
Standard Control	0.20±0.40	20.90±14.90	184.60±23.60
Negative Control	0.02±0.01^{--}	3.00±2.60	115.60±10.03^{--}
Amaranthus viridis	0.03±0.01*	3.39±3.30	233.08±57.56*
Telfairia occidentalis	0.02±0.00	3.71±0.40	391.27±56.47*
Vernonia amygdalina	0.04±0.03*	13.56±11.42*	329.41±87.25*
Pterocarpus santalinoides	0.07±0.02*	7.39±1.24*	313.79±37.58*
Ocimum canum	0.01±0.00**	2.74±1.87	233.49±30.00*
Ocimum gratissimum	0.06±0.03*	20.10±9.86*	376.70±47.20*

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control.

SOD = Superoxide Dismutase

CAT = Catalase

GPx = Glutathione Peroxidase

Table 21: The effect of pre-treatment with 400mg/kgbw of aqueous leaf extracts of some leafy vegetables on the activities of the liver antioxidant enzymes of rats intoxicated with a 2g/kgbw single dose of acetaminophen

SAMPLES	SOD (U/mg)	CAT (U/mg)	GPx (µ/mg)
Normal Control	0.05±0.01	3.00±2.30	397.00±100.00
Standard Control	0.20±0.40	20.90±14.90	184.60±23.60
Negative Control	0.02±0.01^{--}	3.00±2.60	115.60±10.03^{--}
Amaranthus viridis	0.04±0.01*	5.58±4.04	143.53±8.23*
Telfairia occidentalis	0.03±0.00*	4.15±0.78	192.53±31.51*
Vernonia amygdalina	0.03±0.01*	11.25±4.86*	249.12±98.50*
Pterocarpus santalinoides	0.07±0.02*	7.39±1.24*	313.79±37.58*
Ocimum canum	0.09±0.06*	6.54±1.95*	203.83±63.93*
Ocimum gratissimum	0.12±0.17*	11.21±16.03*	171.93±15.47*

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Negative control while $^{++}$ and $^{--}$ indicate values with significant increase and decreases respectively compared to the Normal control.

SOD = Superoxide Dismutase

CAT = Catalase

GPx = Glutathione Peroxidase

4.11: The effect of pre-treatment with 400mg/kgbw of leaf extracts of some leafy vegetables on the the histopathology of livers of rats intoxicated with a 2g/kgbw single dose of acetaminophen

The oral administration of 2g/kgbw dose of acetaminophen over nine (9), hours to pre-treated and untreated rats caused varying degrees of disruption of the hepatocellular architecture. Whilie the untreated animals had poorly preserved hepatocellular architecture,some of the pre-treated animals had well-preserved, moderately preserved to poorly preserved liver architecture. (Plates 8 to 22).

The rats that received no treatment apart from feed and water showed well-preserved hepatic lobular architecture, with normal appearing cords hepatocytes interspersed by hepatic sinusoids (arrow). A normal appearing central vein (CV) is also seen.(Plate 8)

The rats pre-treated with 400mg/kgbw of **Silymarin** showed well-preserved hepatic lobular architecture, with mild diffuse inflammation and increased kupffer cell population (as seen in the circle) and hepatocyte karyopyknosis (arrows) as seen below on plate 9

The rats intoxicated with 2g/kgbw single dose of acetaminophen (without any pre-treatment) showed poorly-preserved hepatic lobular architecture, showing marked sinusoidal dilatation (black arrows) and necrosis (blue arrows) as shown in Plate 10

The rats pre-treated with 400mg/kgbw aqueous leaf extract of *amaranthus viridis*, (Green Amaranth), leaf showed well-preserved hepatic lobular architecture with extensive areas of hepatocytes exhibiting karyopyknosis (arrows) was observed (massive hepatocyte necrosis), see Plate 11.

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Amaranthus viridis*, (Green Amaranth), leaf showed moderately preserved hepatic lobular structure, showing diffuse sinusoidal dilatation with mild chronic inflammation as shown in plate 12

The rats pre-treated with 400mg/kgbw aqueous leaf extract of *Telfeiria occidentalis*, (Fluted pumpkin), leaf showed well-preserved hepatic lobular architecture showing mild chronic inflammatory cell infiltrate (arrows) as shown in Plate 13

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Telfeiria occidentalis*, (Fluted pumpkin), leaf showed severely deranged hepatic lobular architecture showing derangement of the hepatic plate and hepatocyte necrosis and kupffer cells (circled area). Several hepatocytes with reactive nuclei (arrows) are also seen (Plate 14)

The rats pre-treated with 400mg/kgbw aqueous leaf extract of ***Vernonia amygdalina*, (Bitter leaf), leaf** showed hepatic lobular architecture in severe disarray showing extensive areas of haemorrhage, see Plate 15.

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Vernonia amygdalina*, (Bitter leaf), leaf showed moderately-preserved hepatic lobular structure, and hepatocytes with reactive nuclei (arrows). Mild diffuse chronic inflammation is also observed as shown in Plate 16

The rats pre-treated with 400mg/kgbw aqueous leaf extract of *Pterocarpus santalinoides*, (Red sandal wood), leaf showed well-preserved hepatic lobular structure with normal appearing hepatocytes as seen on Plate 17

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Pterocarpus santalinoides*, (Red sandal wood), leaf showed well-preserved hepatic lobular architecture, showing normal appearing hepatocytes. Few apoptotic hepatocytes are seen, Plate 18

The rats pre-treated with 400mg/kgbw aqueous leaf extract of *Ocimum canum*, (Curry), leaf showed well preserved hepatic lobular structure with normal appearing hepatocytes, see Plate 19

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Ocimum canum*,(Curry), leaf showed section of liver tissue that had moderately preserved hepatic lobular structure with minimal diffuse chronic inflammatory cellular infiltrate (arrows) – Plate 20

The rats pre-treated with 400mg/kgbw aqueous leaf extract of *Occimum graissimum*, (Scent leaf), leaf showed moderately-preserved hepatic lobular architecture, showing mild diffuse chronic inflammatory cell infiltrate and a few karyopyknotic hepatocytes (Plate 21)

The rats pre-treated with 400mg/kgbw ethanol leaf extract of *Occimum graissimum*, (Scent leaf), leaf showed well-preserved hepatic lobular architecture, showing diffuse moderate chronic inflammation (blue arrows) and sinusoidal dilatation (black arrows). See Plate 22

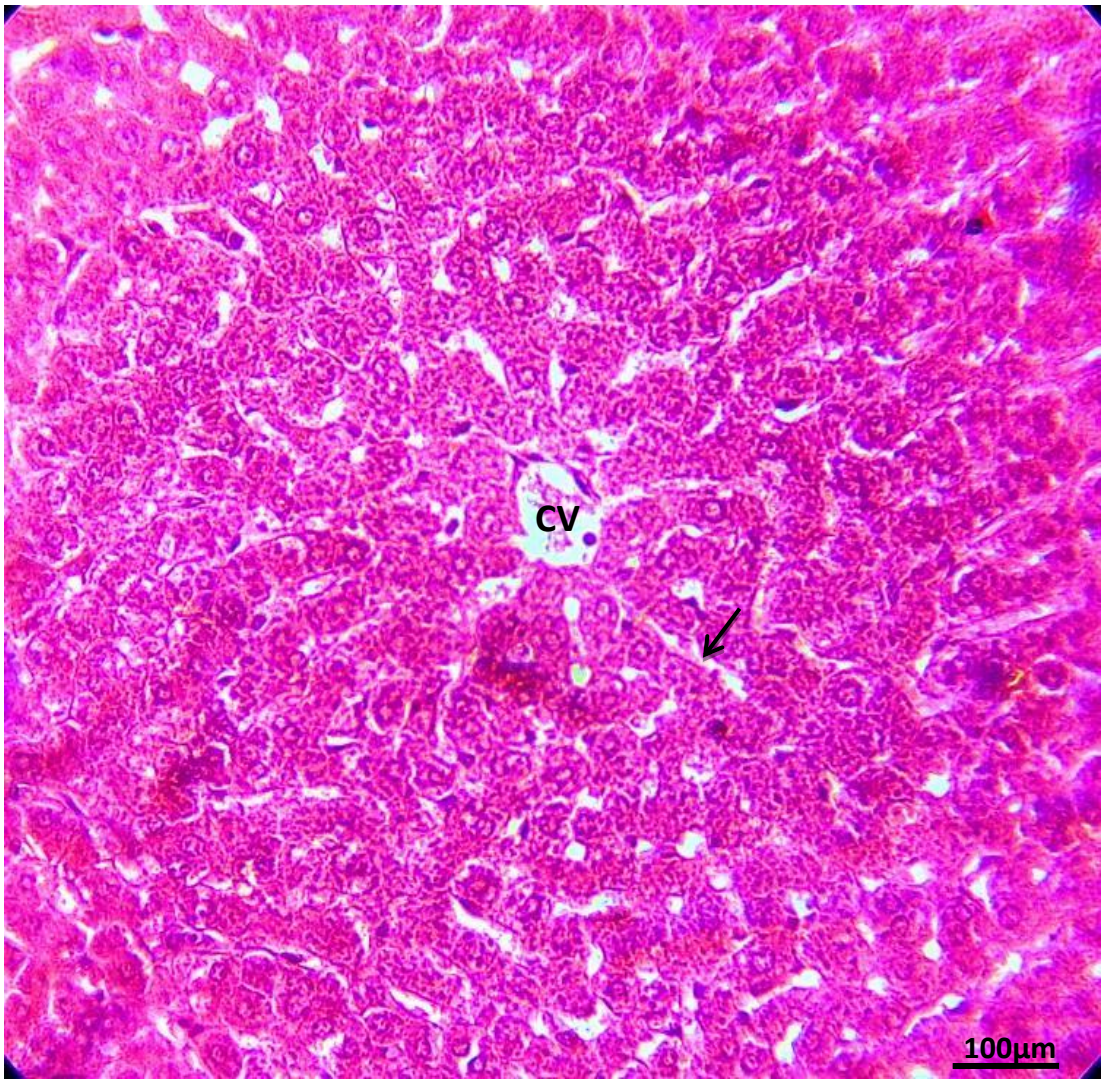


PLATE 8: The histological architecture of livers of rats used as Normal Control (X100)

- ***Haematoxylin and Eosin stain***

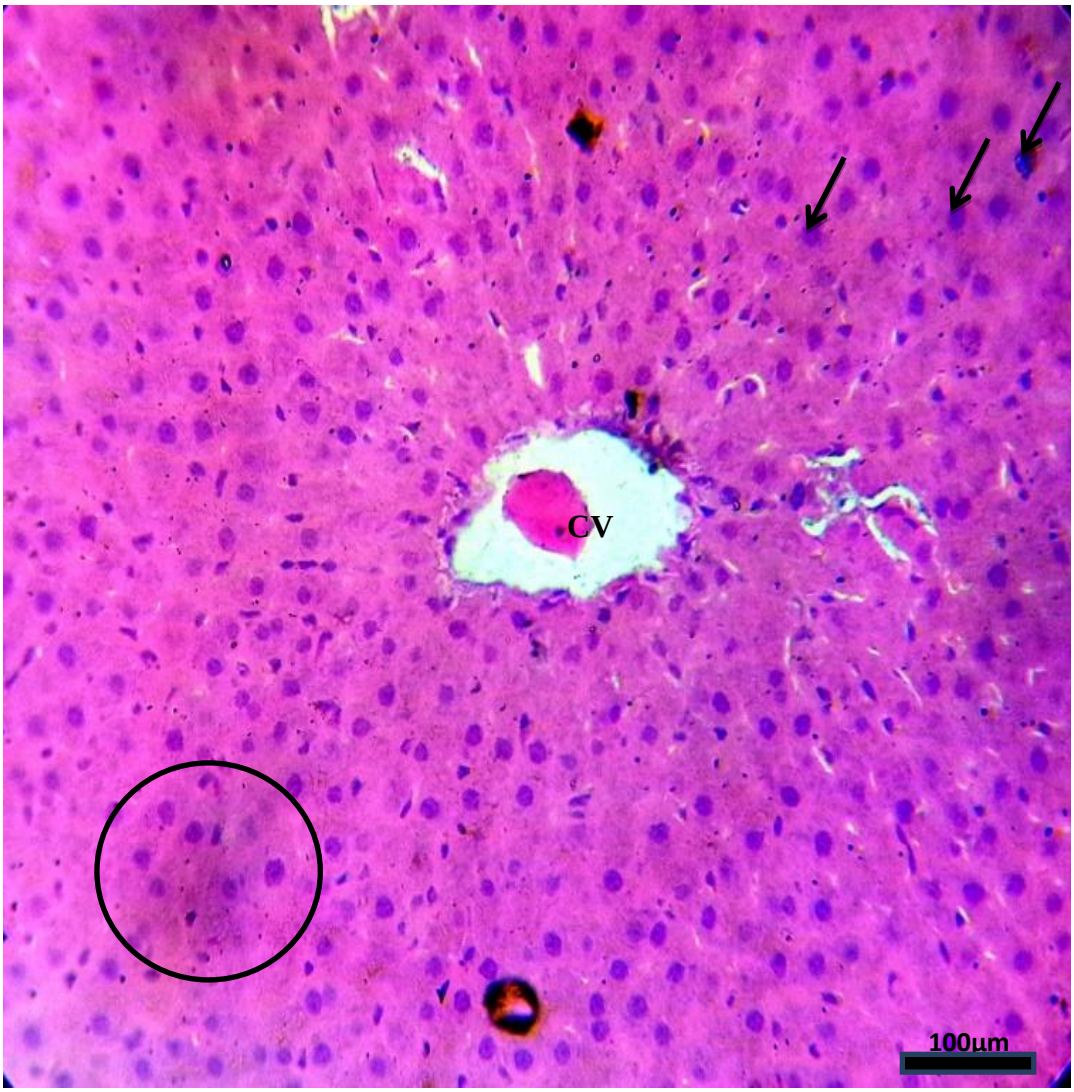


Plate 9: The effect of pre-treatment with Silymarin on the histological architecture of livers of rats administered with a single over-dose of acetaminophen, (X100) - ***Haematoxylin and Eosin stain.*** **CV** = central vein

