# EVALUATION OF DIFFERENT SOLVENT EXTRACTS OF *Vernonia amygdalina* ON SOME BIOCHEMICAL INDICES OF DIABETIC ALBINO RATS.

**BY**

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**CERTIFICATION**

This is to certify that this work "**Evaluation of Different Solvent Extracts of *Vernonia Amygdalina* on Some Biochemical Indices of Diabetic Albino Rats**" was carried out by Alozie, Evans Ugochukwu in partial fulfilment for the award of Master's Degree in Biochemistry, Federal University of Technology Owerri.

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## DEDICATION

This work is dedicated to the Lord Almighty God.

**ACKNOWLEDGEMENT**

I am indebted to Almighty God who has been the source of my life and strength throughout this period.

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Alozie Evans Ugochukwu November, 2023

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## Abstract

*The present study was designed to evaluate of different solvent extracts of Vernonia amygdalina on some biochemical lindices of diabetic albino rats. Five different solvents (having different polarity) were used for the extraction purpose. These solvents include Toluene, Benzene, Methanol , Ethanol and Water. The aim of this study was to determine the antidiabetic effect of Vernonia amygdalina extracts on Adult Male albino rats. The result showed that Ethanol and Methanol extract gave 33.19% and 43.66% blood glucose reduction. Hence, it compared favorably with the standard drug Metformin. Diabetic rats showed a gradually steady increase in body weight within the study period with the exception of the diabetic control and Benzene extract treated group. The diabetic control group showed increase in body weight which reduced at the end of the study period. However, group treated with Benzene extract showed a drastic decrease in body weight after 3 days of treatment. Thereafter, the body weights was gradually being restored but were not normalized. The serum enzyme activity of ALT, AST, ALP in the groups receiving Ethanol, Methanol, Toluene and Benzene extract were significantly reduced when compared to diabetic control group. However, administration of the different solvent extracts of V. amygdalina did not result in a significant($p<0.05$) normalization of altered serum bilirubin concentration. The extracts of Ethanol, Toluene, Benzene and Aqueous also produced varying degree of lipid profile reduction. The Methanol extract; Aqueous and Methanol extracts; Methanol, Toluene and Benzene; Methanol and Toluene; Ethanol, Methanol and Aqueous extracts caused a significant ($p<0.05$) reduction of total cholesterol concentration, Triacyglycerol, HDL-cholesterol, LDL-cholesterol concentration as well as LDL/HDL ratio and Cadiovascular risk ratio respectively. The extracts normalized the concentrations of ascorbicacid, α-tocopherol as well as SOD and GPx activity but offered a significant protection against Glutathione depletion when compared to diabetic control group. The Ethanol and Methanol extracts offered significant reduction in MDA concentration in both liver homogenate and serum when compared to the group treated with the standard drug. Histopathological examination results show mild inflammation and degeneration of the liver and kidney of rats induced with diabetes using120mg/Kg body weight of alloxan which was reversed by V. amygdalina leaf extracts of Methanol and Ethanol. These results therefore, indicated that V. amygdalina leaf extracts used in this study has varying hypoglycemic, hypolipidemic, hepatoprotective and endogenous antioxidant property with methanol showing a stronger protective effect against diabetes induced liver and kidney damage in rats compared to the other solvent extracts.*

***Keywords:*** *Anti-diabetic effect, Vernonia amygdalina, lipid profile, Liver function test, antioxidants, oxidative stress.*

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

The usefulness of plants to man is not only as a source of raw materials for industries, but also as a source of food and medication. From earliest times, plants have provided man with diverse means of healing. Many parts of plants such as fruits, seeds, barks, roots, fruits and flowers have been used as medicaments to cure various diseases that afflict man and other animals (Phyllistin *et. al*., 2000).

In Nigeria, as in other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Ojiako & Nwanjo, 2006).

Most communities especially in the South-South, South-East and South-West geo-political zones broadly use herbal medicines to treat various diseases and ailments which include: asthma, tuberculosis, ulcers, diarrhea, dysentery. (Nwaoguikpe, 2010).

Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases (Naczk & Shahidi, 2006). Increasing the consumption of fruits and vegetables has been recommended by many agencies and health care systems throughout the world (Vivekananthan,, *et. al.,* 2003). Plants are able to produce a large number of diverse bioactive compounds (Suffredini, *et. al.*, 2004).

Plants have been known to contain or possess abundant phytochemicals, antimicrobials and pharmacologically active principles, which include: anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids (Nwaoguikpe, 2010).

*Vernonia amygdalina* Del. popularly known as bitter leaf is a shrub of 2-5 m tall with petiolate green leaves of about 6 mm diameter (Ojiako & Nwanjo, 2006). The leaves are characteristically bitter but the bitterness can be abated by boiling or by soaking in several changes of clean water. The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are a very popular soup vegetable and have even been reported to be consumed by goats in some parts of Nigeria (Ojiako & Nwanjo, 2006).

## 1.2 Statement of the Problem

Despite many scientific breakthroughs in the areas of medicinal research in the developed and developing countries, yet diabetes is still a prevalent killer disease (Obeta & Ani, 2015). A staggering 53.1 million citizens will be affected by the diabetes according to experts who predicted that the incidence of diabetes is set to soar by 64% by 2025 (Rowley & Bezold, 2012). There is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs have undesirable effects (Kwameswara & Appa, 2001). Most diabetic patients especially in Nigeria are grossly faced with inadequate medicine, and cost of managing the disease is high, in addition the use of available antidiabetic drugs like metformin have several side effects, which compounds the existing problems faced by health care-givers (Ibegbu, *et. al.,* 2018). Many experimental studies of *V. amygdalina* Del. have reported that this plant possess anti-oxidant activity (Ayoola *et al*, 2008), anti-diabetic activity (Erasto, *et. al.*, 2009; Taiwo et al, 2009) and liver protective effect (Arhoghro *et. al*, 2009; Adesanoye & Farombi, 2009). There are many techniques to recover antioxidants from plants, such as soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound assisted extraction. However, extraction yield and antioxidant activity not only depend on the extraction

method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Do, *et.al.,* 2014). Different solvent systems have been used to extract antioxidants from plant materials such as fruits, vegetables, legumes, and other foodstuffs. Water, aqueous mixtures of ethanol, methanol, and acetone are commonly used to extract antioxidants from plant foods (Xu & Chang., 2007). And have also studied its effect on alloxan/streptozotocin induced diabetic rats. Still yet the most effective polar solvent extract of V. amygdalina for treatment of the disease has not been investigated. This study will compare five different polar solvent leaf extract of *V. amygdalina* to investigate the efficacy of the extracts on alloxan induced diabetes rats.

## 1.3 Objectives of Study

1. The study compared serum blood glucose levels.
2. The study compared the body weight of the animal.
3. The study evaluated the levels of non-enzymatic antioxidants (vitamin C and E and GSH) in the liver tissue homogenate.
4. The study evaluated the effect of the extract on the activities of enzymatic antioxidants (SOD, GPx) and (MDA) concentration in the liver tissue homogenate and serum of the rat.
5. The study evaluated the effect of the extracts on the liver function parameters (ALT, AST, ALP).
6. The study evaluated the effect of the extracts on the lipid profile parameters (HDL, LDL and Total Cholesterol) of the rats
7. The study evaluated the effect of the extracts on LDL/HDL-cholesterol ratio and cardiovascular risk ratio (CRR).
8. Histopathological studies of the liver and kidney tissue of the rats.

## 1.4 Justification

Herbs have been used in pre-historic times as a form of natural remedy to serveal ailments, of which type 2 Diabetics mellitus has been part of them. Many times people live with this ailemsnts and never develop any form of complications and cant trace reasons to anything tangible. Medically people persons who have developed diabetics are now being advised to diet, exercise along with the insulin and hypoglycemic agents that are recommended. In addition to this, old parents who leaved long with this ailments used Bitter leaf as one of those herbs that proved effective to lower their blood sugar levels. This discovery have long been worked on by several researcher using other polar solvents aside water to extract Bitter leaf, to see if the healing effect on the body might be better. Hence, the present study was designed to evaluate the anti-diabetic effect of polar extracts and drug of VA. This study therefore, will create reawakening information that will serve as a database for both Nigerians at home and in the diaspora to positively affect the consumption of *Vernonia amygdalina.*The study will contribute greatly to the existing body of knowledge on *Vernonia amygdalina* with a view to improving the accessing the medicinal compoenets of the plant while also serving as a baseline for further research.

## 1.5 Scope of Study

This research will evaluate and compare the serum blood glucose levels and the body weight of animal; the non-enzymatic antioxidants such as vitamin C, vitamin E, and GSH in the liver tissue homomgenate; the activities of enzymatic antioxidants such as, superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) concentration; the liver function parameters (ALT, AST, ALP); the LDL/HDL-cholesterol ratio and cardiovascular risk ratio (CRR) and the histopatholgical studies of the lover and kidney tissues of the rats.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Herbs

The utilization of herbal extracts to treat diabetes related illness has increased over the years, according to WHO, due to poverty and lack of access to modern medicine, moderate percentage of world population found in the developing countries depend mostly on plants for primary health care (Ibegbu, *et. al*., 2018).