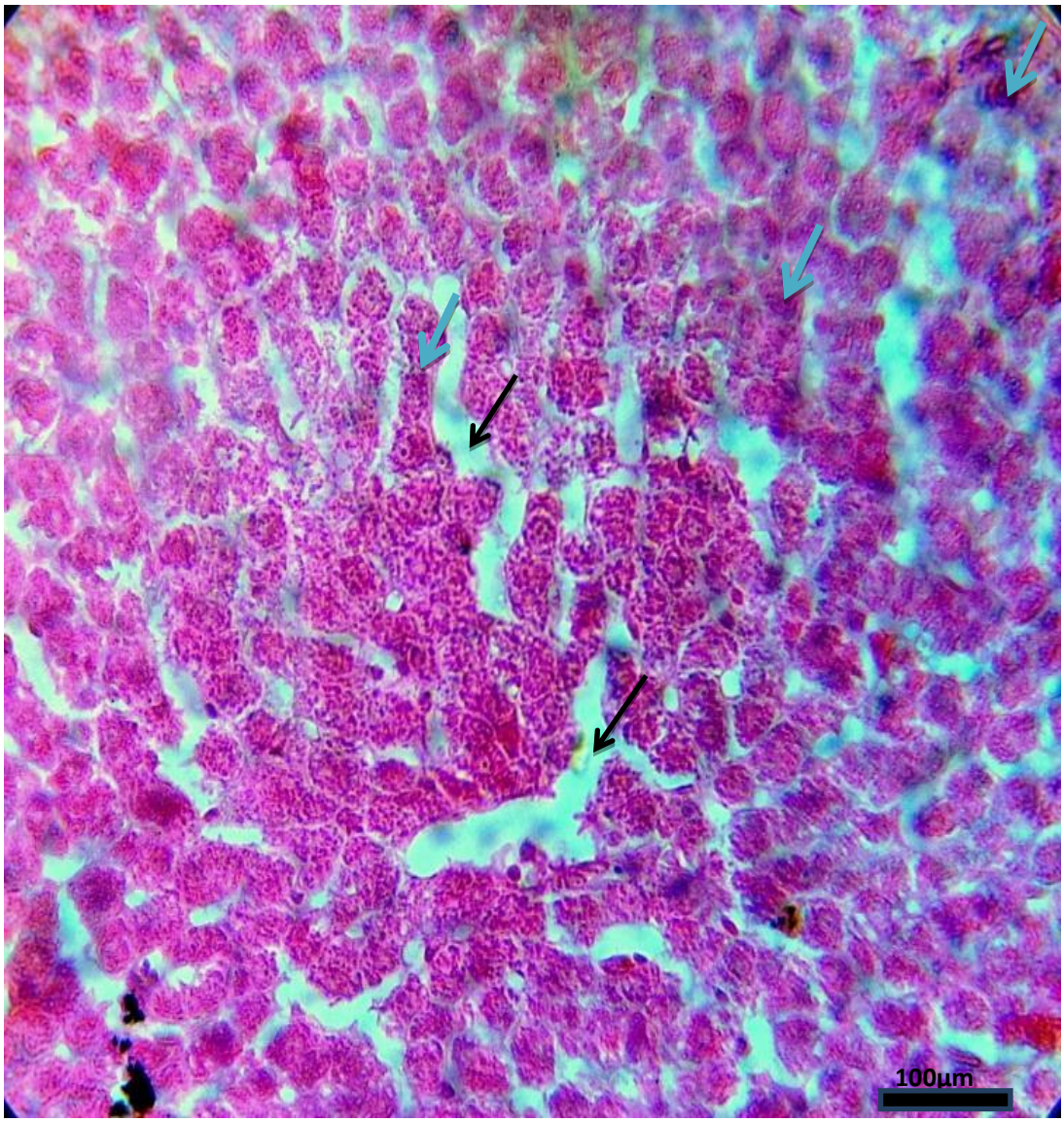


PLATE 10: The effect of a single over-dose of acetaminophen the histological architecture of livers of rats (Negative Control), (X100) - ***Haematoxylin and Eosin stain***

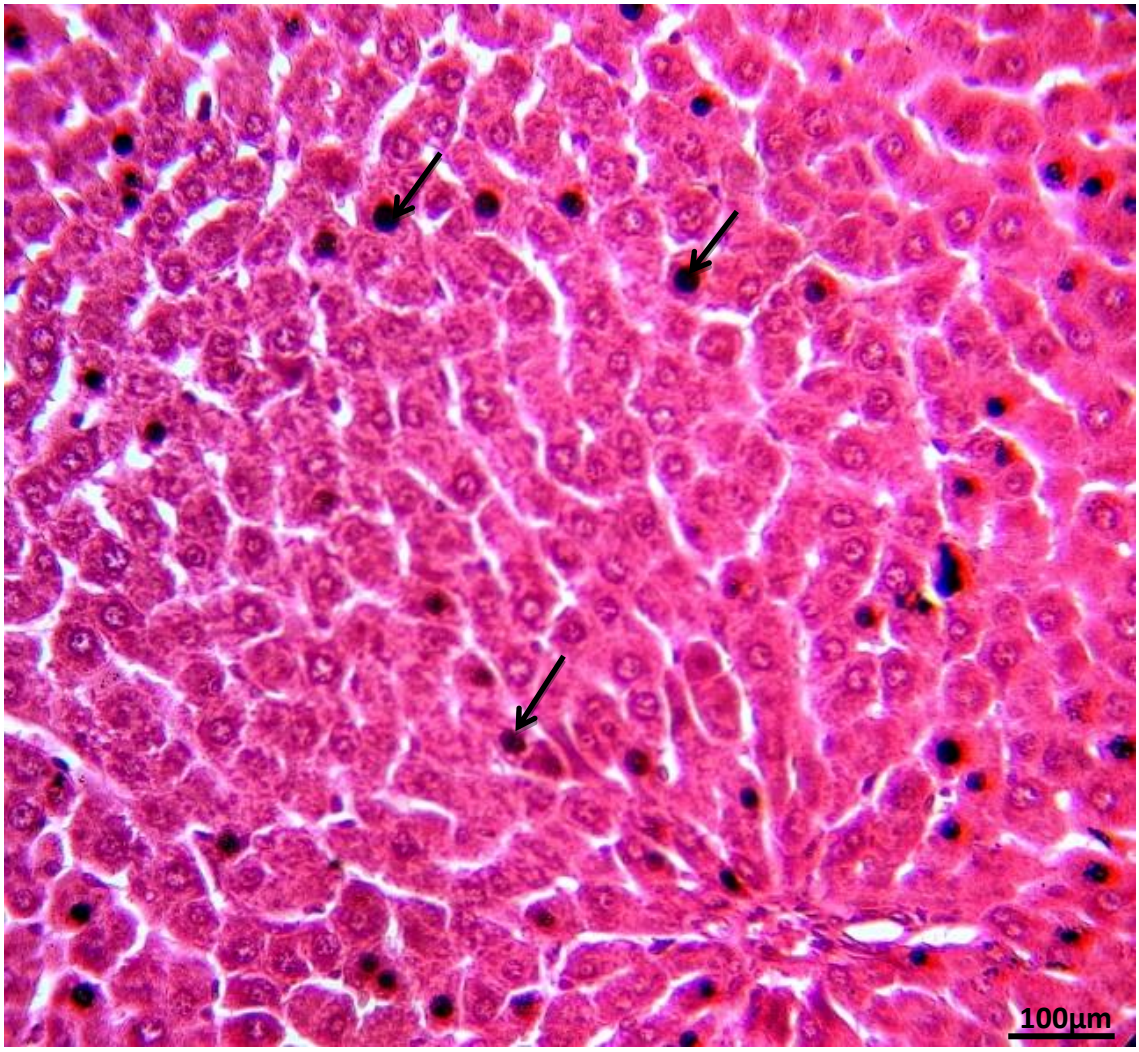


PLATE 11: The effect of pre-treatment with aqueous extract of *amaranthus viridis*, (Green Amaranth), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen, (X100) - ***Haematoxylin and Eosin stain***

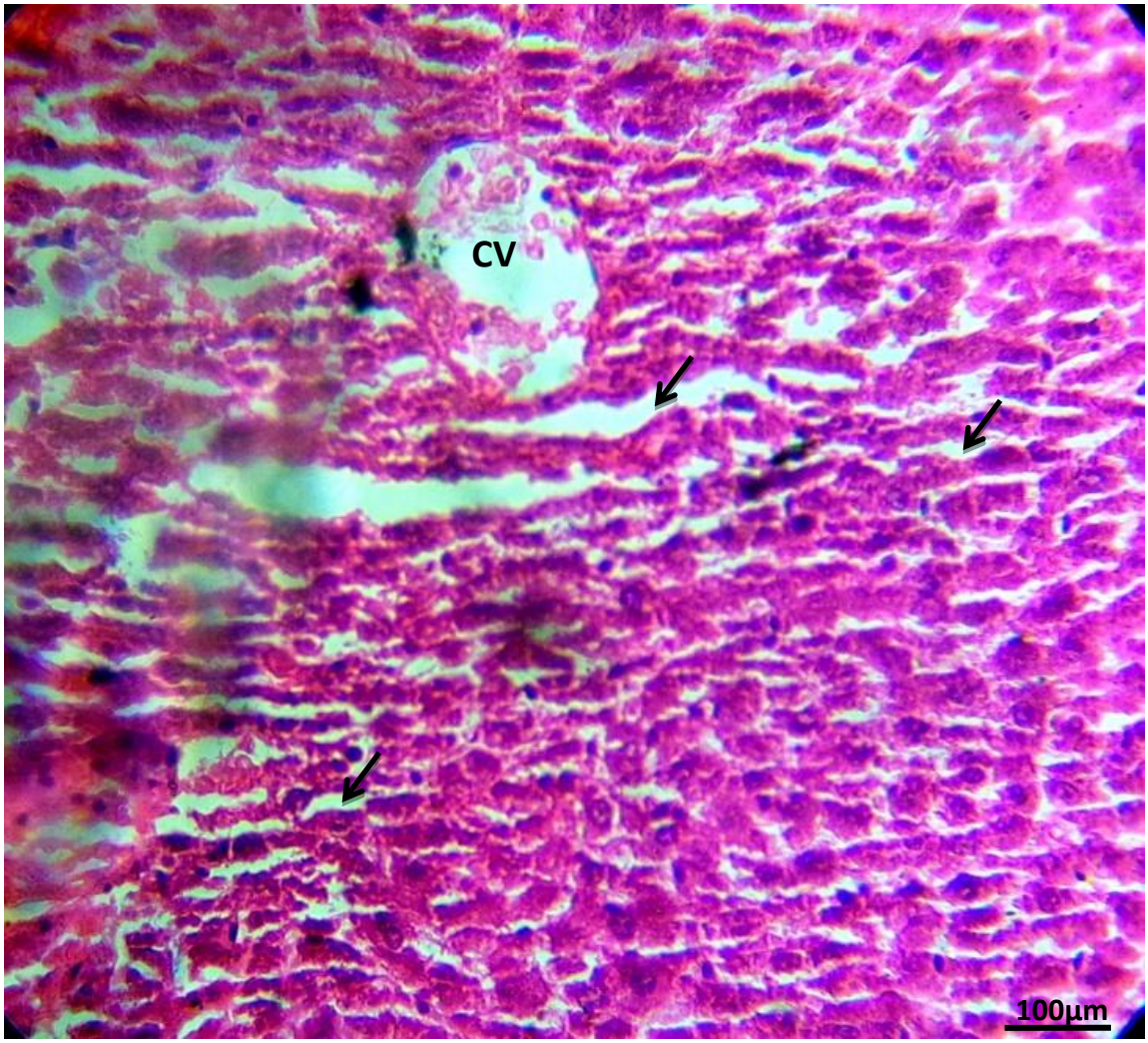


PLATE 12: The effect of pre-treatment with ethanol extract of *Amaranthus viridis*, (Green Amaranth), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

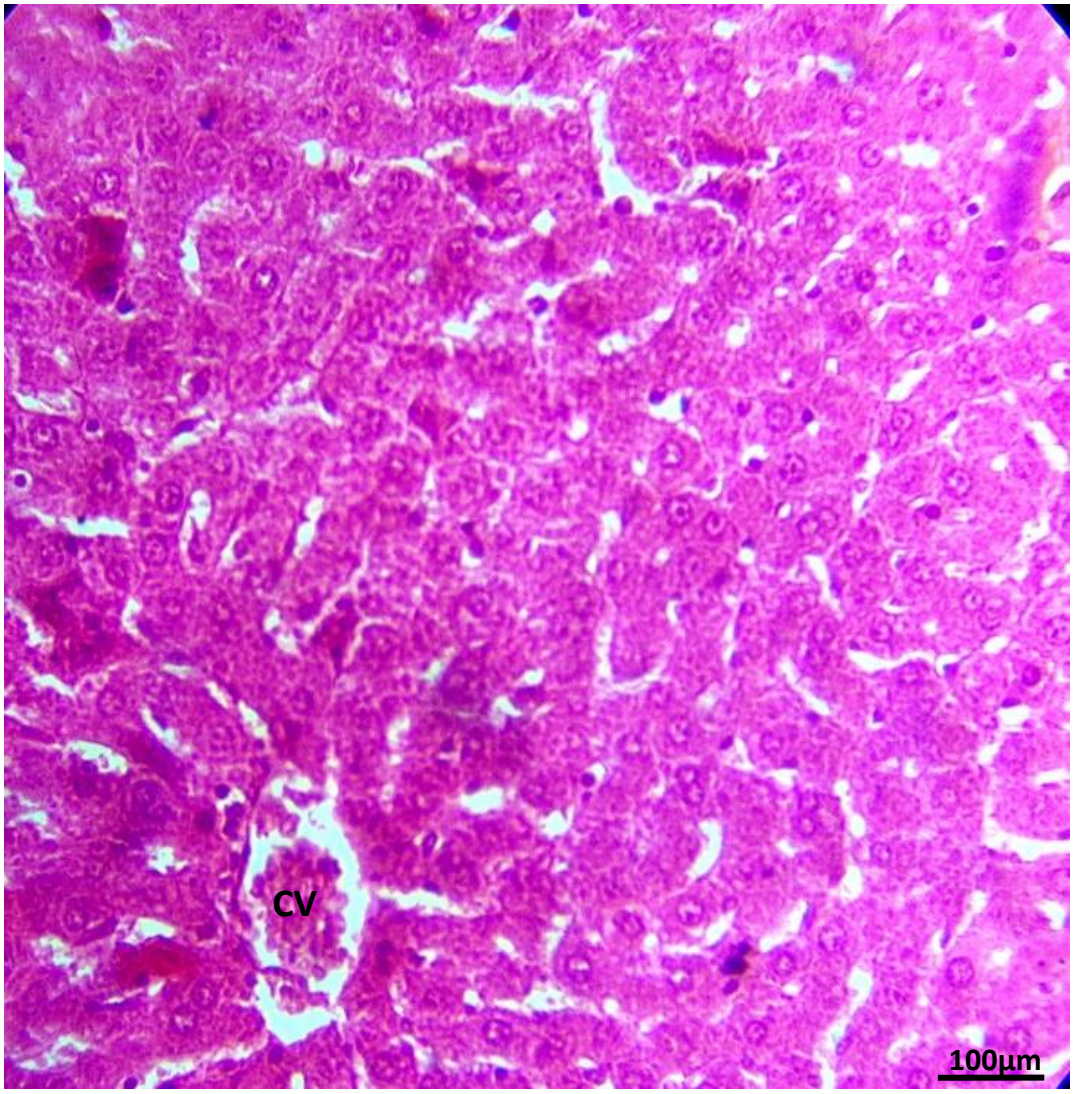


PLATE 13: The effect of pre-treatment with aqueous extract of *Telfeiria occidentalis*, (Fluted pumpkin), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain.***C.V = central vein.