## 2. 2 *Vernonia amygdalina*

The Asteraceae (Compositae) are herbs, shrubs, or less commonly trees and has approximately 1,620 genera and more than 23,600 species. Vernonia is a genus of about 1,000 species of forbs and shrubs of which V. amygdalina is the most prominent specie and one of the pan tropical tribes of the family Asteraceae. Normally, *V. amygdalina* does not produce seeds but its cultivation is usually done by stem planting and mostly grow in tropical areas. This plant is found majorly along the drainage, commercial plantation or forest (Yeap, *et al*., 2010). Several species of Vernonia, including; *V, calvoana, V.amygdalina and V, colorata* are eaten as leaf vegetables. (Ajuru, *et al.,* 2013). Some of its species; *Vernonia cinerea* and *Vernonia anthelmintica* are available in India (Ejiofor, *et.al.,* 2017).

*Vernonia amygdalina*, is a shrub that grows up to three meters high in African tropics and other parts of Africa, particularly, Nigeria, Cameroon, and Zimbabwe (Momoh, *et al.,* 2014). The bark is rusty to dark-brown. The leaves are green with a charastaristic color and bitter taste (Gospel *et al*., 2013).

The bitter taste of VA is as a result of its antinutritional components such as alkaloids, saponins, glycosides and tannins. The reported activity of VA is attributable to the complex active secondary plant compounds that are pharmacologically active (Clement *et al.,* 2014).

In Nigeria, it is known by several local names such as ‘’Ewuro’’ in Yoruba language, ‘’Onugbu’’ in Igbo language, “Oriwo” in Bini language, “Ityuna” in Tiv language, “Chusar doki or fatefate” in Hausa language and “Etidot” in Ibiobio (Clement*et al.,* 2014).

*Vernonia amygdalina* has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins like vitamin A, C and E. (Reginald., 2010).

Several studies carried out on this plant had suggested that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones. These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, antiinflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Oluwaseun, *et al.,* 2017).

The macerated leaves of the plant are consumed as vegetables and condiments while the water extract serves as tonic for the prevention of certain illnesses (Arhoghro, *et al.,* 2009).

**Plate. 2.1: Picture of *Vernonia amygdalina***

**Scientific classification of *Vernonia amygdalina***

Kingdom ⟶ Plantae

Phylum ⟶ Tracheophyta

Class ⟶ Magnoliopsida

Order ⟶ Asterales

Family ⟶ Asteraceae

Genus ⟶ Vernonia

Species ⟶ *Vernonia amygdalina*

## 2.3 Traditional medicine and research

It is a highly appreciated vegetable in west and central Africa where it is commonly used in traditional medicine. It performs both medicinal and nutritive functions (Olowolafe & Olufayo., 2018). The roots and

leaves decoction of VA are commonly used in ethno medicine to treat fevers, hiccups, kidney problems and stomach discomfort among other several uses. It is also used in the treatment of diarrhea, dysentery hepatitis and cough and as a laxative and fertility inducer. In addition, extracts of the plants have been reported to be used in Nigerian herbal homes as tonic, in the control of tick and treatment of hypertension. The root infusion is taken in Nigeria for the treatment of intestinal worms as well as for enteritis and rheumatism. It is known as quinine substitute because it is widely used for the treatment of fevers. Fresh leaves of VA have been reported to have abortifacient and purgative activities. It is used in some part of Africa to prepare cough remedy. The root of VA is used for its antifertility effect and for the treatment of amenorrhoea. The chopped roots of VA are used for the treatment of sexually transmitted diseases in parts of Zimbabwe. The root of VA is used for its antifertility effect and for the treatment of amenorrhoea (Clement, *et al.,* 2014). Teas containing bitter leaf (*V. amygdalina*) are also used throughout West Africa for the management of diabetes and other metabolic diseases associated with the liver (Leonard, *et al.*, 2002). The Medical Traditional Healer Association in Rukararwe, Uganda produced the greenish powder packed in sachet and consume as tea by patients suffering from malaria (Njan, *et al*., 2008). In contrary, Temma people of Sierra Leone called bitter leaf as

'goat killer', this makes the animals to stay away from it due to its bitterness (Yeap *et al.*, 2010).

### 2.4 Plants as sources of natural antioxidants

Despite the fact that humans are equipped with an impressive repertoire of antioxidant enzymes as well as small antioxidant molecules, these agents may not be sufficient enough to normalize the redox status during oxidative stress (Seifried, *et. al., 2007*). Plants, especially medicinal herbs, have been used for the prevention and/or treatment of several diseases since very old times (Ou *et al., 2003*). Plant extracts, such as flavonoids and phenolics, have raised public interest in their potential to act as antioxidants. Natural antioxidants can strengthen the endogenous antioxidant

defense from ROS ravage and restore the optimal balance by neutralizing the reactive species (Soobratte *et .al., 2005*). Moreover, the medicinal plants also exhibit far stronger antioxidant activity and contain significantly higher levels of phenolic compounds than common vegetables and fruits (Cai *et.al.,* 2004). Therefore, the medicinal plants are promising sources of natural antioxidants (Krishnaiah *et. al., 2011*).

Some of the previously isolated constituents in *Vernonia amygdalina* Del. include: sesquiterpene lactones, flavonoids like luteolin, luteolin 7-O-glucosides and luteolin 7-O-glucuronide, steroid glycosides, and vernonioside A, B, A1, A2, A3, B2, B3 and A4 (Farombi& Owoeye, 2011).

**2.4.1 Flavonoids (flavones)**

*Vernonia amygdalina* leaves contain flavones that are antioxidants and can combate carcinogens as well as the ageing process because of its medicinal value. Majority of flavonoids are derivatives of two phenylchroman (flava). They are also representatives of heterocyclic compounds containing in their molecule the functional groups C=O, C=H, as well as a prominent fragment of a quinol ring. It is due to the presence of these groups in their molecules and some sugar components that makes these components to have their common physical and chemical properties. Some of the flavonesthat occur in *Vernonia amygdalina* leaves include luteolin 7-O-β-glucoronoside, luteolin 7-O-β-glucosides and leutolin. The antioxidant activities of these flavonoid compounds isolated from the leaves have been reported using coupled oxidation of β-carotene and linoleic acid. Luteolin 7-O-β-glucoronoside which is said to be the most abundant of the flavones is a highly potent antioxidant in this plant. Flavones play a beneficial role oncertain organs of the body like the smooth muscle, the internal organ vessels and bile ducts (Ekam *et.al.,* 2010).

### 2.4.2 Saponins

Saponin is another highly active compound in *Vernonia amygdalina*. It is made up of complex molecules in which the glycans are triterpeniods or steroidal. Saponins are starch glycosides which have complex sugar components attached to 3 hydroxyl groups. It iis also said to be a white or light brown amorphous powder with a sweet taste, which later has a bitter taste and even acid burning taste. Saponins are supposedly believed to be too irritating to the gastrointestinal tract, to be used internally. The stigmastene type steroidal saponins are vernoniosides and all part of the reason behind the characteristic bitter taste in *Vernonia amygdalina.* Also noted were the dominant stigmastene type saponin, vernonioside D which makes up 35% of the saponins responsible for the characteristics of *Vernonia amygdalina* when it was washed. Experimentally, steroidal saponin in *Vernonia amygdalina* seems to be higher than that found in other plants(Ekam, *et.al.,* 2010).

Other anti-nutritive factors responsible for the bitter taste of VA include tannins, glycosides.

## 2.5 Acute Toxicity Tests

Despite these varied uses of the plant, there has been insufficient information on its exact toxicological potentials on the animal system. There have been reports of the presence of toxic phytochemicals. There are also reports of actual hepatotoxicity in mice, yet there is a report that extracts from the plant are able to inhibit and even reverse carbon tetrachloride-induced hepatotoxicity in rats. (Ojiako & Nwanjo, 2006)

Egharevba et al. (2014) reported that the toxicity limit was insignificant when compared with the highly toxic substances (toxicity at less than 1 mg/kg). Studies on the acute toxicity of the leaf extracts resulted in $LD_{50}$ of 5.1523 g/kg when administered orally and this showed that the extracts were non-toxic (Adiukwu *et al.,* 2012).

Another acute toxicity studies showed an $LD_{50}$ of 500 mg/kg body weight. This indicates that the vegetable when consumed in high quantities may, indeed, elicit hepatotoxicity as observed in small animals like mice by Igile and co-workers. Traditionally, however, the vegetable is macerated and the dilute extract administered for therapeutic purposes while in nutritional applications, the leaves are soaked and washed severally in clean water prior to its use in soup making. Some times, the washed vegetables are sun-dried and then used installmentally. Such processing methods have been shown to detoxify several leafy vegetables that are ordinarily very toxic in their raw state, including the more popular vegetable *Telfaria occidentalis* (fluted pumpkin). *V. amygdalina* leaves may be toxic (just like several other vegetables) if consumed in very large quantities but the potential danger is not higher than has been observed for other common vegetables that are routinely consumed in Africa in even larger quantities. (Ojiako & Nwanjo, 2006)

## 2.6 Effect of Solvent Polarity on the Extraction of Components

In order to extract different phenolic compounds from plants with a high degree ofaccuracy, various solvents of differing polarities must be used. Moreover, scientists have discovered that highly polar solvents, such as methanol, have a high effectiveness as antioxidants. Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents is as follows:

Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water.
It has been reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol**(**Ammar *et al,* 2017).