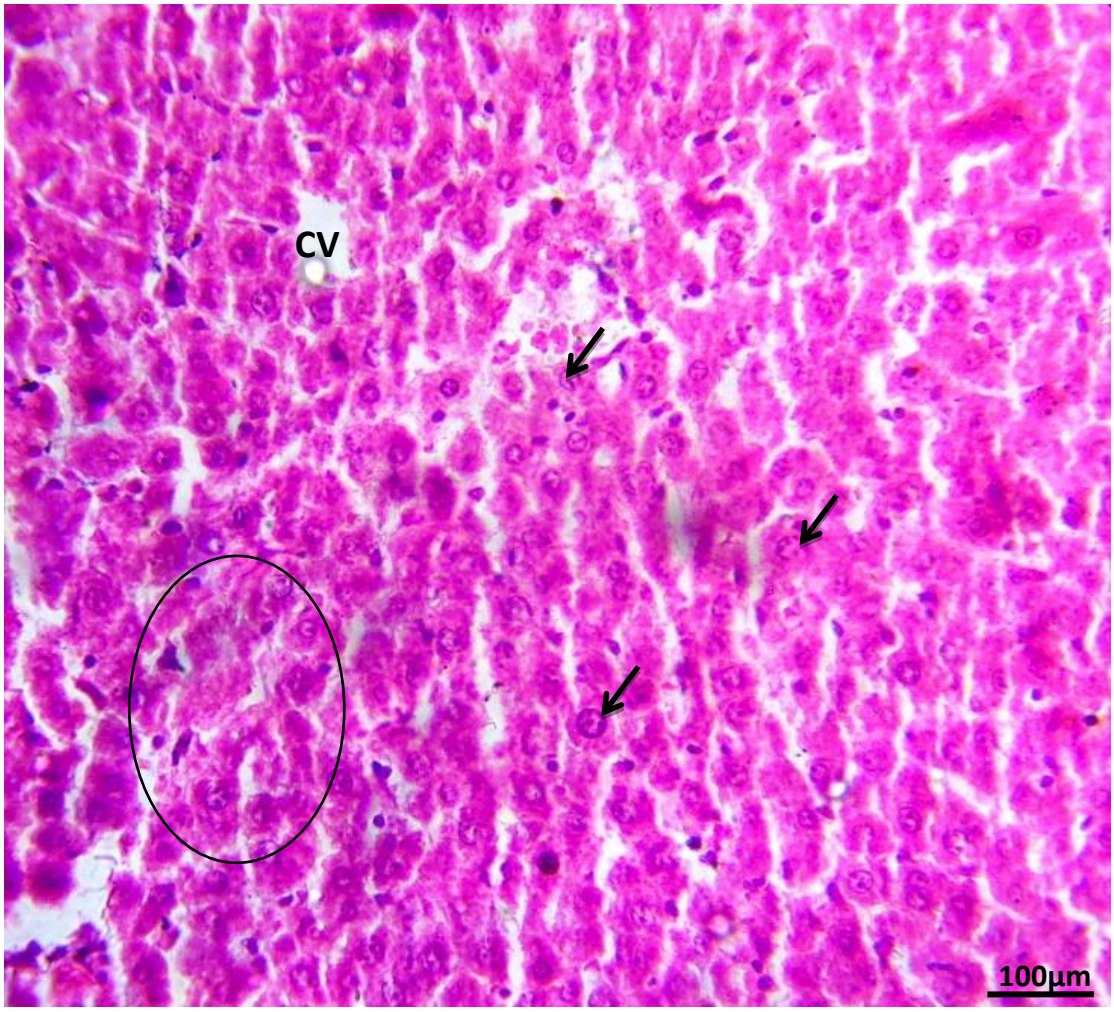


PLATE 14: The effect of pre-treatment with ethanol extract of *Telfeiria occidentalis*, (Fluted pumpkin), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

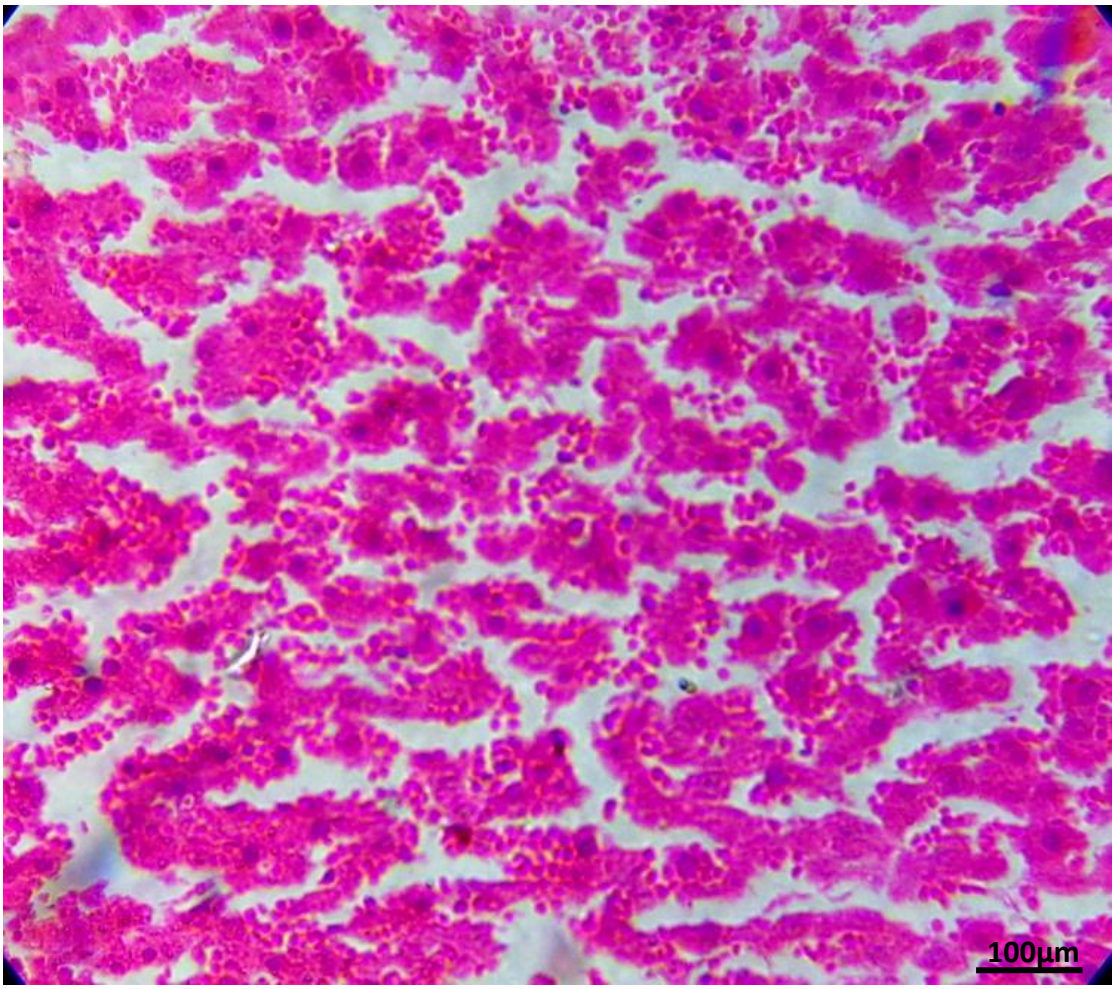


PLATE 15: The effect of pre-treatment with aqueous extract of *Vernonia amygdalina*, (Bitter leaf), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

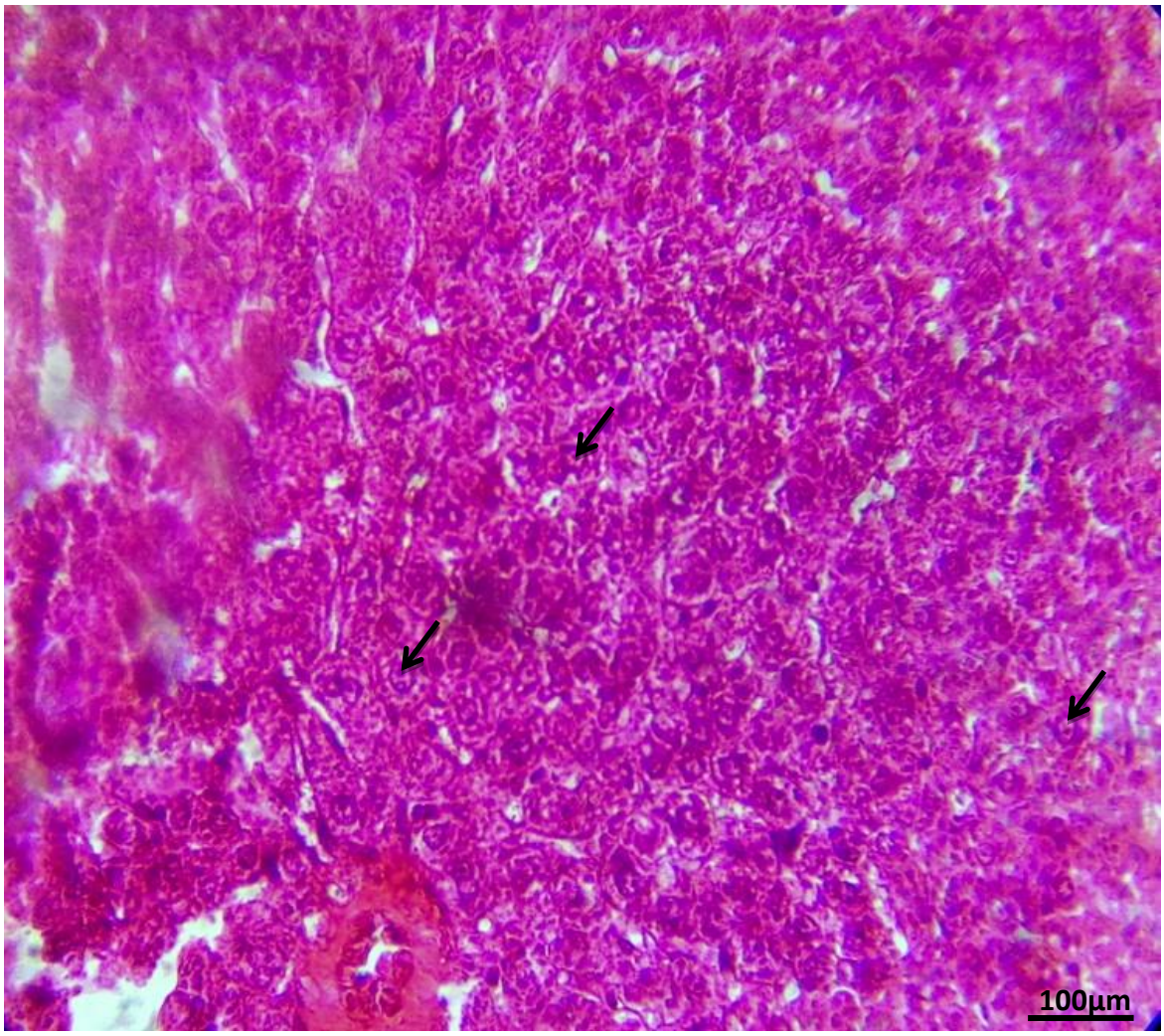


PLATE 16: The effect of pre-treatment with ethanol extract of *Vernonia amygdalina*, (Bitter leaf), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

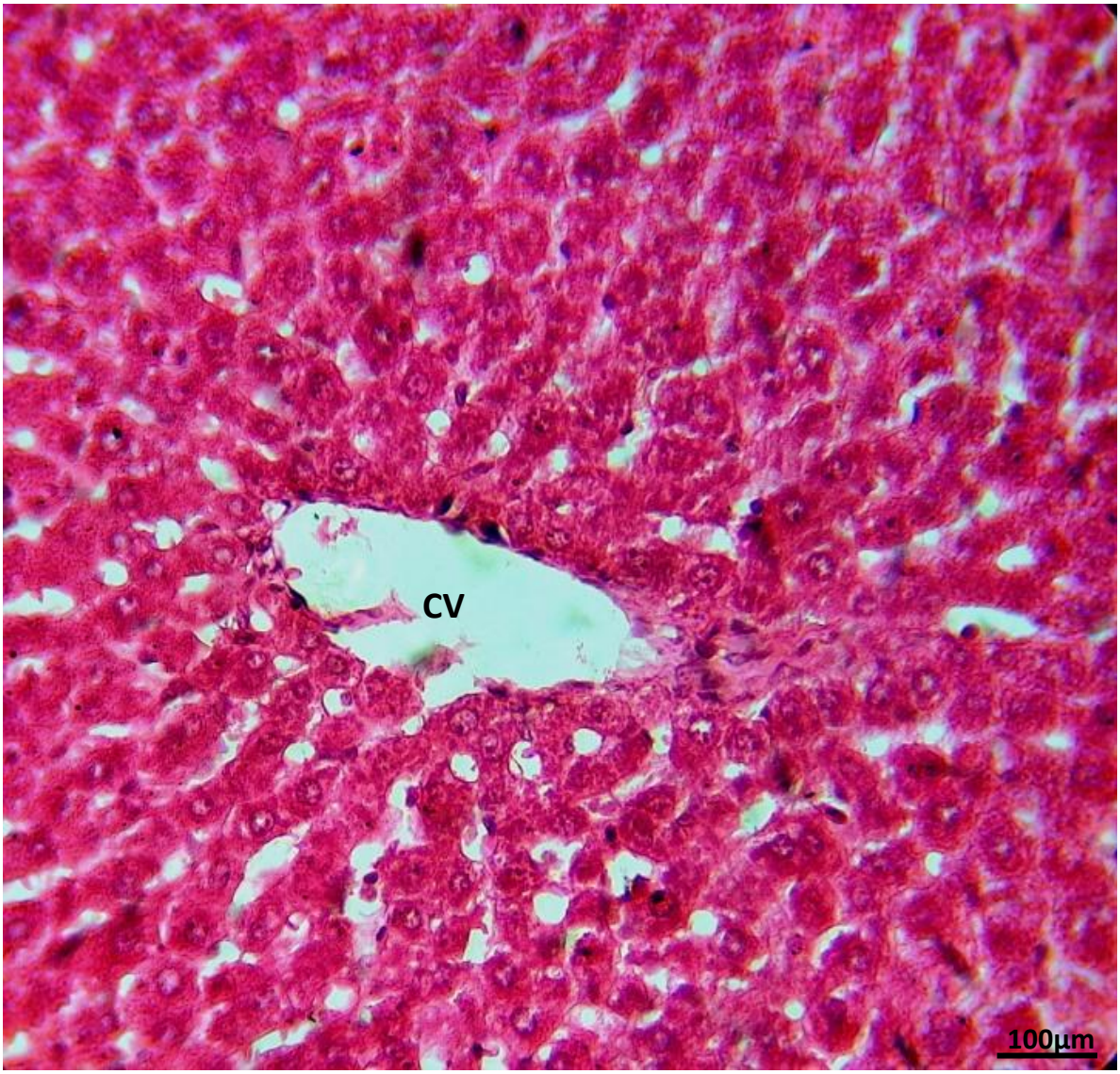


PLATE 17: The effect of pre-treatment with aqueous extract of *Pterocarpus santalinoides*, (Red sandal wood), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

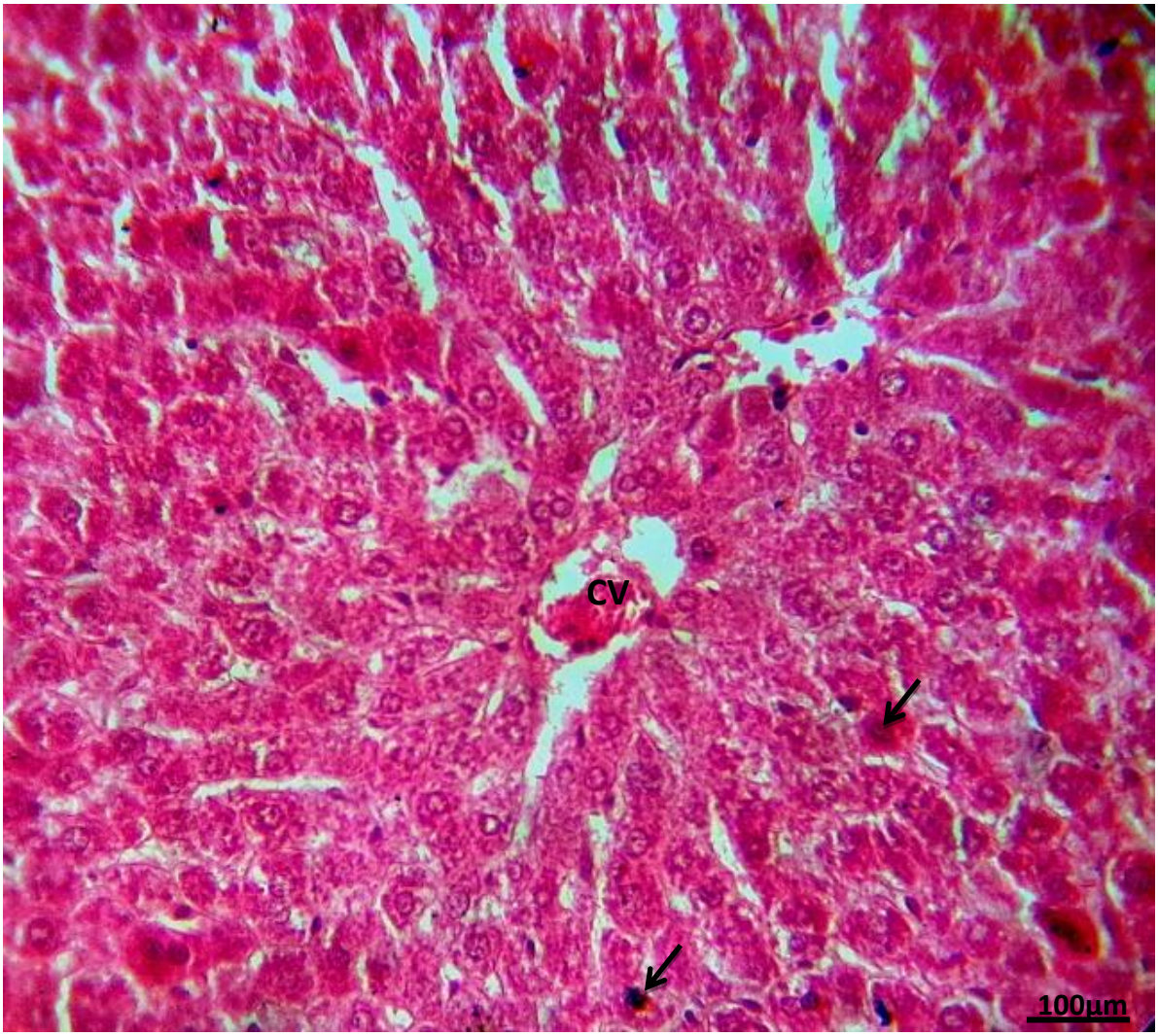


PLATE 18: The effect of pre-treatment with ethanol extract of *Pterocarpus santalinoides*, (Red sandal wood), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

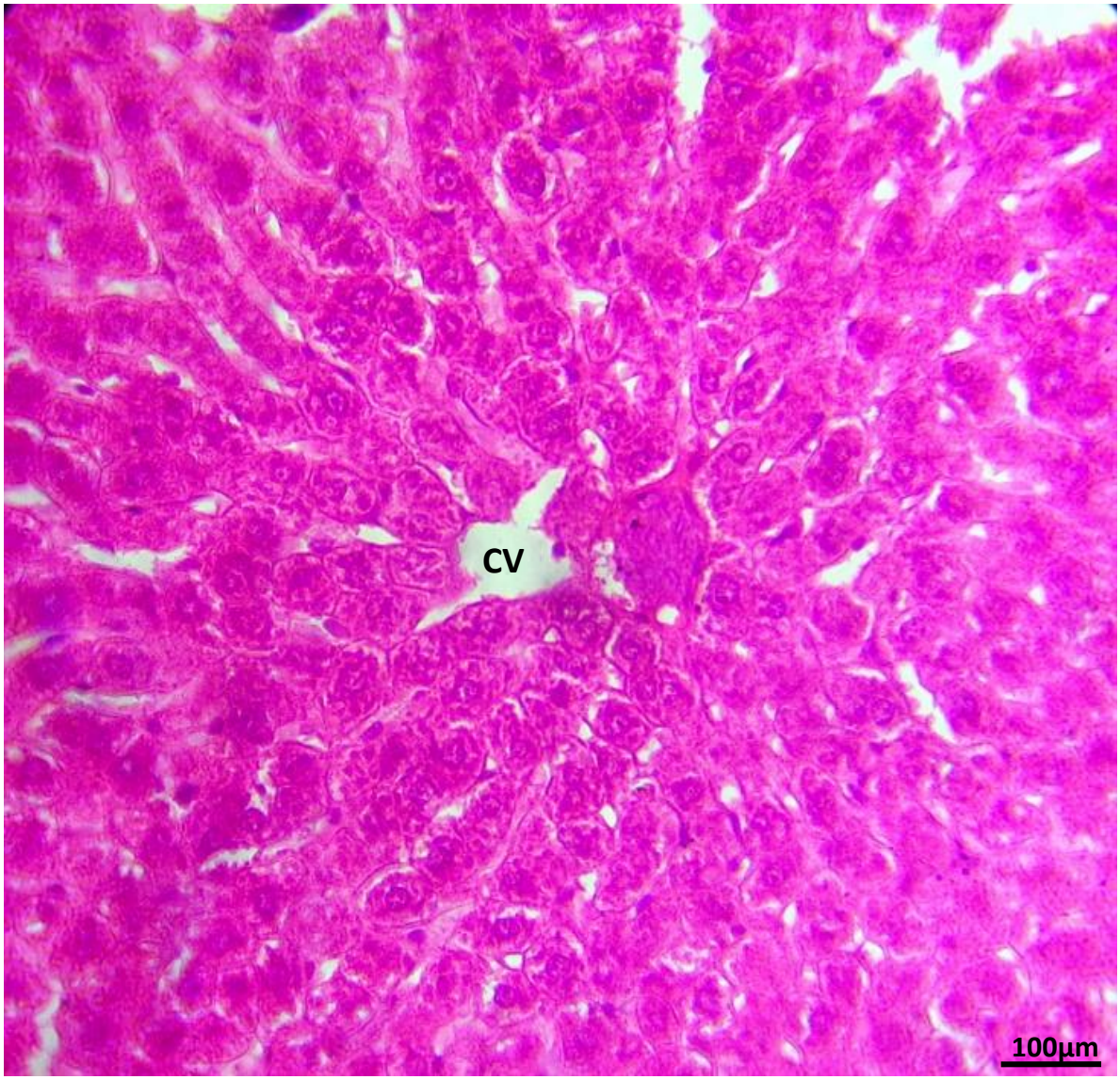


PLATE 19: The effect of pre-treatment with aqueous extract of *Ocimum canum*, (Curry), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

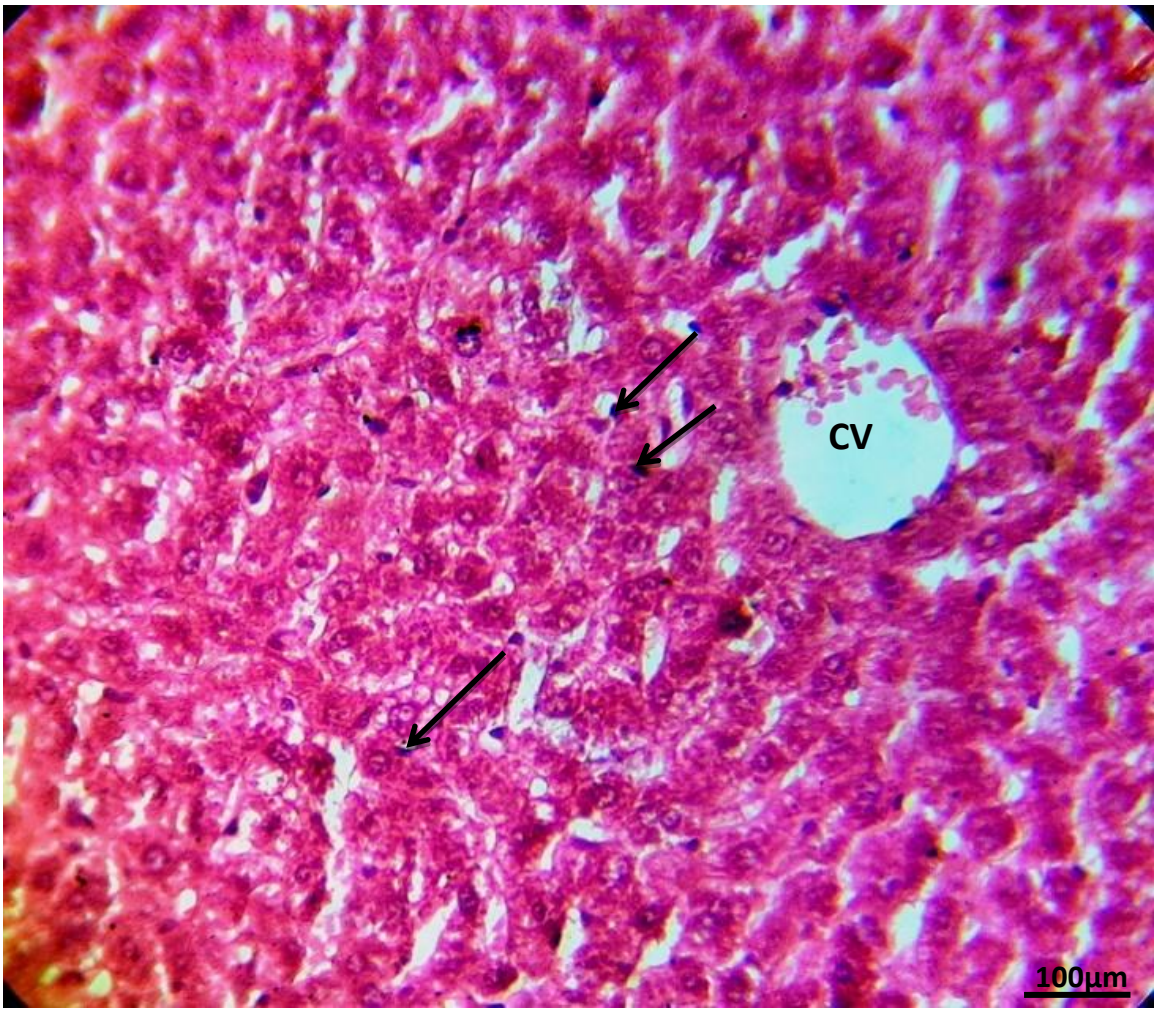


PLATE 20: The effect of pre-treatment with ethanol extract of *Ocimum canum*, (Curry), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