**Polarity of Solvents**

Water
Acetic acid
Ethyleneglycol **Polar**
Methanol
Ethanol
Isopropanol
Pyridine
Acetonitrile
Nitromethane
Diehylamine
Aniline
Dimethylsulfoxide
Ethylacetate
Dioxane
Acetone
Dicholoroethane
Tetrahydrofuran
Dichloromethane
Chloroform
Diethylether
Benzene
Toluene
Xylene
Carbontetrachloride
Cyclohexane
Petroleum ether
Hexane
Pentane **Non-Polar**

## 2.7 Oral Antidiabeteic Drug

Four categories of oral antidiabetic agents are available namely; insulin secretagogues, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors (Nolte & Karam, 2001). In addition, we also have the Dipeptidyl-peptidase-4 (DPP-4) inhibitors, Glucagon – like peptide – 1 (GLP-1) agonist, Sodium glucose – 2 (SGLT2) inhibitors (Diabetic drugs, 2018).

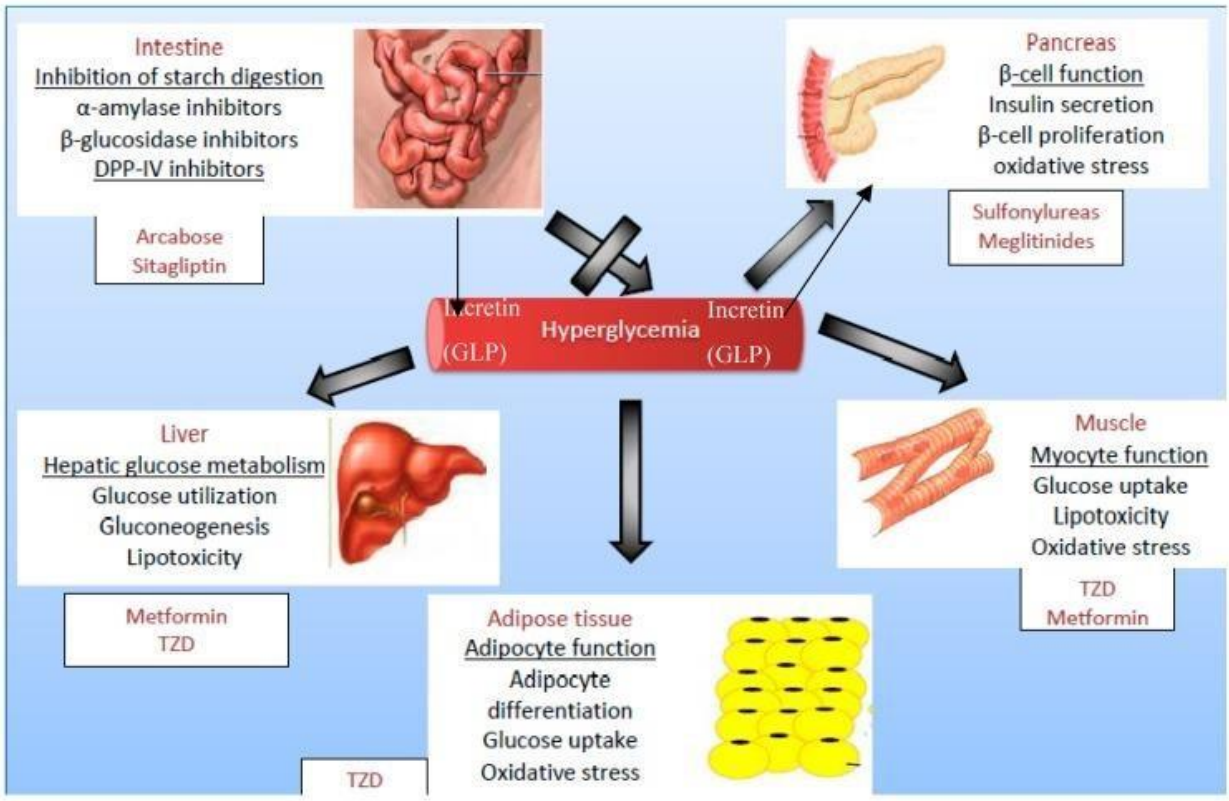
Three types of biguanides are being used in the treatment of diabetes namely phenformin, buformin and metformin. The use of the first two mentioned was discontinued in the United States of America due to its association with lactic acidosis (Nolte & Karam, 2001).

Metformin originates from the French lilac, *Galega officinalis* L., a perennial herb known for centuries to reduce the symptoms of diabetes. The active compound is galegine, a guanidine derivative. Metformin's clinical trails were successfully completed in 1995 and its use approved in the United States of America. The full extent of the mechanism of the action of biguanides is unknown, but its blood glucose-lowering action does not depend on the presence of functioning pancreatic beta cells. Proposed mechanisms of action includes direct stimulation of glycolysis in the tissue, and the increase of glucose removal from the blood; reduced hepatic gluconeogenesis; slowing of glucose absorption from the gastrointestinal tract; with increase glucose to lactate conversion by enterocytes and the reduction of plasmaglucagon levels (Nolte & Karam, 2001). Metformin acts as an insulin sensitizing agent, probably through activation of adenosine monophosphate dependent (AMP) kinase in liver and muscle tissue. Metformin is often associated with weight loss making it a preferred, first line agent for management of overweight patients with type 2 diabetes

Metformin has not been linked to serum enzyme elevations during therapy and is an exceeding rare cause of idiosyncratic clinically apparent acute liver injury (LiverTox, 2012).

## 2.8 Target Organs in Diabetes Treatment

Most conventional and herbal treatments are targeted towards specific organs or metabolic pathways as shown in figure below. These treatments either activate chemicals that enhance insulin secretion or suppress hepatic glucose output. The potency of the documented medicinal plants used for the treatment of diabetes has been attributed to the presence of their phytochemicals. These phytochemicals are synthesised by plants to protect themselves from internal stresses such as free radicals, and external stresses from insects and pests; this property of plants explains their potential to cure diseases and their benefits in traditional medicine. The phytochemicals of these plants have been reported, and their mechanism of action has been suggested (Samuel & Graeme, 2018)




Summary of therapeutic targets for the management of diabetes mellitus. TZD =

Thiazolidinedione. DPP-IV: Dipeptidyl peptidase IV; GLP: Glucagon-like peptide 1. (Samuel & Graeme,2018).

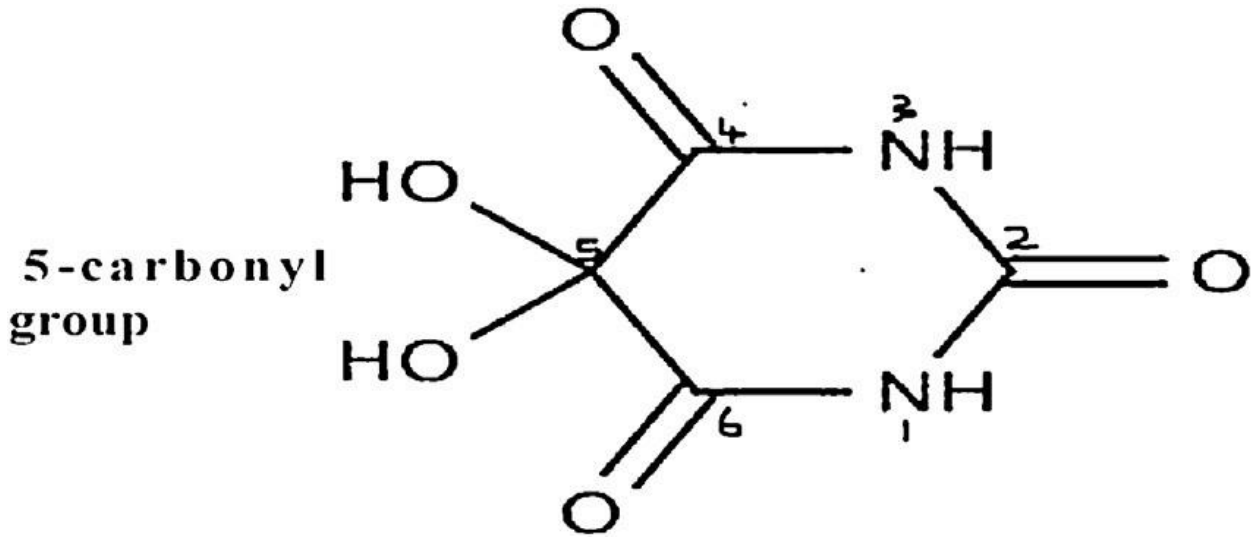
## 2.9 Diabetogenic Agents

The five major diabetogenic agents are chemicals, biological agents, peptides, potentiators, and steroids but most commonly used chemicals agents are alloxan and streptozotocin (Vineeta & Janeshwer, 2014).

## 2.10 Alloxan

Alloxan which is chemically known as 5, 5-dihydroxyl pyrimidine- 2, 4, 6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti-insulin serum; alloxan and streptozotocin (STZ) are the most widely used in diabetes studies. (Osasenaga, *et.al.,* 2017)

The drug has been noted to its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species and route of administration. Moreover, alloxan has been demonstrated to be non-toxic to the human beta-cells, even in very high doses, because humans have different glucose uptake mechanisms as compared to rodents (Vineeta & Janeshwer, 2014).




**Chemical structure of alloxan.**

Alloxan is a very unstable chemical compound with a molecular shape resembling glucose. Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane. The alloxan molecule is structurally so similar to glucose that the GLUT2 glucose transporter in the beta cell plasma membrane accepts this glucomimetic and transports it into the cytosol. Alloxan does not inhibit the function of the transporter, and can therefore selectively enter beta cells in an unrestricted manner. (Lenzen, 2008)

Alloxan induces a multiphasic blood glucose response when injected into an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultra-structural beta cell changes ultimately leading to necrotic cell death (Ighodaro, *et.al.,* 2017).