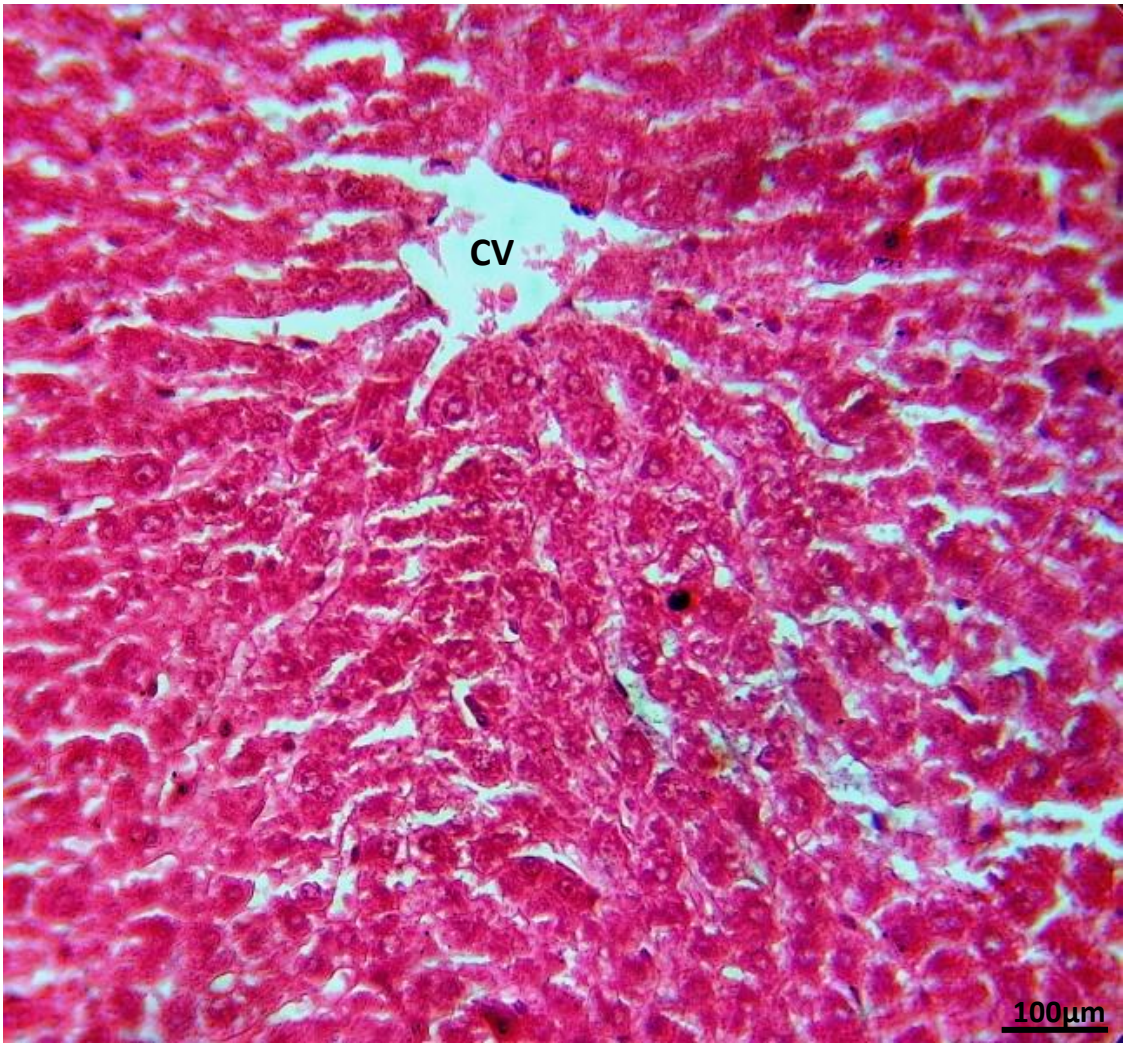


PLATE 21: The effect of pre-treatment with aqueous extract of *Occimum graissimum*, (Scent leaf), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen. (X100) - ***Haematoxylin and Eosin stain.***

C.V = central vein.

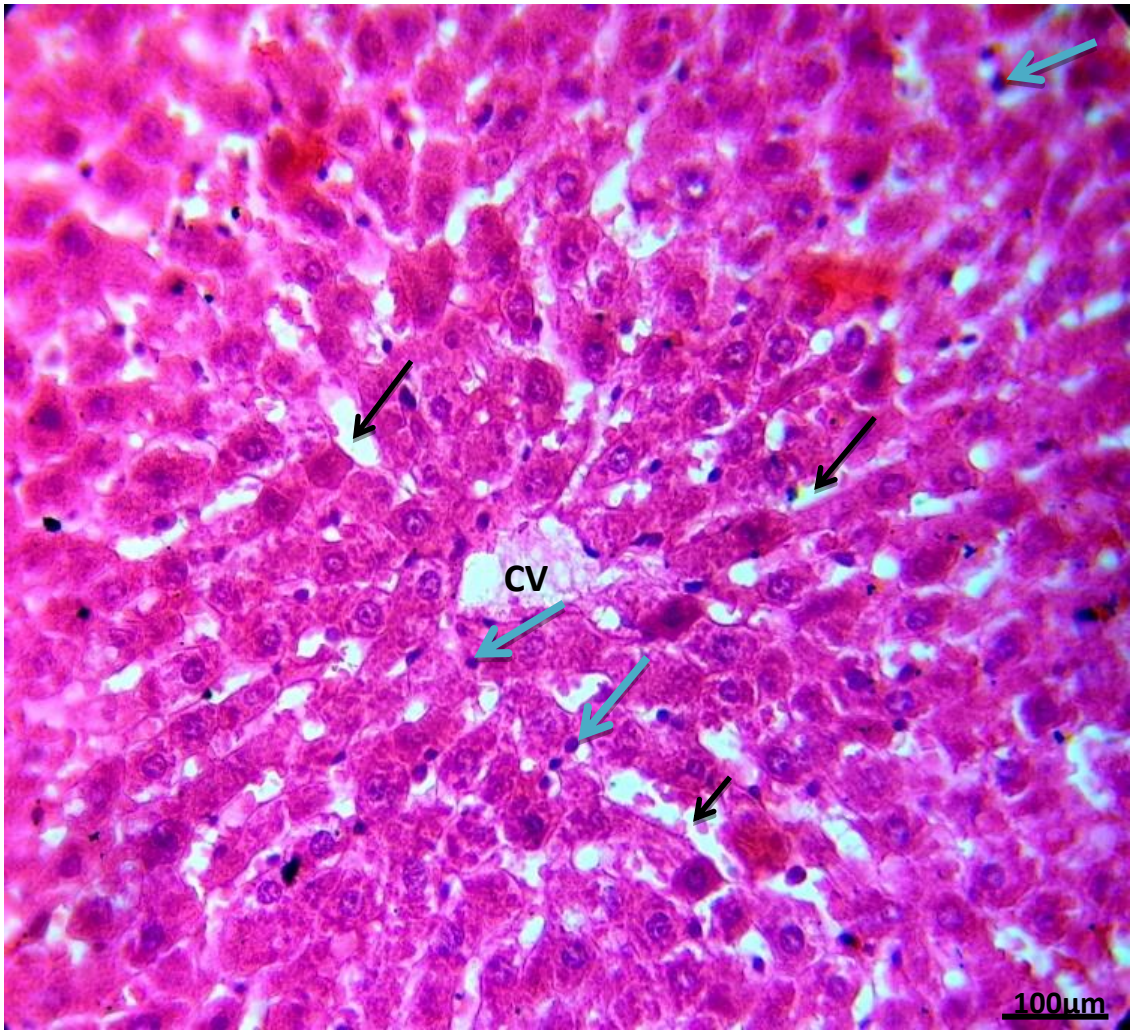


PLATE 22: The effect of pre-treatment with ethanol extract of *Occimum graissimum*, (Scent leaf), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) - ***Haematoxylin and Eosin stain*.**

C.V = central vein.

CHAPTER FIVE

DISCUSSION

The results from this research showed that the moisture contents of the vegetables under study ranged between 8.34 ± 0.04% for *T. occidentalis* to 14.19±0.03 in *T. santalinoides.* This compares with 10.74% obtained by Ndukwe and Ipkeama (2013) for *Pterocarpus santalinoides,* 12.21% reported by Javid *et al*., (2009) for *Amaranthus viridis* and 10% by Asaolu *et al.,* (2012), for *Amaranthus viridis,* in a study conducted on Nigerian Leafy Vegetables. Moisture content of the leafy vegetables is one of the indices that determine their shelf life. It is common knowledge that the risk of putrefaction is predicated on the proliferation of microbes (bacteria), evidenced by the growth of moulds or self-digestion by enzymes in the feed when moisture in the feed is more 15%. The relatively low moisture content of the dried leaf samples (maximum of 14.19% (in *Pterocarpus santalinoides*) would hinder the growth of microorganism and shelf-life would be long. Ash content was relatively high with values ranging from 11.12 ± 0.01 % for *Ocimum gratissimum* to 17.77 ± 0.07% for *Amaranthus viridis*. These values indicate that these vegetables may be considered as good sources of minerals when compared to values (2 – 10 %) obtained for cereals and tubers (FAO, 1986). It is also comparable to the findings of Oulai *et al.,* (2014), where the ash content of some vegetable spices was found to be between 8.59 ± 1.34 % for *A. hybridus* to 25.67 ± 1.12 % for *C. patendra*. The crude fibre level of between 2.15 ± 0.00% for *Ocimum gratissimum* and 9.54 ± 0.01 for *Amaranthus viridis* fall short of what was found by Oulai *et al.,* (2014), in vegetable spices, which ranged from 12.11 - 33 %. High level of crude fibres in leafy vegetables would be advantageous for their active role in the regulation of intestinal transit, increasing dietary bulk due to their ability to absorb water (Jenkin *et al*., 1986). Ndukwe and Ipkeama (2013) reported 9.48% crude fibre content for *Pterocarpus*

santalinoides, A fibre content of between 4.02% and 12.08% had been reported in leafy vegetables, (Asaolu *et al.,* 2012; Javid *et al.,* 2009; Mosha *et al.,* 1995) The dry matter of *Vernonia amygdalina* has been found to contain 6.5 to 29.2% of crude fibre (Alabi *et al*., 2005a; Antia *et al*., 2006; Oboh, 2006; Okoli *et al*., 2003a). These plants are good sources of crude fibre and when adequately consumed, (dietary fibre) can lower the serum cholesterol level, heart disease, hypertension, constipation, diabetes and breast cancer (Ekumankama, 2008; Ishida *et al.,* 2000). Fibre cleanses the digestive tract, by removing potential carcinogens from the body and prevents the absorption of excess cholesterol (Smith, 1985). Fibre also binds to cancer-causing chemicals, keeping them away from the cells lining the colon, providing yet another line of protection from colon cancer (Ensminger and Ensminger, 1996). Crude ash content of between 11.12% (in *Pterocarpus santalinoides*) and 17.36 (in *Occimum gratissimum*) were recorded in this research. Ndukwe and Ipkeama (2013) reported 7.83% crude fibre content for *Pterocarpus santalinoides*. A value as low as 5.02% was found in Indian spinach by Asaolu *et al.,* (2012) and as high as 22.8% ash content was reported for *Amaranthus viridis* by Javid *et al.,* (2009). While Asaolu *et al.,* (2012), stated that the ash contents of *Vernonia amygdalina* was 9.56%, Indian spinach 5.02%, *Occimum gratissimum* 13.01%, *Amaranthus hybridus* 15.55%, *Telfeiria occidentalis* 8.56%. Javid *et al.,* (2009), reported 22.84% for *Amaranthus viridis* and 22.15% for *C.* Album. The ash content is a reflection of the amount of mineral elements present in a sample. Ash in feeds is useful for judging nutritional characteristics of the feed because ash has generally constant element composition by feed material type as long as the feed does not contain earth and sand, etc. Ash content in feeds of plant origin is not very good as a nutritional indicator because: it varies The values obtained (11.97 – 1414.54%) for lipids in these vegetables contradicts the findings of many authors which showed that leafy vegetables are poor sources of lipids Oulai

et al., 2014). *Pterocarpus santalinoides* has exceptionally low crude lipid content of 1%. However, it is important to note that diet providing 1 – 2 % of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yields to cardiovascular disorders such as atherosclerosis, cancer and aging (Kris-Etherton *et al*., 2002). Therefore, the consumption of these *Pterocarpus santalinoides* in large amount may be recommended to individuals suffering from obesity. The crude proteins content ranged between 10.14 ± 0.01 % and 25.09 ± 0.07%. The proteins content of *A. viridis* (25.09 ± 0.07%), *Telfeiria ocidentalis* (23.36 ± 0.08%) and *V. amygdalina* (22.85 ± 0.05%) were higher than that reported for some high value leafy vegetables such as *Momordica balsamina* (11.29 %) and *Moringa oleifera* (20.72 %) (Asaolu *et al*., 2012) and some leafy vegetable spices (13.25 ± 0.13 % and 21.96 ± 0.30%), (Oulai et *al.,* 2014). Plant foods which provide more than 12 % of their calorific value from proteins have been shown to be good source of proteins (Ali, 2009). This suggests that all the leafy vegetables investigated are good sources of proteins and could play a significant role in providing cheap and available proteins for rural communities. Assuming complete protein absorption, 100 g of the studied leaves would respectively contribute for about 18.6 to 30.92 % of the daily protein requirement (71 g/day) of pregnant and lactating mothers (FND, 2005). The lowest carbohydrate contents of 1.25% (*Telferia ocidentalis*) and highest of 9.09 ± 0.15 (*Pterocarpus santalinoides*) was found in the leaves. This is comparable to the results of Asaolu *et al.,* (2012) which showed *V. amygdalina,* (8.65%), Indian spinach (7.51%), Bush-buck (5.81), Scent leaf (1.22), *A. hybridis* (3.36) and *Telfeiria occidentalis* (1.16). Thus, the calorific value is in agreement with general observation that vegetables have low energy values (Lintas, 1992).

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that both the aqueous and ethanol leaf

extracts were rich in tannins, aqueous extracts all have saponins while the ethanol extracts were rich in phenolics. These bioactive compounds have been found to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). Alkaloids inhibit or activate the enzymes alcohol dehydrogenase, and the phosphatases inhibit the phosphodiesters which normally destroy cAMP, involved in breakdown of fats and carbohydrates. The net result is the alteration of the storage of carbohydrates and fats. The non-sugar part of saponins have been reported to have a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases. (Phytochem.2017). The absence of flavonoids in all the leaf extracts in the present study is in contrast with the opinions of Abdul *et al.*, (2013) who noted the presence of flavonoids in all the medicinal plants studied, Edeoga *et al.,* (2005), in some Nigerian medicinal plants, Ndukwe and Ipkeama (2013) and Bothon, *et al* (2014), carried out their researches in southern Nigeria and southern Benin Republic and reported the presence of flavonoids in some of these leafy vegetables. The present research and previous researches results were different probably due to the change in location. According to studies by Borges *et al,* (2013) environmental factors such as temperature, soil mineral availability and nutritional stress could affect the presence and quantity of secondary metabolites. For instance, Marschner (1997) reported that the deficiency of Manganese (Mn) impairs lignification therefore phenolic compounds accumulates while its deficiency results to decrease in phenolic compounds such as flavonoids. High light intensity favours the accumulation of phenolic compounds while the opposite were reported for flavonoids (Ghasemzadeh and Ghasemzadeh, 2011). The Middle belt of Nigeria, particularly Keffi, where the samples for this research were sourced, is known to have a lengthier and higher light intensity than West African coastal Region. It was found that some of these investigated plants contained

steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). The presence of terpenoids in these extracts was confirmed in some of the extracts and some of the plants are widely used in herbal medicine. The plants studied here can be seen as a potential source of useful drugs.

The *in vitro* antioxidant assay of the aqueous leaf extracts of these plants showed a progressive increase in DPPH radical scavenging properties with increase in the concentration on the extracts. This suggests that the bioavailability of these phytochemicals that leads to the antioxidant properties is a factor in their *in vivo* activities. It can therefore be adduced that the higher the concentration of the bioactive constituents in the extracts, the higher the antioxidant potential of the sample in question. This assertion did not apply to the ethanol extracts which showed a non sequential pattern of increase in DPPH-scavenging activity. The DPPH-scavenging activity of the ethanol leaf extracts of these plants may have been affected by the presence of some phytochemicals, not identified in the study, but extracted by the solvent (ethanol). In other words ethanol may have extracted some bioactive materials that antagonized the *in vitro* antioxidant activity of the plant materials.

The oral administration of 2g/kgbw single dose of acetaminophen to the rats (negative control), that were not pre-treated with either the aqueous or ethanol leaf extracts of the plants, induced liver injury. This was evidenced by the significant ($p<0.05$) increase of the serum activities of the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) of the rats in the negative control group (see Tables 14 and 15). Al-Mamary, (2002) reported that the aminotransferase are abundant in the liver and are released into the bloodstream following hepatocellular damage, making them sensitive markers of liver damage. Therefore, a marked increase in the serum ALT and AST activities

is indicative of liver damage. Serum levels of aminotransferases are used as an indicator of damage to the liver structural integrity because these enzymes are cytoplasmic in location and are released into the circulating blood only after structural damage. (Okediran *et al*, 2014). The pre-treatment of the animals (rats), with 400mg/kgbw of either aqueous or ethanol leaf extracts of *Amaranthus viridis, Telfeiria occidentalis, Pterocarpus santalinoides, Vernonia amygdalina, Ocimum canum* and/or *Ocimum gratissimum* gave protection against toxicity and oxidative stress arising from a 2g/kgbw single dose of acetaminophen over nine hours (9hrs). Generally, the aqueous and ethanol leaf extracts of *Amaranthus viridis, Telfeiria occidentalis, Pterocarpus santalinoides, Vernonia amygdalina, Ocimum canum* and/or *Ocimum gratissimum*led to decrease of the serum activities of ALT and ALP of the animals pre-treated with them prior to the intoxication with acetaminophen, but markedly increased the serum activity of AST. However, the decreases in the activities of ALT and ALP in the serum of the rats pre-treated with these extracts were not as much as that caused by Silymarin. The order of activities of ALT for the aqueous extracts and Silymarin was Sil <Va< To < Og < Av <Ps < Oc, while that for the ethanol leaf extract was Sil < Ps < Og < Va< To < Oc < Av. (see Tables 14 and 15). The extracts also markedly increased the serum albumin and total protein of the pre-treated rats with the preservation of the hepatic lobular architecture. All these are indications of some degree of protection of the liver by the aqueous and ethanol leaf extracts of the plants under study. It can therefore, be suggested that these plants produced effects which may contribute to the preservation of cellular GSH levels in the acetaminophen-intoxicated rats, which further provides cellular defence both as a hydroxyl radical scavenger (Ekor, *et al.*, 2006), and also a detoxifying agent against NAPQI, the toxic intermediate of acetaminophen in a GST- catalysed reaction.

A marked reduction in total serum protein and abnormal increase in serum bilirubin had been reported in hepatotoxicity, (Olamide and Mattew, 2013; Olorunnisola *et al.* 2011; Martin and Friedman, 1992). A decrease in total protein and album shows that the ability of the liver to synthesize/metabolize protein (example albumin), may have been impaired, hence indicative of liver damage. The administration of 400mg/kgbw of these extracts improved the serum concentrations of both the albumin and the total protein, (see Tables 16 and 17).

N-acetyl-p-benzoquinoneimine (NAPQI), is an oxidative product of acetaminophen metabolism that binds covalently to the sulphydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver causing hepatotoxicity (Kanchana and Mohammed Sadiq, 2011). Results from the present study also provides evidence of the induction of oxidative stress nine hours (9hrs), following acute acetaminophen (APAP), intoxication. The induced oxidative stress as found in this study is evident in the decreased activities of the SOD, CAT and the GPx of the animals in the negative control group as compared to the normal control group, resulting in decrease in cellular defence against oxidative damage. Only the decrease in the activities of SOD and GPx however, were significant when compared to the normal control. The increased formation of superoxide may have led to hydrogen peroxide formation and peroxidation reactions by Fenton-type mechanism as found in James, *et al.,* (2003). Ekor *et al., (*2006) reported that after seven hours, following Paracetamol (PCM) intoxication, there was a rise in GST activity, indicating increased GST-catalysed conjugation of PCM toxic metabolite NAPQI, with GST, that led to the depletion of cellular GSH level. The histological profile of the livers of the rats in the negative control group showed poorly preserved hepatic lobular architecture, sharply demarcated hepatocyte, necrosis and some exhibiting peri-portal sinusoidal congestion (Plate 10), marked sinusoidal dilatation which is a confirmation of liver

injury. The lipid profile parameters (triacylglycerol, low density lipoprotein, high density lipoprotein and cholesterol) concentration in the serum of the rats in this study showed no significant change, probably due to the short time interval (9hrs) between the intoxication and the sacrifice. Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) are the primary intracellular defence mechanism to cope with increased oxidative stress. They eliminate superoxide anion and hydrogen peroxide that may oxidise cellular substrates and the prevent free radical chain reactions (Ekor, *et al.,* 2006). Although only the activities of the SOD and GPx were significantly ($p<0.05$) reduced by the action of acetaminophen, in this study, all the antioxidant enzymes activities assayed in this study (CAT, SOD and GPx), were induced by the actions of the leaf extracts during the hepatic damage produced by the acute acetaminophen toxicity, (aqueous leaf extract of *Ocimum canum* significantly decreased the activity of the SOD). The protection may be due to the antioxidant properties of these plants, which stem from their phytochemical components. Researchers have found that phytochemicals have the potential to reduce oxidative damage to cells and there are likely health effects of phytochemicals that researchers have not yet recognized (Densie Webb, 2013; Huang, *et al.*, 1992). However, it is not known whether the health benefits are the result of individual phytochemicals, the interaction of various phytochemicals, the fibre content of plant foods, or the interaction of phytochemicals and the vitamins and minerals found in the same foods.It was found in this study that *Amaranthus viridis, Telfeiria occidentalis, Pterocarpus santalinoides, Vernonia amygdalina, Ocimum canum* and/or *Ocimum gratissimum* are rich sources of phytochemicals, (see Table 12).The bioavailability of phytochemicals varies greatly and can range from less than 0.03% of what's consumed (certain flavonols) to 50% (isoflavones) (Gordon, 2012). While evidence is limited regarding how phytochemicals are stored, research suggests there are no long-term stores of

polyphenols in the body(Erdman, *et al.,* 2007). Aside from inherent differences in the bioavailability of these compounds, absorption also is affected by the gut microflora and individuals' genetic makeup, both of which vary greatly (Da Costa, *et al.,* 2012). In addition, processing, such as steaming, drying, freezing, and boiling, can reduce the levels of some phytochemicals found in the final food product (Mahn, 2012). The generation of superoxide radicals was reported to be inhibited by tannins and related compounds, (Chung, *et al.,* 1998). In this research, it was found that all the leaf extracts contain tannins, while phenols, alkaloids and saponin were sparsely distributed among the extracts and these could be the bioactive constituents that lead to the antioxidant properties of these plants. Alkaloids Inhibit or activate the enzymes alcohol dehydrogenase, and the phosphatases, inhibit the phosphodiesters which normally destroy cAMP, involved in breakdown of fats and carbohydrates. The net result is the alteration of the storage of carbohydrates and fats. The aqueous leaf extracts of *Amaranthus viridis* (Av), *Vernonia amygdalina* (Va), *Ocimum gratissimum* (Og) and *pterocarpus santalinoides* (Ps) significantly ($p<0.05$) increased the SOD activities of the rats pre-treated with them (in the order Av<Va<Og<Ps) and the increase induced by the four extracts was greater than that effected by the standard drug (Silymarin), as shown on Table 20. The aqueous leaf extract of *Ocimum canum* (Oc) also did not give a positive result on the SOD activity. The aqueous leaf extracts of *Amaranthus viridis, Telfeiria occidentalis, Pterocarpus santalinoides, Vernonia amygdalina* and *Ocimum gratissimum* increased the activity of Catalse in the order of their listing, with Ps, Va and Og inducing a significant ($p<0.05$) increase in activity compared with the negative control. The group pre-treated with Sylimarin had a higher increase in the CAT activity when compared to the ones pre-treated with the extracts. There was a generally significant increase of the GPx activity of all the animals pre-treated with the aqueous leaf extracts of all the plants studied,

when compared to the negative control. The increase in activities is also higher than the one induced by the pre-treatment with Silymarin. Pre-treatment with ethanol leaf extracts of all the plants under this study elicited significant ($p<0.05$) increase in SOD activity of the rats although, the values were lower than what was obtained with pre-treatment with Silymarin (Sil). The order of increase was Silymarin > To >Va> Av > Ps > Oc > Og. CAT activity was not raised by pre-treatment with ethanol leaf extracts of *Telfeiria occidentalis* and *Amaranthus viridis.* Pre-treatment with ethanol leaf extracts of *Ocimum canum, Pterocarpus santalinoides, Ocimum gratissimu* and *Amaranthus viridis* raised significantly the activity of CAT in the order of their listing above. However, none of the ethanol leaf extract was able to attain the same level of activity induced by the pre-treatment with Silymarin. The activities of the GPx of the rats were markedly raised by the administration of the ethanol leaf extracts of the plants under study, even more than what was achieved by the standard drug. The order of increase was Ps >Va> OC >To > Sil >Og >Av (see Table 21).

Phenolic compounds including simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive substances occurring widely in food plants. Many phenolic compounds in plants are good sources of natural antioxidants. Phenol was found in all but the aqueous leaf extracts of *Telfeiria occidentalis* and *Ocimum canum.* All the extracts containing phenol elicited significant ($p<0.05$) increases in the activities of the antioxidant enzymes (SOD, CAT and GPx), but led not to marked positive influence on the SOD and CAT activities of rats pre-treated with aqueous leaf extracts of *Telfeiria occidentalis* and *Ocimum canum* although the GPx activity level was significantly increased in these rats (Table 21).

From the foregoing, it could be suggested that the plants under study possess great deal of antioxidant potentials which can account for the preservation of the livers' lobular

architecture (Figures 11 to 22), resulting in the low activities of ALT and ALP (Tables 14 and 15) in the serum of the pre-treated rats compared to the control group. All the effects stated above are dependent on the solvent of extraction and the metabolism (bioavailability) of the bioactive constituents in the plant materials. The ethanol as a solvent gave higher yields of the bioactive compounds but this did not translate to higher efficiency in hepatoprotection asthe aqueous extract gave better protective effect on the liver. The aqueous leaf extract of *V. Amygdalina* and *Pterocarpus santalinoides* gave relatively better protection than other extracts.

Conlussion

The present study provides evidence that *Amaranthus viridis, Telfeiria occidentalis, Pterocarpus santalinoides, Vernonia amygdalina, Ocimum canum* and/or *Ocimum gratissimum*have antioxidantpotentials and offered protection to the hepatic cells. Therefore, they can be good sources of raw materials for the prevention and treatment of liver diseases and other pathological conditions associated with oxidative stress.

Recommendations

Pre-administration of ethanolic and aqueous leaf extracts of *Ocimum canum, Telfeiria occidentalis, Amarranthus viridis, Pterocarpus santalioirdes, Vernonia amygdalina* and *Ocimum gratissimum* showed some degree of hepatoprotectionin rats, thus encouraging and supporting, their use as edible vegetables and for therapy respectively. However, it is important to do an effective dose-response study to identify the dose range at which the leaf extracts are beneficial and at which dose they are toxic and to clearly identify the specific compounds with the Hepatoprotective properties in the leaf extracts of the plants.

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APPENDICES

Apendix 1: The Chemicals/Reagents/Kits

S/NO	CHEMICALS (ANALAR)
1	Ethanol
2	Sucrose
3	Sodium Carbonate
4	Gallic Acid
5	DPPH
6	Nitro Blue Tetra Zolium
7	EDTA
8	NaCN
9	Riboflavin
10	Deoxyribose
11	$FeCl_3$
12	H_2O_2
13	KH_2PO_4
14	Potassium hydroxide
15	Sodium nitroprusside
16	Sulphanilamide
17	H_3PO_5
18	Napthylethylene diamine dihydrochloride
19	Potassium ferricyanide
20	Trichloroacetic acid
21	$FeCl_2$
22	Tris-HCl
23	Ascorbate
24	Thiobarbituric acid
25	TCA
26	Sodium dodecyl sulphate
27	Potassium dichromate
28	Glacial acetic acid
29	Adrenaline
30	Reduced Glutathione
31	DTNB

Apendix 2: Preparation of Reagents:

- **Dichromate Solution (5%)**

$K_2Cr_2O_7$ (5 g) was dissolved in 80 ml of distilled water and made up to 100 ml with same.

- **Hydrogen peroxide(0.2M)**

H_2O_2 (0.67 g) was mixed with distilled water in a 100 ml volumetric flask and the solution made up to the mark with same.

- **Dichromate/acetic acid**

This reagent was prepared by mixing 5% solution of $K_2Cr_2O_7$ with glacial acetic acid (1:3 by volume) and it is stable for about one month.

- **Phosphate buffer (0.01M, pH 7.0)**

$Na_2HPO_4.12H_2O$ (3.5814 g) and 1.19 g $NaH_2PO_4.2H_2O$ dissolved in 900 ml of distilled water. The pH adjusted to 7.0 and distilled water added to make up to 1 litre.

- **Carbonate buffer (0.05 M, pH 10.2)**

$Na_2CO_3.10H_2O$ (14.3 g) and 4.2 g of $NaHCO_3$ were dissolved in 900 ml of distilled water. The pH was adjusted to 10.2 and then made up to 1 litre.

- **Adrenaline (0.3 mM)**

Adrenaline (0.0137 g) was dissolved in 200 ml distilled water and then made up to 250 ml. This solution was prepared just after the experiment.

- **Phosphate buffer (0.1 M, pH 7.4)**

  a. First 0.1M $Na_2HPO_4.12H_2O$ was prepared by dissolving 7.1628 g in 200 ml of distilled water.

  b. 0.1M $NaH_2PO_4.2H_2O$ was prepared by dissolving 1.5603 g in 100 ml of distilled water.

  Finally 0.1M phosphate buffer was prepared by adding 200 ml of (a) to 100 ml of (b) and the pH adjusted to 7.4 with drops of concentrated HCl or NaOH as the case may be. This is stable indefinitely unless mold forms. If crystals develop during storage at 4^oC, heating may dissolve these.

- **Ellman's Reagent [5′, 5′-Dithiobis- (2-nitrobenzoate) DTNB]**

  This was prepared by dissolving 40 mg of Ellman's reagent in 0.1M Phosphate buffer and made up to 100 ml.

- **Precipitating Solution**

  Prepared from 1.67 g metaphosphoric acid, 0.2 g disodium or dipotassium ethylenediamine tetraacetic acid (EDTA) and 30 g NaCl per 100 ml of distilled water and stored at 4^oC. It is stable for 3 weeks. The EDTA was added to prevent difficulties that might arise where water supply contains appreciable concentration of metallic ions.