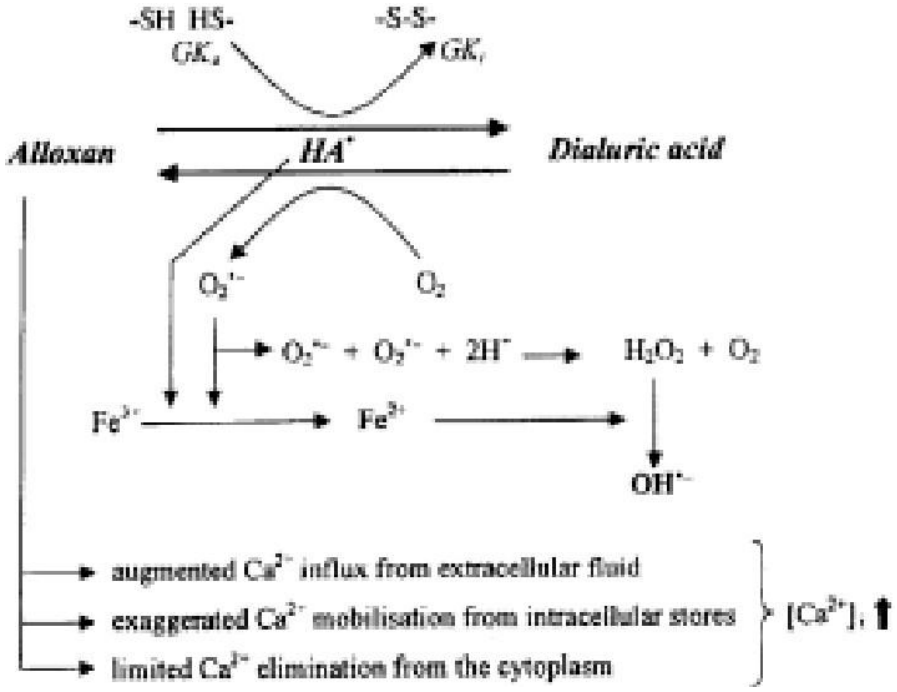
Glucose also provides complete protection against all toxic effects of alloxan both in vivo and in vitro. This universal protection is achieved through the prevention of glucokinase inhibition and the preservation of the antioxidative defence mechanisms of the beta cell. The nonmetabolisable glucose analogue 3-O-methylglucose also provides protection, but does this exclusively through

the prevention of alloxan uptake into the beta cell via the GLUT2 glucose transporter. The protection offered by glucose explains why fed animals are less sensitive to alloxan induced diabetes compared with fasted animals with relatively lower blood glucose level (Lenzen, 2008)

## 2.12 Mechanism of action

Alloxan treatment evokes a sudden rise in insulin secretion in the presence or absence of glucose and this insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used. Further, important feature of alloxan action in pancreas is preceded by its rapid uptake by pancreatic beta cells. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups. Alloxan reacts with two - SH groups in the sugar binding site of glucokinase and results in inactivation of the enzyme. As a result dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle and generates reactive oxygen species (ROS) and superoxide radicals. The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions and also undergo dismutation to yield hydrogen peroxide ($H_2O_2$). As a result, highly reactive hydroxyl radicals are formed in the presence of ferrous and $H_2O_2$. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. In the beta cells alloxan causes DNA fragmentation and damage. Antioxidants like superoxide dismutase, catalase and the non enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity. In addition cytosolic free elevated $Ca^{2+}$ has also been reported to constitute an important step in the diabetogenic action of alloxan. The calcium influx results from the ability of alloxan to open voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of $Ca^{2+}$ ion further contributes to supraphysiological insulin release that

along with ROS eventually causes damage of beta cells of pancreatic islets (Vineeta & Janeshwer, 2014).




**The mechanism of alloxan-induced reactive oxygen species generation in B cells of rat pancreas. GKa, GKi – glucokinase active and inactive, respectively; HA· - alloxan radicals; [Ca2+]I – intracellular calcium concentration.**

## 2.13 Alloxan model of diabetes mellitus

1. Alloxan is very rapidly destroyed in the body. Its half – life is less than 1 minute. Hence, its action is most unpredictable unless it is administered by rapid intravenous injection.

   Slow rate of intravenous injection reduces the efficacy of the agent.
2. Very young animals have a high resistance to the diabetogenic effect of alloxan.
3. Damage to the renal tubules by alloxan also alters blood glucose level which may also influence the results of study.

4. The susceptibility to both toxic and diabetogenic dose varies widely not only in different species but also among animals of the same species.
5. Rats were more sensitive to a constant does of alloxan after a fore period on a high fat diet and less sensitive after a high carbohydrate and protein diet.
6. The spontaneous recovery is particularly evident in rats and mice, in which the majority of animals become normoglycemic. The recovery from the diabetes is believed to be the consequence of either a multiplication of B cells from the duct epithelium or the endocrine portion of the pancreas. This is the reason why mild diabetes produced by alloxan recovers spontaneously in rats.
7. Lower doses of alloxan (90-140 mg/kg, i.p.) can result in auto-reversion to normal state. It has been found that in many research studies, the dose of alloxan used to induce diabetes was suboptimal. In these studies, it was found that alloxan induces very mild diabetes. However, in these animals, revert back to the blood glucose values reverted back to normal in a week.
8. B cells of islets of Langerhans in rats show regenerative capacity.
9. Human islets tissue is exceedingly resistant to the degenerative effects of alloxan. Alloxan diabetes differs strikingly from other types of experimental diabetes in the course and nature of the lesions which are developed (Dinesh & Raj, 2011).

## 2.14 Diabetes mellitus

According to Urmila & Goyal, 2013 Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling, pathways, and the defect usually results from pancreatic β-cell deficiency and/or a deficiency of insulin.

Diabetes mellitus (DM) can also be seen as a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both (Ozougwu, *et. al.*, 2013). Diabetic complication involves vital organs such as the heart, kidney, liver, eye and blood vessels etc (Oluwole *et al*., 2011).

Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes (Akram & Hisham, 2015**)**.

Common symptoms of diabetes mellitus include frequent urination, excessive thirst, intense hunger and fatigue irritability, blurred vision, wounds that do not heal quickly or adequately, sexual dysfunction in men, and gum infections (Samuel & Graeme,2018). Acute complications include diabetic ketoacidiosis, hyperosmolar hyperglycaemic state or death (Kitabchi, *et. al*, 2009). Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome (Akram& Hisham, 2015**)**.

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of

atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (American Diabetes Association, 2010).

Infectious diseases are more prevalent in individuals with DM. Some infections almost always affect only diabetic persons, such as malignant external otitis, rhinocerebral mucormycosis, and gangrenous cholecystitis.(Juliana*et. al*, 2012)

People with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease (Fox *et. al.* 2007). Persons with DM come up with big babies making conception a bit complicated (Gestational diabetes).

DM can arise from other diseases or due to drugs such as genetic syndromes, surgery, malnutrition, infections, and corticosteroids intake (Waqas, *et. al*., 2017)

Diabetes is one of the biggest global public health problems: the prevalence is estimated to increase from 425 million people in 2017 to 629 million by 2045, with linked health, social, and economic costs (International Diabetes Federation, 2017).

The prevalence of diabetes mellitus in Nigeria has been reported to have increased from 2.2% in 1997 to 5.0% by 2013, diabetes mellitus is amongst the leading cause of mortality in Africa. Unlike in Africa, diabetes mellitus prevalence in India has reached a pandemic level, with number of diabetic patients reaching over 62 million this reflects the global burden of the disease (Ibegbu, *et.al.*, 2018)

### 2.16 Classifications of Diabetes

Diabetes can be classified into the following general categories:

1. Type 1 diabetes or insulin dependent diabetes (due to b-cell destruction, usually leading to absolute insulin deficiency)

2. Type 2 diabetes or non-insulin dependent diabetes mellitus (due to a progressive insulin secretory defect on the background of insulin resistance)

3. Prediabetes is a term used to refer to subjects who have a high risk of future type 1 diabetes or type 2 diabetes, with the understanding that not all subjects who meet the definition for prediabetes will coming down with diabetes (Genuth *et.al.,* 2018).

4. Gestational diabetes mellitus (GDM) is any degree of glucose intolerance with onset or first recognition during pregnancy. GDM can classify as A1GDM and A2GDM. Gestational diabetes managed without medication and responsive to nutritional therapy is diet-controlled getational diabetes or A1GDM. On the other shand, gestational diabetes managed with meidcatin to achieve adequate glycemic control is A2GDM (Quintanilla Rodriguez & Mahdy, 2023).

5. Diabetes insipidus (DI) is a disorder of water homeostasis that is characterized by excretion of large volumes of hypotonic urine either due to the deficiency of the hormone arginine vasopressin [AVP, also knownas antidiuretic hormone (ADH)]. Or due to resistance to the action of AVP on its receptors in the kidneys. It is typicall classified into 3 forms: 1 Central DI, 2. Nephrogenic DI and Gestational DI (Di Iorgi *et.al*., 2012; Garrahy *et.al.,* 2019).

6. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation)**.** (American Diabetes Association, 2015)

## 2.17 Type 2 Diabetes mellitus

This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive (American Diabetes Association, 2010). Type 2 DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure (Olusegun &Lateefat, 2012).

The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors (Zimmet, et.al., 2001). Type 2 diabetes is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, overeating, lack of exercise, and stress, as well as aging. (Kohei, 2010). A number of lifestyle factors are known to be important to the development of type 2 DM. These are sedentary lifestyle, cigarette smoking and generous consumption of alcohol (Hu, *et. al.*, 2001). T2DM factors can be irreversible (Waqas, *et. al.*, 2017). Environmental toxins may contribute to the recent increases in the rate of type 2 DM. A weak positive correlation has been found between the concentration in the urine of bisphenol A, a constituent of some plastics, and the incidence of type 2 DM (Lang, *et. al.*, 2008).

Obesity, which generally results in impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type II diabetes are obese (Nolte & Karan, 2001) and will ultimately require multiple anti-diabetic agents to maintain adequate glycaemic control (Gerich, 2001).

The increased consumption of sugar-sweetened beverages containing fructose increases the likelihood of being overweight or obese thus increasing the risk and prevalence of type II diabetes (Fabiyi-Edebor & Fasanmade, 2019).

Whereas few studies have found strong association of T2DM with high intake of carbohydrates and fats. Many studies have reported a positive association between high intake of sugars and development of T2DM (Khatib, 2004). High intake of red meat, sweets and fried foods, contribute to the increased the risk of insulin resistance and T2DM (Panagiotakos, 2005).

**Comparison of Type 1 and Type 2 diabetes (**Dean & McEntyre, 2004)

| | **Type 1 diabetes** | **Type 2 diabetes** |
|---|---|---|
| Phenotype | Onset primary in childhood and adolescence | Onset predominantly after 40 year of age * |
| | Often thin or normal weight | Often obese |
| | Prone to ketoacidosis | No ketoacidosis |
| | Insulin administration required for survival | Insulin administration not required for survival |
| | Pancreas is damages by an autoimmune deficiency | Pancreas is not damaged by an autoimmune attack |
| | Absolute insulin deficiency | Relative insulin deficiency and/or insulin resistance |
| | Treatment: insulin injections | Treatment: (1) healthy diet and increased exercise; (2) hypoglycemic tablets; (3) insulin injections |
| Genotype | Increased prevalence in relatives | Increased prevalence in relatives |
| | Identical twins studies: <50% concordance | Identical twins studies: usually above 70% concordance |
| | HLA (Human leucocyte antigene)associations: Yes | HLA associations |

## 2.18 Signs and Symptoms

The classic symptoms of diabetes such as polyuria, polydypsia and polyphagia occur commonly in type 1 diabetes, which has a rapid development of severe hyperglycaemia and also in type 2 diabetes with very high levels of hyperglycaemia. Severe weight loss is common only in type 1

diabetes or if type 2 diabetes remains undetected for a long period. Unexplained weight loss, fatigue and restlessness and body pain are also common signs of undetected diabetes. Symptoms that are mild or have gradual development could also remain unnoticed (Ramachandran, 2014).

## 2.19 Pathophysiology

**Insulin** is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, adipose tissue and muscle, except smooth muscle, in which insulin acts via the insulin-like growth factor-1 (IGF-1). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

The body obtains glucose from three main sources: the intestinal absorption of food; the breakdown of glycogen, the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body (Shoback & Gardner, 2011). Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen.

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells as well as decreased rate of breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Barrett, 2012).

If the amount of insulin available is insufficient, or if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (Shoback & Gardner, 2011).

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) (Murray, 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia) (Shoback & Gardner, 2011).

## 2.20 Diagnosis

| **Measure** | **American Diabetes Association** | | **World Health Organization** | |
|---|---|---|---|---|
| | Diabetes | Prediabetes | Diabetes | Impaired Glucose |
| Fasting plasma glucose | ≤126 mg/dl | 100-125 mg/dl/(IFG) | ≤126 mg/dl | 100-125 mg/dl/(IFG) |
| 2 – Hr plasma glucose (during an OGTT with a loading dose of 75g) | ≤200 mg/dl | 140-199mg/dl/(IGT) | ≤200mg/dl | 140-199 mg/dl/(IGT) |
| Casual (random) plasma glucose (In a patient with classic hyper glycemic symptom) | ≤200 mg/dl | | ≤200mg/dl | |
| Glycated hemoglobin | ≤6.5% | 5.7-6.4% | ≤6.5% | |

Major diagnostic criteria for diabetes and prediabetic or at – risk states. Data are adapted from the

American Diabetes Association. Adapted from Salomaa & Diabetes Care. Bearse *et al.,* 2004; Hove *et.al.,* 2004

## 2.21 Myths and Misconceptions among Patients with Diabetes Mellitus

In other regions of the world, various myths and misconceptions have been responsible for some avoidable indirect cost of the burden of the disease, for example a report in India showed more than 43.9% of a population believes wrongly that "High sugar intake causes diabetes", likewise Bertran et al. reported that a population of Arab American diabetics believes anything that is bitter is good for diabetes. Some believe that taking insulin injection signify the final stages of type 2 diabetes not knowing that the medication type given to patients is determined by the stage of the disease and what is most appropriate per situation. (Olamoyegun, *et. al.,* 2018)

## 2.22 Complication of Type 2 Diabetes

The complications of type 2 diabetes are divided into macrovascular and microvascular disease. These complications are a result of injury to the blood vessels. Moreover, if the blood vessels are small, they are called microvascular and if they are large blood vessels, they are called macro vascular complications. The main macro-vascular complications include coronary heart disease (CHD), stroke (cardiovascular accident), myocardial infarction (MI), peripheral arterial disease (PAD) heart failure (CHF). The micro-vascular complications include nephropathy, retinopathy, and neuropathy, and these lead to renal failure, blindness, and lower extremity amputation. (Charles, 2017)

## 2.23 Epidemiology

Diabetes happens to be a worldwide problem and has also become an increasing health burden. The majority of those who are affected are the middle-income populations living in Asia, Africa and South America (Medagama & Bandara., 2014). Diabetes causes one death every 6 sec with death cases (5 million), a rate higher than all deaths from human immunodeficiency virus (HIV) (1.5 million), tuberculosis (1.5 million) and malaria (0.6 million) combined (Kesavadev, *et.al.*,2017).

According to the International Diabetes Federation (IDF), the prevalence of diabetes globally is estimated to rise from 382 million in 2013 to 592 million by 2035 (Guariguata, *et al.,* 2014). This increase cuts both ways and is driven in part by improved medical advancement that promotes longer lifespan in the general population. This has increased the aging population, which in turn has contributed to the increase in type 2 diabetes among seniors ages 65 and older. Almost 12 million seniors or 25.9% have type 2 diabetes in the United States (CDC, 2012). The increase or growth in minority ethnic groups with higher rates of pre-diabetes and type 2 diabetes (family history), obesity, sedentary lifestyles, socio-economic status, place of residence, increasing age, and diets that are rich in fats have all been recognized as the major drivers for the increased prevalence of type 2 diabetes in the U.S. and the world (Charles, 2017).

The WHO 2004 report estimated that 1.7 million people in Nigeria had diabetes, with the projection that the number will triple by 2030 (World Health Organization, 2004).

Coronavirus infections are proven to have a huge effect on the management of diabetes mellitus because they aggravate inflammation and alter immune system responses, leading to difficulties in glycaemic control. SARS-CoV-2 infection also increases the risk of thromboembolism and is more likely to induce cardiorespiratory failure in patients with diabetes mellitus than in patients

without diabetes mellitus (Lim, *et.al.,* 2020). A higher mortality rate was recently suggested in patients with COVID-19 who had preexisting diabetes (Lana & Marcello, 2020).

### 2.24 Prevention and control of Diabetes Type 2

To combat the growing global diabetes epidemic, the American Diabetes Association has called for increased use of lifestyle interventions as part of primary care (ADA, 2017). The main reasons for occurrence of insulin resistance is associated with physical inactivity and or a sedentary lifestyle of the sufferer, which in turn increases postprandial hyperglycemia (PPHG)(Nikhil, *et. al.,* 2017).

In particular, several trials suggest that vegetarian and vegan low-fat diets are superior to conventional diets for lowering glucose, HbA1c, and insulin levels; decreasing diabetes medication dosages; and reducing diabetes complications, such as neuropathy, in T2D patients.

This is corroborated by prospective cohort studies, such as the Nurses' Health Study and Adventist Health Study-2, which report that plant-based diets reduce the risk of developing type 2 diabetes by about half. Moreover, among populations such as the Seventh-day Adventists, the prevalence of diabetes among vegans is as low as 0.22 times that among non-vegetarians. Plant-based diets have also been found to improve lipids, renovascular health, and oxidative stress levels in adults with T2D more effectively than conventional diets. Plant-based diets likely mediate these effects through multiple mechanisms, including through improvements in body weight and visceral fat [e.g. insulin sensitivity and beta-cell function; incretins and gastrointestinal hormones; oxidative stress; phytochemical intake [including polyphenols and plant sterols]; fiber and prebiotic intake, which positively modulate gut microbiota; lower oxidant intake (such as heme iron); and increased wellbeing.