- **Trichloroacetic acid (TCA, 30%)**

  TCA (9 g) was dissolved in distilled water and made up to 30 ml with same.

- **Thiobarbituric acid (0.75%)**

  This was prepared by dissolving 0.225 g of thiobarbituric acid (TBA) in 0.1 M HCl and made up to 30 ml with same.

- **Tris-KCl buffer (0.15 M, pH 7.4)**
- KCl (1.12 g) and 2.36 g of Tris base were dissolved separately in distilled water and made up to 100 ml with same. The pH was then adjusted to 7.4.
- **5% (w/v) Ferric Chloride Solution:**

  A weighed sample ferric chloride 5.0g) was dissolved in 100ml of distilled water.

- **Ammonium Solution:**

  187.5ml of the stock concentrated ammonium solution was diluted in 31.25ml of distilled water and then made up to 500ml with distilled water.

- **45% (v/v) Ethanol:**

  45ml of absolute ethanol was mixed with 55ml of distilled water.

- **AluminiumChloride Solution:**

  0.5g of aluminium chloride was dissolved in 100ml of distilled water.

- **Dilute Sulphuric Acid:** 10.9ml of concentrated sulphuric acid was mixed with 5ml of distilled water and made up to 100ml.
- **Lead Sub Acetate Solution:** 45ml of 15% lead acetate
- **Wagner's Reagent:** A weighed sample of iodine crystals (2.0g) and potassium iodide (3.0g) were dissolved in 100ml of distilled water.
- **Mayer's Reagent:** A weighed sample of mercuric chloride (13.5g) was dissolved in 50ml of distilled water. Also, 5.0g of potassium iodide was dissolved in 20ml of

distilled water. The two solutions were mixed and the volume made up to 100ml with distilled water.

- **Dragendorff's Reagent:** A weighed sample of bismuth carbonate (0.85g) was dissolved in 100ml of glacial acetic acid and 40ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0g of potassium iodide in 20ml of distilled water. Both solutions were mixed to give a stock solution.
- **Molisch Reagent:** A weighed sample of -naphthol (1.0g) was dissolved in 100ml of absolute ethanol.
- **2% (v/v) Hydrochloric Acid:** 2.0ml of concentrated hydrochloric acid was diluted with some distilled water and made up to 100ml.
- **1% (w/v) Picric Acid:**A weighed sample of picric acid (1.0g) was dissolved in 100ml of distilled water.
- **Preparation of standard garlic acid and tannic acid solution**

The stock solution of garlic acid and tannic acid was prepared by dissolving 10.0 mg of each in 100.0 mL methanol, creating a 100 μg/mL solution. This solution was diluted with the solvent as needed to prepare different standard solutions (2, 4, 6, 8, 10, 12, 14, 16 and 20μg/mL)

Apendix 3: Histopathogy Report

Group 1A:(1) Section of liver tissue showing a well preserved hepatic lobular structure with normal appearing hepatocytes **(2)** Well preserved hepatic lobular architecture with minimal chronic inflammatory cellular infiltrate. **(3)** Moderately preserved normal appearing hepatic lobular architecture showing glycogen-laden hepatocytes, mild sinusoidal dilatation and minimal chronic inflammatory cellular infiltrate. **(4)** Moderately preserved normal appearing hepatic lobular architecture showing glycogen-laden hepatocytes, mild sinusoidal dilatation and a diffuse chronic inflammatory cellular infiltrate. **(5)** Well-preserved hepatic lobular architecture showing typical hepatocytes. ***Haematoxylin and Eosin stain*.**

Group 1B:(1) Section of liver tissue showing a moderately preserved hepatic lobular structure with minimal diffuse chronic inflammatory cellular infiltrate **(2)** Moderately preserved hepatic lobular architecture with minimal diffuse chronic inflammatory cellular infiltrate. Occasional karyopyknotic hepatocytes are also observed. **(3)** Moderately preserved hepatic lobular architecture showing minimal diffuse chronic inflammatory cellular infiltrate. **(4)** Moderately preserved hepatic lobular architecture showing mild derangement of the hepatic plate. Mild diffuse chronic inflammatory cellular infiltrate is also observed. **(5)** Hepatic lobular architecture in slight disarray showing moderate diffuse chronic inflammatory cellular infiltrate. ***Haematoxylin and Eosin stain*.**

Group 1C:(1) Section of liver tissue showing a well-preserved hepatic lobular structure with normal appearing hepatocytes**(2)**Hepatic lobule in severe disarray. Hepatocytes exhibit anisocytosis and anisokaryosis, and there is a mild diffuse chronic inflammatory cellular infiltrate. **(3)**Well-preserved hepatic lobular architecture showing minimal diffuse chronic

inflammatory cellular infiltrate.**(4)**Severely deranged hepatic lobular architecture showing derangement of the hepatic plate and hepatocyte necrosis.**(5)** Hepatic lobular architecture in slight disarray showing some hepatocyte necrosis. ***Haematoxylin and Eosin stain*.**

Group 2A:(1) Section of liver tissue showing a well-preserved hepatic lobular structure with normal appearing hepatocytes. There is moderate dilatation of the sinusoids, with increased kupffer cell population and mild diffuse chronic inflammatory cell infiltrate. A few apoptotic hepatocytes are seen.**(2)**Well-preserved hepatic lobular architecture, showing normal appearing hepatocytes. Few apoptotic hepatocytes are seen. **(3)**Poorly-preserved hepatic lobular architecture showing massivehepatocyte necrosis and a few apoptotic hepatocyte exhibiting karyopyknosis. **(4)**Moderately deranged hepatic lobular architecture showing derangement of the hepatic plate and hepatocyte necrosis.**(5)** Hepatic lobular architecture in slight disarray showing some hepatocyte necrosis and mild diffuse chronic inflammation. ***Haematoxylin and Eosin stain*.**

Group 2B:(1) Section shows a well-preserved hepatic lobular structure with moderate oedematous dilatation of the sinusoids.**(2)**Well-preserved hepatic lobular architecture, showing islands of hepatocytes ballooning degeneration and hyper-pigmented nuclei. **(3)**Well-preserved hepatic lobular architecture showing centrilobular sinusoidal congestion with numerous hyper-pigmented hepatocyte nuclei.**(4)**Severely deranged hepatic lobular architecture showing derangement of the hepatic plate and hepatocyte necrosis. Several hepatocytes with reactive nuclei are also seen.**(5)** Hepatic lobular architecture in slight disarray showing glycogen laden hepatocytes and sheets of hepatocytes undergoing ballooning degeneration. Centrilobularmacrovesicular fatty change is also observed. ***Haematoxylin and Eosin stain*.**

Group 2C:(1) Section shows a poorly-preserved hepatic lobular structure with hepatic plate in disarray. Increased kupffer cell population and hepatocytes showing reactive nuclei are also observed.**(2)**Well-preserved hepatic lobular architecture, however there sharply demarcated islands (zone 2) of hepatocyte necrosis and increase in fibrous connective tissue component. **(3)**Well-preserved hepatic lobular architecture showing mild chronic inflammatory cell infiltrate.**(4)** Moderately preserved hepatic lobular structure showing diffuse sinusoidal congestion with haemorrhagic necrosis of hepatocytes. ***Haematoxylin and Eosin stain*.**

Group 3A:(1) Section shows a poorly-preserved hepatic lobular structure with hepatic plate in disarray. Mild diffuse chronic inflammation and sinusoidal haemorrhage are observed.**(2)**Poorly-preserved hepatic lobular architecture. Coagulative necrosis.**(3)**Moderately-preserved hepatic lobular architecture showing massive hepatocyte necrosis.**(4)** Moderately preserved hepatic lobular structure showing diffuse sinusoidal dilatation with mild chronic inflammation.**(5)**Moderately preserved hepatic lobular structure showing diffuse sinusoidal dilatation with mild chronic inflammation ***Haematoxylin and Eosin stain*.**

Group 3B:(1) Section shows a well-preserved hepatic lobular structure, showing hepatocytes with reactive nuclei. Mild diffuse chronic inflammation and sinusoidal dilatation are observed.**(2)**Well-preserved hepatic lobular architecture. Extensive areas of hepatocytes exhibiting karyopyknotis are observed (massive hepatocyte necrosis). **(3)**Moderately-preserved hepatic lobular architecture showing significant sinusoidal dilatation and increased kupffer cell population. **(4)** Moderately preserved hepatic lobular structure showing diffuse

sinusoidal dilatation with numerous karyopyknotic hepatocytes. ***Haematoxylin and Eosin stain*.**

Group 3C:(1) Section shows a moderately-preserved hepatic lobular structure, showing hepatocytes with reactive nuclei. Mild diffuse chronic inflammation is also observed.**(4)**Well-preserved hepatic lobular architecture. Sharply demarcated areas of centrilobular hepatocyte necrosis are observed. ***Haematoxylin and Eosin stain*.**

Group 4A:(1) Section shows a poorly-preserved hepatic lobular architecture, showing hepatocytes with reactive nuclei. Periportal focal chronic inflammation and sinusoidal congestion are observed.**(3)**Well-preserved hepatic lobular architecture. Extensive areas of radiating centrilobular vascular congestion with several pigment-laden macrophages.**(4)**Hepatic lobular architecture in severe disarray showing extensive areas of haemorrhage.**(5)** Moderately preserved hepatic lobular structure showing mild diffuse chronic inflammatory cell infiltrate and numerous hepatocytes exhibiting ballooning degeneration and necrosis. ***Haematoxylin and Eosin stain*.**

Group 4B:(1) Section shows a moderately-preserved hepatic lobular architecture, showing mild diffuse chronic inflammatory cell infiltrate and a few karyopyknotic hepatocytes.**(2)**Poorly-preserved hepatic lobular architecture, showing mild sinusoidal dilatation and mild chronic inflammation. **(3)**Wel-preserved hepatic lobular architecture showing centrilobular radiating sinusoidal dilatation and several karyopyknotic hepatocytes.**(4)** Poorly preserved hepatic lobular structure showing mild diffuse chronic inflammatory cell infiltrate and numerous hepatocyte karyopyknosis and necrosis.**(5)** Hepatic

lobular architecture in moderate disarray, showing extensive areas of centrilobular congestion necrosis evidenced by pale-staining hepatocytes. ***Haematoxylin and Eosin stain*.**

Group 4C:(1) Section shows a well-preserved hepatic lobular architecture, showing diffuse moderate chronic inflammation and sinusoidal dilatation.**(3)** Section shows a moderately-preserved hepatic lobular architecture, showing diffuse moderate chronic inflammation and sinusoidal dilatation. **(4)** Section shows a well-preserved hepatic lobular architecture, showing diffuse moderate chronic inflammation and sinusoidal dilatation. **(5)** Moderately preserved hepatic lobular structure showing mild diffuse chronic inflammatory cell infiltrate and numerous hepatocytes macrovessicular fatty change and necrosis. ***Haematoxylin and Eosin stain*.**

Group 5A:(1) Section shows a well-preserved hepatic lobular architecture, showing normal appearing hepatocytes and a minimal diffuse chronic inflammatory infiltrate.**(3)** Section shows a well-preserved hepatic lobular architecture showing normal appearing hepatocytes, with mild chronic inflammatory cell infiltrate and mild sinusoidal dilatation. **(4)** Section shows a moderately-preserved hepatic lobular architecture, showing mild sinusoidal dilatation. **(5)** Well-preserved hepatic lobular structure showing mild periportal chronic inflammatory cell infiltrate. ***Haematoxylin and Eosin stain*.**

Group 5B:(3) Section shows a well-preserved hepatic lobular architecture, showing normal appearing hepatocytes and a minimal diffuse chronic inflammatory infiltrate.**(4)** Section shows a well-preserved hepatic lobular architecture showing normal appearing hepatocytes, with mild diffuse chronic inflammatory cell infiltrate and mild sinusoidal dilatation. ***Haematoxylin and Eosin stain*.**

Group 5C:(1) Section shows a poorly-preserved hepatic lobular architecture, showing moderate diffuse chronic inflammatory infiltrate.**(3)** Section shows a well-preserved hepatic lobular architecture showing sharply demarcated patchy areas of hepatocyte necrosis and sinusoidal congestion. **(4)** Section shows a well-preserved hepatic lobular architecture, showing mild diffuse inflammation with increased kupffer cell population and hepatocyte anisokaryosis. **(5)** Poorly-preserved hepatic lobular structure showing mild chronic inflammatory cell infiltrate significant sinusoidal dilatation and haemorrhage. ***Haematoxylin and Eosin stain*.**

Group 6A:(1) Section shows a poorly-preserved hepatic lobular architecture, showing mild diffuse chronic inflammatory infiltrate and sinusoidal dilatation.**(2)**Section shows a moderately preserved hepatic lobular architecture exhibiting centri-portal sinusoidal congestion and sharply demarcated hepatocyte necrosis **(3)** Section shows a poorly-preserved hepatic lobular architecture showing marked sinusoidal dilatation and necrosis. **(4)** Section shows a poorly-preserved hepatic lobular architecture, showing mild diffuse inflammation with increased kupffer cell population and hepatocyte karyopyknosis and necrosis. **(5)** Moderately-preserved hepatic lobular structure showing mild diffuse chronic inflammatory cell infiltrate and sinusoidal dilatation. ***Haematoxylin and Eosin stain*.**

Apendix 4: Statistical Analysis

STATISTICAL ANALYSIS												
	ALB	ALP	TP	ALT	AST	T. BIL	D. BIL	IND. BIL	T. CHOL	HDLP	LDLP	T.G
Aqueous vs Normal pvalue	0.155121579	0.015347445	0.451811423	0.074050954	0.000045	0.001057565	0.063632226	0.006163	0.155383	0.642002	0.136556	0.952962
Aqueous vs std pvalue	0.003214403	0.127174095	0.95300863	0.00220208	0.000170604	0.155940263	0.106166461	0.21002	0.471519	0.182003	0.499846	0.647094
Aqueous vs negative pvalue	0.001310732	0.005409971	0.16726638	0.072060418	0.000018	0.005490469	0.052359849	0.003036	0.542879	0.185118	0.388953	0.488564

SAMPLES	ALB	ALP	TP	ALT	AST	T. BIL	D. BIL	IND. BIL	T. CHOL	HDLP	LDLP	T.G
Ethanolic vs Normal p value	0.547555392	0.593931729	0.269606574	0.091846328	0.003218652	0.00002	0.004491636	0.001137	0.165779	0.996758	0.021499	0.002038
Ethanolic vs std pvalue	0.023657433	0.641407235	0.960637404	0.807340865	0.003564556	0.065918331	0.015190942	0.099931	0.958796	0.482093	0.538466	0.006166
Ethanolic vs negative pvalue	0.014552612	0.568074259	0.052461326	0.005056809	0.000987674	0.000752996	0.004314318	0.000696	0.508067	0.176103	0.445097	0.021765

SAMPLES	ALB	ALP	TP	ALT	AST	T. BIL	D. BIL	IND. BIL	T. CHOL	HDLP	LDLP	T.G
std AND NORMAL pvalue	0.011886017	0.455585167	0.823714552	0.006078105	0.897072823	0.159813854	0.478317284	0.722499	0.405886	0.314253	0.725351	0.254987
negative and normal pvalue	0.004708144	0.372843809	0.023806439	0.033941842	0.057894646	0.963859795	0.287106528	0.718747	0.069555	0.020867	0.511908	0.026039

	ALB	ALP	T.P	GPT	GOT	T.BIL	D.BIL	IND.BIL	T.CHOL	HDLP	LDLP	T.G	
5A1	10	342	59.01	47.6	73.9	22.3	3.7	18.6	1.75	0.39	1.12	0.54	
5A2	1.9	77	68.23	31.3	50.1	10.4	5.2	5.2	2.12	0.421	1.1	1.34	
5A3	4	217	70.07	25.1	60.5	13	1.6	11.4	2.63	0.259	1.83	1.19	
5A4	8	284	61.49	34.7	144	11.4	5.5	5.9	1.91	1.34	0.3	0.63	
5A5	10	40	62.87	17.6	32.4	2.5	1	1.5	0.96	0.259	0.65	0.11	
	6.78	192	64.33	31.26	72.2	11.92	3.4	8.52	1.874	0.534	1	0.76	AVERAGE
	3.6663	130.3	4.654	11.218	42.9	7.075	2.046	6.6533	0.6091	0.457	0.576	0.5	STNDEV
	0.5581	0.919	0.423	0.0234	0.52	0.716	0.146	0.4673	0.7952	0.792	0.966	0.21	NEGATIVE
	0.4112	0.793	0.054	0.0984	0.3	0.672	0.12	0.9421	0.7062	0.362	0.911	0.45	STANDARD
	0.1951	0.692	0.19	0.984	0.72	0.864	0.367	0.8595	0.0806	0.34	0.147	0.13	POSITIVE
6A1	6	300	62.35	200	97.9	16.2	6.2	10	1.18	0.32	0.25	1.34	
6A2	6	205	64.07	113	51.8	12.1	5.1	7	2.78	0.622	1.63	1.17	
6A3	8	202	61.87	80.2	40.9	22.2	16.1	6.1	2.67	0.344	1.51	1.8	
6A4	20	85	69.07	53.1	45.2	2.6	1.5	1.1	1.54	0.405	0.79	0.78	
6A5	4	132	83.43	76.1	52.4	15	9.2	5.8	1.76	0.681	0.74	0.75	NEGATIVE
5B1	4	149	81.45	33.5	38.4	13.1	6.4	6.7	1.55	0.414	0.99	0.99	
5B2	18	239	73.41	44	108	9	3.4	5.6	1.57	0.423	0.72	0.96	
5B3	14	304	61.57	35.3	82.7	10.5	5.1	5.4	1.26	0.259	0.49	1.14	
5B4	24	30	64.08	25.4	42.3	14.7	1.9	12.8	1.07	0.259	0.25	1.25	
5B5	4	80	70.5	17.4	45	15.3	6.2	9.1	0.89	0.259	0.14	1.53	POSITIVE
5C1	6	88	88.4	20.6	45.8	12.7	6.4	6.3	0.78	0.278	0.05	1.01	
5C3	6	290	73.69	14.5	51.8	5.3	3.7	1.6	2.67	0.259	1.88	1.18	
5C4	2	124	68.17	18.7	30.8	25.9	9.4	16.5	1.53	0.259	0.89	0.84	STANDARD

	ALB	ALP	TP	ALT	AST	T.BIL	D.BIL	IND.BIL	T.CHOL	HDLP	LDLP	T.G	
3C1	20	80	90.96	39.3	61.1	21.3	9.5	11.8	5.02	0.523	4.18	0.7	
3C2	8	104	75.52	13.8	79.6	12.2	1	1.2	1.4	0.259	0.76	0.85	
3C4	8	130	68.97	34.7	83.5	21.8	7.9	13.9	1.82	0.349	1.28	0.43	
	12	104.6667	78.48333	29.26667	74.73333	18.43333	6.133333	8.966667	2.746667	0.377	2.073333	0.66	AVER
	6.928203	25.00667	11.29053	13.59056	11.96676	5.404011	4.517005	6.807594	1.979933	0.134209	1.84286	0.212838	S.D

	SOD	Catalase	GPX
3C1	0.026539	15.82642	138.0545
3C2	0.032766	6.146925	325.8748
3C4	0.018877	11.76952	283.4336
aveage	*0.026061*	*11.24762*	*249.121*
std	0.006957	4.860806	98.49941

4A1	4	160	73.1	5.29	105	13.8	1.9	11.9	1.662	0.259	1.13	0.61	
4A5	8	48	49.23	12.8	131	9.8	3.4	6.4	0.98	0.259	0.35	0.82	
	6	104	61.165	9.045	118	11.8	2.65	9.15	1.321	0.259	0.74	0.715	AVER
	2	56	11.935	3.755	13	2	0.75	2.75	0.341	0	0.39	0.105	S.D

4A1	*0.030347*	*2.592366*	*289.308*
4A2	0.081564	19.20474	226.5222
4A3	0.03218	7.114933	340.3525
4A4	0.036462	8.061787	464.0658
4A5	0.009292	30.81759	326.7785
average	0.037969	13.55828	329.4054
std	0.026537	11.42088	87.25382

ALP	TP	ALT	AST	T.BIL	D.BIL	IND.BIL	T.CHOL	HDLP	LDLP	TG	SOD	CAT	GPX	GROUP
243	85.77	63.9	167	10	1	4	2.39	0.25	1.14	0.21	0.01675	0.957368	223.2197	1A
217	83.73	63.6	133	10	1.6	4.4	1.48	0.259	0.81	0.6	0.005292	4.682186	267.276	1A
143	63.2	71.5	141	6.6	1	5.6	1.55	0.403	1.1	0.43	0.008339	2.574038	209.9802	1A
201	77.56667	66.33333	147	8.866667	1.2	4.666667	1.806667	0.304	1.016667	0.413333	0.010127	2.737864	233.492	AVERAGE
51.88449	12.48364	4.476978	17.77639	1.962991	0.34641	0.832666	0.506392	0.085855	0.180093	0.195533	0.005935	1.867805	29.99736	STDEV
ALP	TP	ALT	AST	T.BIL	D.BIL	IND.BIL	T.CHOL	HDLP	LDLP	TG	SOD	CAT	GPX	
140	72	69.5	171	1.5	1.9	0.5	3.3	0.259	2.22	0.44	0.140563	4.302455	182.959	1B
147	76	74.7	82.8	4.8	4.1	0.5	3.13	0.32	1.88	0.61	0.114161	7.912201	152.9443	1B
100	77	28.9	174	4.6	1	1.5	2.53	0.259	1.98	0.37	0.017742	7.406514	275.5915	1B
129	75	57.7	142.6	3.633333	2.333333	0.833333	2.986667	0.279333	2.026667	0.473333	0.090822	6.54039	203.8316	AVERAGE
25.35744	2.645751	25.07668	51.81004	1.850225	1.594783	0.57735	0.404516	0.035218	0.174738	0.123423	0.064651	1.954532	63.93225	STDEV

SAMPLES	ALB	ALP	TP	ALT	AST	T. BIL	D. BIL	IND. BIL	T. CHOL	HDLP	LDLP	T.G
1C1		54	99.99	43.3	90.3	1.9	0.9	1	1.25	0.355	0.6	0.66
1C2	12	42	78.98		100	5.1	4.4	0.7	4.32	0.259	2.98	2.4
1C3	12	47	65.92	57	109				4.61	0.402	3.4	1.8
1C4	16		73.59	44.1	115	4.9	2.1	2.8	1.19	0.259	0.68	0.56
1C5	16		62.25	62.6	99.8				1.27	0.478	0.43	0.81
AVERAGE	14	47.666667	76.146	51.75	102.82	3.9666667	2.4666667	1.5	2.528	0.3506	1.618	1.246
SD	2.3094011	6.0277138	14.838892	9.577926	9.4916806	1.7925773	1.7785762	1.1357817	1.7714457	0.0944368	1.4455172	0.8128222
Normal p va	0.1551216	0.0153474	0.4518114	0.074051	0.000045	0.0010576	0.0636322	0.0061633	0.1553832	0.6420016	0.1365562	0.9529621
std p	0.0032144	0.1271741	0.9530086	0.0022021	0.0001706	0.1559403	0.1061665	0.2100203	0.4715192	0.1820033	0.499846	0.6470937
negative p	0.0013107	0.00541	0.1672664	0.0720604	0.000018	0.0054905	0.0523598	0.0030358	0.5428785	0.1851177	0.3889525	0.4885635
2A1			74.41	12	158	3.8	2.3	1.5	1.27	0.259	0.71	0.67
2A2	16	220	75.72	16.6	134		3.5	3.7			1.74	

2A3	8		67.16	40	206	2.1	0.7	1.4	1.6	0.259	1.081	0.58
2A4	20	152	73.56		233	2	1.1	0.9	2.44	0.513	1.71	0.5
2A5	20	231	94.88	11.5		3.1	1.9	1.3	1.45	0.259	1	0.43
AVERAGE	16	201	77.146	20.025	182.75	2.75	1.9	1.76	1.69	0.3225	1.2482	0.545
SD	5.6568542	42.790186	10.44741	13.513049	44.924937	0.8582929	1.0954451	1.1081516	0.5178803	0.127	0.4567124	0.1034408
Normal p va	0.5475554	0.5939317	0.2696066	0.0918463	0.0032187	0.00002	0.0044916	0.0011373	0.1657793	0.9967577	0.0214987	0.0020375
std p	0.0236574	0.6414072	0.9606374	0.8073409	0.0035646	0.0659183	0.0151909	0.099931	0.9587962	0.4820932	0.5384659	0.0061661
negative p	0.0145526	0.5680743	0.0524613	0.0050568	0.0009877	0.000753	0.0043143	0.0006957	0.5080669	0.1761032	0.4450966	0.0217652
5B	18	149	81.45	33.5	38.4	13.1	6.4	6.7	1.55		0.99	
5B	14	239	73.41	44	42.3	14.7	3.4	5.6	1.57		0.72	0.96
5B	24	304	70.5	35.3	45	15.3	5.1	5.4	1.26	0.259	0.49	1.14
5B									1.07	0.259		1.25
5B							6.2	9.1	0.89	0.259		1.53
AVERAGE	18.666667	230.66667	75.12	37.6	41.9	14.366667	5.275	6.7	1.268	0.259	0.7333333	1.22
SD	5.033223	77.835296	5.6717458	5.6151581	3.318132	1.1372481	1.3744696	1.6990193	0.2970185	0	0.2502665	0.2387467
Std	0.011886	0.4555852	0.8237146	0.0060781	0.8970728	0.1598139	0.4783173	0.7224988	0.405886	0.3142531	0.7253508	0.2549872

negative	0.0047081	0.3728438	0.0238064	0.0339418	0.0578946	0.9638598	0.2871065	0.7187474	0.0695552	0.0208672	0.5119081	0.0260395
5C	6	88	88.4	20.6	45.8	12.7	6.4	6.3	0.78	0.278	0.05	1.01
5C	6	290	73.69	14.5	51.8	5.3	3.7	1.6	2.67	0.259	1.88	1.18
5C	2	124	68.17	18.7	30.8	25.9	9.4	16.5	1.53	0.259	0.89	0.84
AVERAGE	4.6666667	167.33333	76.753333	17.933333	42.8	14.633333	6.5	8.1333333	1.66	0.2653333	0.94	1.01
SD	2.3094011	107.74662	10.457114	3.1214313	10.816654	10.435197	2.8513155	7.6173049	0.9516827	0.0109697	0.916024	0.17
6A	6		62.35			16.2	6.2		1.18	0.32	0.25	1.34
6A	6	205	64.07	113	51.8	12.1	5.1	7	2.78	0.622	1.63	1.17
6A	8	202	61.87	80.2	40.9	22.2		6.1	2.67	0.344	1.51	0.8
6A			69.07	53.1	45.2				1.54	0.405	0.79	0.78
6A	4	132		76.1	52.4	15	9.2	5.8	1.76	0.681	0.74	0.75
AVERAGE	6	179.66667	64.34	80.6	47.575	16.375	6.8333333	6.3	1.986	0.4744	0.984	0.968
SD	1.6329932	41.307788	3.2917473	24.674008	5.5174722	4.2476464	2.1221059	0.6244998	0.706739	0.1659286	0.5766108	0.2693882

SAMPLE NAME	SOD (ɥ/mg)	CATALASE (ɥ/mg)	GPX (ɥ/mg)
1C1	0.089622	6.805296	272.8523
1C2	0.062541	8.818627	321.7987
1C3	0.053723	6.5535	346.7187
1C4			
1C5			
AVERAGE	0.06862867	7.392474333	313.7899
SD	0.01870773	1.241484546	37.57880946
positive control	0.08412202	0.032877615	0.21902899
std control	0.01303739	0.303138873	0.004199482
negative control	0.08741281	0.059226586	0.000910257
2A1	0.034619	4.870939	
2A2	0.032479	1.142464	155.7015
2A3	0.025236	0.676316	197.4166
2A4	0.022617		97.82972
2A5	0.020403	0.61516	
AVERAGE	0.0270708	1.82621975	150.31594
SD	0.00620255	2.04342699	50.0113973
positive control	0.08355831	0.462574938	0.002738507
std control	0.21482389	0.013742933	0.463872365
negative control	0.01711281	0.527960476	0.304247952
5B	0.025871	0.866682	361.7183
5B	0.060454	4.252955	335.7404
5B			
5B	0.035256	5.669118	344.3989
5B	0.044811	1.351051	

AVERAGE	0.041598	3.0349515	347.2858667
SD	0.01475841	2.306460434	13.22738653
STD	0.07379407	0.027996218	0.000142119
NEGATIVE	0.91833239	0.991233832	0.0000173
5C	0.016199	13.83373	
5C		10.77484	161.0941
5C	0.023825	6.015918	170.6735
5C	0.023944		192.8944
5C			
AVERAGE	0.02132267	10.20816267	174.8873333
SD	0.00443762	3.93959243	16.3135546
6A	0.050207		
6A	0.041468	6.042973	
6A	0.036153		114.015
6A		1.507037	106.5174
6A		1.490335	126.3804
AVERAGE	0.04260933	3.013448333	115.6376
SD	0.00709618	2.623658613	10.0304195