Lifestyle interventions that combine plant-diets with regular exercise may be even more effective. In the late 1990's, Canvasback Missions, Inc., recognized the need for such interventions in the RMI (Republic of the Marshall Islands) and conducted two lifestyle intervention programs with excellent results, using a modified version of the NEWSTART wellness program operated by the Guam Seventh-day Adventist Clinic. NEWSTART is an abbreviation or acronym that is interpreted thus; Nutrition, Exercise, Water, Sunshine, Temperance, Air, Rest, and Trust in divine power respectively. The program emphasizes a whole-food, plant-rich diet with moderate exercise and is used to treat T2D, obesity, and other chronic diseases. Although no studies have been published on the intervention, Guam physicians have found it effective and continue to refer their patients to wellness centers (Davis, *et. al.,* 2019).

The alimental α-glucosidase and α-amylase inhibitors acting in gut moderately deter the enzymatic breakdown of soluble starches, which have been recognized as a natural and safe method in controlling PPHG (postprandial hyperglycemia). Only few studies regarding the enzyme inhibitory activity of fermented food products (i.e. douche, koumiss, kefir, viili etc.) are available. Work in this direction regarding this topic is in progress at different research organizations. Various traditional African vegetables shows significant inhibition against α- amylase (i.e. >70%). Japanese and Chinese vegetables were also screened in an enzyme immobilized system for their α-glucosidase inhibitory activities and except for tomato all other raw vegetables (i.e. radish, cabbage, onion, cucumber, and carrot) exhibited an inhibitory effect on α-glucosidase than boiled samples. Likewise, brinjal, onion, mushroom *Ganodermalucidum*, garlic, and sea cucumber have also been tested (*in vitro* and *in vivo*) for their α- glucosidase and α-amylase inhibition role. The cereal grains constitute the most important part of human diet playing an important role in diabetes management via inhibition of related digestive enzymes. These include millets (i.e. finger,

sorghum, foxtail etc.), Bengal gram, wheat, beans, and several others. Additionally, spices also exert several beneficial physiological effects, including the antidiabetic influence. Commonly used spices in Indian cuisine such as fenugreek, fennel powder, cardamom powder, mustard, ginger, cinnamon, and turmeric have been explored for anti-diabetic activity. Additionally, the fruits consist of different bioactive compounds and antioxidants, improving resistance of the body involving traditional remedies treating the disorders of diabetes, obesity and or diabesity. Research indicated that fruits such as apples, watermelon, berries, grapes, sugarcane, mulberry, jamun, pineapple, mango, maple, Indian gooseberry, chinese yam, kiwifruit, and more possess strong inhibitory activity against α-glucosidase and α-amylase under *in vitro* as well as *in vivo*, thereby exhibiting the role in prevention of diabesity (Nikhil, *et. al.*, 2017).

### 2.25 Oxidative stress and antioxidant protection mechanisms

Oxidative stress occurs when this balance is disrupted by excessive production of reactive oxygen species (ROS) (Li *et. al..*, 2016). ROS are produced by neutrophils and macrophages during the process of respiratory burst in order to eliminate. They also serve as stimulating signals of several genes which encode transcription factors, differentiation, and development as well as stimulating cell-cell adhesion, cell signalling, involvement in vasoregulation, fibroblast proliferation, and increased expression of antioxidant enzymes. However over and/ or uncontrolled production of ROS is deleterious (Brahm Kumar Tiwari, *et. al..*, 2013).

Hyperglycemia and free fatty acid intake are among the causes for oxidative stress conditions (Evans, *et al..*, 2002). Oxidative stress caused by hyperglycemia in diabetes may impair insulin signaling, leading to insulin resistance. Pancreatic beta-cells are especially sensitive to ROS and RNS, because their natural enzymatic antioxidant defenses are lower compared to other tissues

such as liver. Moreover, they lack the ability to adapt their low enzyme activity levels in response to stress such as high glucose or high oxygen (Lazo-de-la-Vega-Monroy & Fernández-Mejía., 2013). Glucose enters to the beta-cell in an insulin independent fashion, because besides providing energy, glucose sensing in the beta-cell is crucial for insulin secretion. It has been suggested that hyperglycemia can generate chronic oxidative stress by the glucose oxidation pathway (Robertson *et al..*, 2003), leading to an excess in mitochondrial superoxide production, which further activates uncoupling protein-2 (UCP-2). This protein lowers ATP/ADP relationship through proton leak in the beta-cell, which reduces insulin secretion (Lazo-de-la-Vega-Monroy & Fernández-Mejía., 2013). Weak defence system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress (Brahm Kumar Tiwari, *et. al..*, 2013).

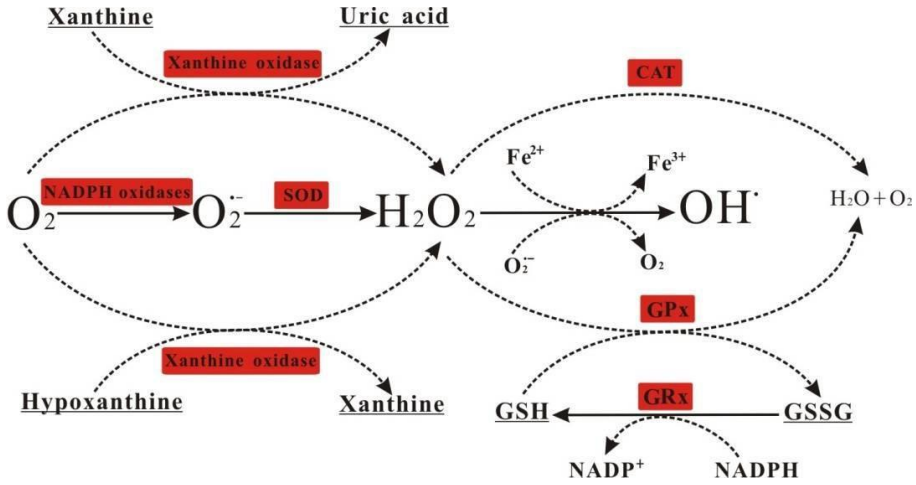
Antioxidants are central to the redox balance in the human body. The term 'antioxidant' refers to any molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage a target molecule (Li, *et. al.,* 2016). Humans have several mechanisms to counteract oxidative stress, either by producing antioxidants from endogenous antioxidant systems or externally supplied through exogenous antioxidants (Figure 2). The endogenous antioxidant systems, including enzymatic and non-enzymatic antioxidants, play a crucial role in maintaining optimal cellular functions. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx). SOD, the first line of defense against free radicals, catalyzes the dismutation of $O_{2-}$• to $O_2$ and to the less-reactive species $H_2O_2$ by reduction. In humans there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn- SOD, and extracellular SOD (EC-SOD). The $H_2O_2$ is transformed into water and oxygen by CAT or GPx. The selenoprotein GPx enzyme removes $H_2O_2$ by

using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power.

The non-enzymatic antioxidants are also divided into endogenous (metabolic) and exogenous (nutrient) antioxidants. Endogenous antioxidants are produced by metabolism in the body, such as lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metalchelating proteins, transferrin. Exogenous antioxidants are compounds that cannot be produced in the body, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids. These exogenous antioxidants must be provided by foods or supplements via diet (Li *et. al.,* 2016)

Vitamins C, E, and A constitute the non enzymatic defense against oxidative stress, by regenerating endogenous antioxidants. Vitamin C has a role in scavenging ROS and RNS by becoming oxidated itself. The oxidized products of vitamin C, ascorbic radical and dehydroascorbic radical are regenerated by glutathione, NADH or NADPH. In addition, vitamin

C can reduce the oxidized forms of vitamin E and gluthatione (Garcia-Bailo, *et. al.,* 2011). Vitamin E is a fat-soluble vitamin which may interact with lipid hydroperoxides and scavenge them. It also participates, together with vitamin C, in gluthatione regeneration by interaction with lipoic acid (Evans, *et. al.,* 2002). Vitamin A has a plethora of cellular actions. Besides modulating gene expression, cell growth and differentiation, this vitamin may also act as antioxidant, although the mechanisms of action in this role are not fully deciphered deciphered (Lazo-de-la-Vega-Monroy & Fernández-Mejía, 2013).




**A schematic diagram showing the production of free radicals via different routes and the interaction between intracellular antioxidants.** SOD=superoxide dismutase; CAT= catalase; GPx=glutathione peroxidase; GRx= glutathione reductase; GSH=reduced GSH (L-γ-glutamyl-Lcysteinyl-glycine); GSSG=oxidized GSH.

# CHAPTER THREE

## Materials and Methods

### 3.1 Materials

#### 3.1.1 Chemicals/ Reagents

Alloxan (Sigma-Aldrich No USA), Ethanol, Toluene, Benzene, Methanol, normal saline, formalin ALT test kit (Randox), AST test kit (Randox), AST test kit (Randox), Alkaline phosphatase test kit, Bilirubin test kit (Biosystems), Cholesterol test kit (Biosystems), HDL-Cholesterol test kit (Biosystems), Triglycerides test kit (Biosystems), LDL-Cholesterol test kit (Biosystems ), protein test kit ( Randox) and Albumin test kit.

#### 3.1.2 Equipment and apparatus

Digital spectrophotometer model 390 (Turner®, USA), weighing balance (Mettler-Wagen, Switzerland), water-bath (Grant, England), bench centrifuge (Clay Adams, USA), Soxhlet extractor, Syringe, intubator, automatic pipettes (Teco diagnostics, USA), Digital pH meter (Labtech India), glucometer (accu-chek active), glucose strips.

### 3.2 Methods

#### 3.2.1 Experimental Animals:

Fifty (50) adult male albino rats (*Rattus norvegicus*) (120-350 g) obtained from a certified source was used for the study. The rats were allowed to acclimatize inthe Animal friend farms Owerri, Imo State, where they will be housed throughout in the experimental cage. They were fed with standard rat feed, with water *ad libitum*, but starved for 12 hr prior before commencement of the experiment.

### 3.2.2 Preparation of V. *amygdalina* Del extracts:

The sample of bitter leaf (*Vernonia amygdalina Delile*) was collected in May, 2021 from Anguldi, Zawan Bukuru metropolis, Plateau State, Nigeria. The sample was then authenticated by Mr. O.E. Agyeno at the Herbarium Unit of the Department of Plant Science and Biotechnology, Faculty of Natural Science, University of Jos, Nigeria with Voucher number (JUHN21000359).

The whole plants were collected an dried under a shed for 7 days at ambient temperature (25°C). The dried leaves were subjected to pulverization to get coarse powder. The coarsely powder whole plant (1kg) of V. *amygdalina* Delwere used for extraction with 2.0 L polar solvents (A=Toluene, C=Methanol and D=Ethanol and) in a soxhlate apparatus while similar amount (B=Benzene and E=Water) was etracted by cold maceration for 48hrs. The extract were evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w) (Mohammed *et al*., 2010).

### 3.2.3 Induction of experimental diabetes:

The groups of the experimental animal that were diabetic groups were fasted for 12hours, after which the initial blood glucose levels were measured using Accu-check Active Glucometer. Alloxan monohydrate was used to induce diabetes in experimental rat groups. Diabetes was induced by injecting a dose of 120mg/kg b.wt of alloxan monohydrate dissolved in sterile normal saline intravenously within the tail region of the rats. The alloxan induced rats were monitored for 3 days with free access to food and water. On the $4^{th}$ day the rats were fasted for 12hrs and their blood glucose levels were determined using glucometer. Rats with blood glucose level ranging from 200-400mg/dl were used for the experiment.

### 3.2.4 Extract and Drug Administration

Before use, the extracts was reconstituted in normal saline (vehicle) and administered orally via gastric intubation at a particular dose of X mg kg $^{-1}$ b.w. for each single extract treatment. An antidiabetic drug (i.e Metformin) was administered to the animals based on their body weights to the control was given normal saline.

### 3.2.5 Experimental design

The rats were divided into eight groups comprising five animals in each group (n=5). Therewere a total of forty adult male albino rats and the groups were as follows;

**Group 1**= control that was given normal saline (vehicle)

**Group 2**= diabetic control rats that was given alloxan at a dose of 120mg/kg b.w.

**Group 3**= diabetic rats that was giventoluene extract of *V. amygdalina* (200mg kg$^{-1}$ b.w.)

**Group 4**= diabetic rats that was givenbenzene extract of*V. amygdalina* (200mg kg$^{-1}$ b.w.)

**Group 5**=diabetic rats that was givenmethanol extract of*V. amygdalina* (200mg kg$^{-1}$b.w.)

**Group 6**= diabetic rats that was given ethanol extract of*V. amygdalina* (200mg kg$^{-1}$ b.w.)

**Group 7**= diabetic rats that was given water extract of*V. amygdalina* (200mg kg$^{-1}$ b.w.) **Group 8**= diabetic rats that was given anti-diabetic drug, Metformin (200mg kg$^{-1}$ body wt day$^{-1}$) using an gavage tube for 14 days.

All treatmentwas given orally to experimental rats using a gavage tube using a single dose daily; and alo fed with normal feed ad clean water *ad libitum*.After 14 days of treatment, the animals fasted overnight; rats were sacrificed by light anesthesia using dichloromethane. A portion of blood was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 min and the clear sera aspirated off for biochemical evaluation using commercial kits. The liver and kidney were then quickly removed, washed in ice-cold, isotonic saline and blotted individually on ashfree

filter paper. The tissues were then homogenized in 0.1 M Tris-HCl buffer, pH 7.4 using a Potter-Elvejham homogenizer at 4°C with a diluting factor of 4, the crude tissue homogenate will then be centrifuged at a speed of 9000 rpm for 15 min at room temperature and the supernatant was kept at -20°C for biochemical analysis. The homogenate were used for the estimations of tissue enzymes activities.

### 3.2.6 Assay of biochemical parameters

#### 3.2.6.1 Determination of Glucose Concentration

Glucose concentration was determined using glucose oxidase method by Trinder, (1969), with the aid of a glucometer ( Accu-chek active).

##### 3.2.6.1.1 Principle:

It is based on the reaction of glucose and oxygen in the presence of glucose oxidase to yield gluconic acid and hydrogen peroxide. The hydrogen peroxide formed subsequently reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. The hydrogen peroxide oxidizes the dyes in a reaction mediated by peroxidase producing a blue coloured product, with the intensity of the colour read from the accu-chek active glucometer.The colour intensity is proportional to the glucose concentration of the sample.

### 3.2.7 Assay of Lipids

#### 3.2.7.1 Determination of the Concentration of Total Cholesterol

Serum total cholesterol concentration was determined using the enzymatic (cholesterol esterase/ oxidase/peroxidase) method of Allain *et al*., (1974).

**3.2.7.1.1 Principle:**

Free and esterified cholesterol in the sample originates by means of the coupled reactions described below, to form a coloured complex that absorbs at 500nm.

Cholesterol ester + $H_2O$→ Cholesterol + fatty acid

Cholesterol + $^1O_2$ + $H_2O$→ Cholesterol + $H_2O_2$

$2H_2O_2$ +4- aminoantipyrine + phenol→ Quinoneimine +$4H_2O_2$

**3.2.7.1.2 Procedure:**

The reagents were brought to room temperature and pipetted into test tubes as follows;

| **Reagents** | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| Cholesterol | - | 10µl | - |
| Sample | - | - | 10µl |
| Reagent A(35mmol/l sodium chelate, 28mmol/l phenol, cholesterol esterase>0.2U/ml, cholesterol oxidase>0.4U/l, peroxidase>0.8U/ml; 0.5mm0l/l 4aminoantipyrene, pH7.0) | 1.0ml | 1.0ml | 1.0ml |

The test tubes were mixed thoroughly and incubated for 10minutes at room temperature.

The absorbance (A) of samples or standard was read against the reagent blank at 500nm in the spectrophotometer.

**Calculations;**

Total cholesterol in the sample is given by;

$$\mathbf{C_{sample}} = \frac{A_{sample}}{A_{standard}} * \mathbf{C_{standard}}$$

Where;

$C_{sample}$ = concentration of cholesterol in the sample

$C_{standard}$ = concentration of the standard

$A_{sample}$ = absorbance of the sample

$A_{standard}$ = absorbance of the standard

Total cholesterol was measured in mg/dl

**3.2.7.2.1 Determination of Triacylglycerol Concentration**

The glycerol phosphate oxidase/peroxidase method described by Bucalo and David (1973) and Fossati and Principe (1982) was used.

**3.2.7.2.2 Principle;**

Triacylglycerol in the sample originates by means of the coupled reactions described below, to form a coloured complex that can be measured by spectrophotometry.

Triglycerides + $H_2O$ → Glycerol + fatty acid

Glycerol + ATP → Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + $O_2$→ Dihydroxyacetonephosphate + $H_2O_2$
$2H_2O_2$ +4-aminoantipyrene + chlorophenol → Quinoneimine + $4H_2O$

**3.2.7.2.2 Procedure:**

| **Reagents** | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| TG standard (glycerol 200mg/dl) | - | 10µl | - |
| Sample (serum) | - | - | 10µl |
| Reagent A(45mmol/l glycerol kinase>1.5µmol/ml, glycerol-3-phosphate oxidase>4µmol/ml, peroxidase>0.8µmol/ml, 4-aminoantipyrene 0.75mmol/l, ATP 0.9µmol/l, pH7.0 | 1.0ml | 1.0ml | 1.0ml |

The test tubes were mixed thoroughly by vortexing and incubated for 15mins.

The absorbance (A) of samples or standard was read against the reagent blank at 500nm in the spectrophotometer.

**Calculation**;

The triacyglycerol concentration of the sample was calculated thus;

$$\mathbf{C_{sample}} = \frac{A_{sample}}{A_{standard}} * \mathbf{C_{standard}}$$

Where;

$C_{sample}$ = concentration of triacylglycerol in the sample

$C_{standard}$= concentration of triacylglycerol in the standard
$A_{sample}$ = absorbance of sample

$A_{standard}$ = absorbance of standard

Triacylglycerol was measured in mg/dl

### 3.2.7.3 Determination of High Density Lipoprotein (HDL)-Cholesterol

Serum HDL-cholesterol concentration was determinedusing the phosphotungstate/Mg-cholesterol oxidase and peroxidase method of Grove (1979) & Burstein *et al*., (1980).

#### 3.2.7.3.1 Principle:

Very –low- density lipoprotein (VLDL) and LDL in the sample was precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL. The HDL-Cholesterol was measured spectrophotometrically by means of the coupled reactions described below;

Cholesterol esters + $H_2O$→Cholesterol + fatty acid

Cholesterol + ½ $O_2$ + $H_2O$→ Cholesterol + $H_2O_2$

$2H_2O_2$ + 4-aminoantipyrene +phenol → Quinoneimine + $4H_2O$

Procedure;

The reagents were brought to room temperature and pipetted into labelled test tubes thus;

| | |
|---|---|
| Sample (serum) | 0.2ml |
| Reagent-A (0.4mmol/l phosphotungstate and 20mmol/l magnesium chloride) | 0.5ml |

The test tubes were mixed thoroughly by vortexing and left to stand for 10minutes at room temperature. They were centrifuged at 4000rpm for 10minutes.

Supernatants were separated from the precipitate and pipetted into labeled test tubes and the procedure continued as follows;

| **Reagents** | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| Distilled water | 50µl | - | - |
| HDL-Cholesterol standard | - | 50µl | - |
| Sample supernatant | - | - | 50µl |
| Reagent B(35mmol/l phosphate buffer, 0.5mmol/l sodium cholate, cholesterol esterase>0.2U/ml, cholesterol oxidase>0.1U/l, peroxidase>1.0U/ml; 0.5mmol/l 4-aminoantipyrene, 4mmol/l dichlorophenolsulphanate), pH7.0 | 1.0ml | 1.0ml | 1.0ml |

The contents of the test tubes were thoroughly mixed and incubated for 30minutes at room temperature. The absorbance (A) of samples or standard was read against the reagent blank at 500nm in the spectrophotometer.

HDL-Cholesterol in the sample was calculated using the formula;

$$\mathbf{C}_{\mathbf{supernatant}} = \frac{A_{sample}}{A_{standard}} * \mathbf{C}_{\mathbf{standard}}$$

Where;

$C_{supernatants}$ = concentration of HDL-Cholesterol in the supernatant

$C_{standard}$ = concentration of HDL-cholesterol in the standard

$A_{sample}$ = absorbance of sample

$A_{standard}$ = absorbance of standard

HDL-Cholesterol was measured in mg/d

**3.2.7.4 Determination of the Concentration of Low-Density Lipoprotein (LDL)-Cholesterol** LDL concentration was determined according to the method of Assman *et al*., (1984).

**3.2.7.4 Principle**;

LDL in the sample precipitates with polyvinyl sulphate. Their concentration is calculated from the difference between the total cholesterol and the cholesterol in the supernatant (HDL) after centrifugation.

**3.2.7.4.1 Procedure**;

The reagents after being brought to room temperature were pipetted into labeled test tubes as follows:

| | |
|---|---|
| Sample | 0.4ml |
| Reagent A(3g/l polyvinylsulphate, 3g/l polyethylene glycol) | 0.2ml |

The test tubes were mixed thoroughly by vortexing and left to stand for 15minutes at room temperature, after which they were centrifuged at 4000rpm for 15minutes.

The supernatants were carefully collected into labelled test tubes and the process was continued as follows;

| **Reagents** | **Blank** | **standard** | **Sample** |
|---|---|---|---|
| Distilled water | 20µl | - | - |

| | | | |
|---|---|---|---|
| Cholesterol standard | - | 20µl | - |
| Sample supernatant | - | - | 20µl |
| Reagent A (35mmol/l sodium cholate, 28mmol/l phenol, cholesterol esterase>0.2U/ml, cholesterol oxidase >0.4U/ml, peroxidase >0.8U/ml; 0.5mmol/l 4-aminoantipyrene), pH7.0 | 1.0ml | 1.0ml | 1.0ml |

The test tubes were mixed thoroughly by vortexing and incubated for 30 minutes at room temperature.

The absorbance (A) of samples or standard was read against the reagent blank at 500nm in the spectrophotometer.

LDL in the sample was calculated as thus;

$$C_{supernatant} = \frac{A_{sample}}{A_{standard}} * C_{standard} * \text{Dil factor}$$

Where;

$C_{supernatant}$ = concentration of LDL-cholesterol in the supernatant

$C_{standard}$ = concentration of LDL-Cholesterol in the standard

$A_{sample}$ = absorbance of sample

$A_{standard}$ = absorbance of standard LDL-Cholesterol

was measured in mg/dl.

**LDL cholesterol** = Total cholesterol – Triglyceride/5 – HDL cholersterol (mg/dl)

**LDL/HDL – cholesterol ratio** = LDL-C/HDL-C

**Cardio Risk Ratio (CRR)** = TC/HDL-C

### 3.2.8 Liver Function Tests

The activities of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and Serum alkaline phosphatase (ALP) were assayed with sandard laboratory procedures.

#### 3.2.8.1 Assay of Serum Alanine Aminotransferase (ALT) Activity

Alanine Aminotransferase (ALT) Activity was determined using the colorimetric method of Reitman & Frankel (1957).

**3.2.8.1.1Principle**: ALT catalyse the conversion of L-alanine and α-Ketoglutarate to pyruvate and glutamate. In reaction II below, lactate dehydrogenase (LDH) catalyses the oxidation of the reduced cofactor to the oxidized form. The rate of decrease in absorbance of the reaction mixture at 540nm, due to oxidation of the reduced cofactor is directly proportional to the ALT activity.

α -Ketoglutarate + L- alanine $\xrightarrow{ALT}$ L-glutarate + Pyruvate - - - I

Pyruvate + NADHLDH $\xrightarrow{}$ Lactate + $NAD^+$ - - - II

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4dinitrophenylhydrazine.

**3.2.8.1.2Procedure**: The blank and sample test tubes were set up in duplicates. An amount of 0.1 ml of serum was pipetted into the sample tubes. To these were added 0.5 ml buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37 $^0$C and pH 7.4. an amount 0.5 ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to both tubes while 0.1 ml of sample was added to sample blank tube. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25

$^0$C. About 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 540nm.

**3.2.8.1.3 Calculation:** The activity was calculated as follows:

$$\text{Activity if ALT (IU/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3300$$

**3.2.8.2 Assay of Serum Aspartate Aminotranfserase (AST) Activity**

Aspartate aminotransferase activity was determined using the colorimetric method of Reitman & Frankel (1957).

**3.2.8.2.1 Principle**: AST catalyses the reaction of α –Ketoglutarate and L-aspartate to L-glutamate and oxaloacetate. In reaction II below, malate dehydrogenase (MDH) catalyse the oxidation of NADH to $NAD^{+.}$ The rate of decrease in absorbance of the reaction mixture at 546nm, due to oxidation of NADH is directly proportional to the AST activity. Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4dinitrophenylhydrazine.

α –Ketoglutarate + L- aspartate $\xrightarrow{\text{AST}}$ L- aspartate + oxaloacetate - - - I

Oxaloacetate + NADH + $H^+$ $\xrightarrow{\text{MDH}}$ Malate + $NAD^+$ - - - II

**3.2.8.2.2 Procedure**: The blank and sample test tubes were set up in duplicates. About 0.1ml of serum was pipetted into the sample tubes. An amount of 0.5 ml of Reagent 1 was pipetted into both sample and blank tubes. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37$^0$C and pH 7.4. An amount of 0.5 ml of Reagent 2 containing 2, 4-

dinitrophenylhydrazine was added into all the test tubes followed by 0.1 ml of sample into the blank tubes. The tubes were mixed thoroughly and incubated for exactly 20 minutes at $25^0$C. An amount of 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 546 nm.

**3.2.8.2.3 Calculation:** The activity was calculated as follows:

$$\text{Activity if AST (IU/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3300$$

**3.2.8.4 Assay of Serum alkaline Phosphatase (ALP) Activity**

Alkaline phosphatase activity was determined using the colorimetric method of Babson *et al*. (1966).

**3.2.8.4.1 Principle**: The principle is based on the reaction involving serum alkaline phosphatase and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns pink that can be determined spectrophotometrically.

P-nitrophenylphosphate + $H_20$ ---------➏ ALP ---------➏ $PO_4^{2-}$ + P-nitrophenol (pink at pH=9.8)

**3.2.8.4.2 Procedure**: The blank and sample test tubes were set up in duplicates. 0.05 ml of sample was pipette into the sample test tubes. 0.05 ml of distilled water was pipetted into the blank tube. 3.0 ml of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405 nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one minute intervals.

**3.2.8.4.3 Calculation**: Alkaline phosphatase was calculated as follows:

$$\text{Activity of ALP (IU/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standand}} \times 3300$$

Alkaline phosphatase activity was determined using the colorimetric method of Babson *et al*. (1966).

**Assay of Serum Bilirubin (BIL) Concentration**

Principle: Determination of BIL concentration was based on the Jendrassik and Grof's Method (1938). Conjugated bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex (azobilirubin compound). The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffein- benzoate accelerator which releases the albumin bound bilirubin. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized Sulphanilic Acid → Azobilirubin Compound

Procedure: The reagent was brought to room temperature and was pipetted into labeled test tubes as follows:

**Bilirubin analytical procedure**

| | Sample Blank | Sample |
|---|---|---|
| Reagent 1 – (Sulphanilic acid – 29mmol/l + Hydrochloric) Acid 0.17 | 200µl | 200µl |
| Reagent 2 – (Sodium Nitrate – 38.5mmol/l) | | 1drop(50µl) |
| Reagent 3 – (Caffein – 0.26mol/I) + Sodium benzoate - 0.52mol/l) | 1000µ | 1000µl |
| Sample | 200µl | 200µl |
| Mix incubate for 10 min at 20 – 25 ℃ | | |
| Reagent 4 – (Tartrate – 0.93mol/l + NaOH – 1.9N) | 1000µl | 1000µl |
| Mix, incubate for a further 5 – 30 minutes at 25 ℃ | | |
| Insert the curvette into the $R_x$ Monza analyzer | | |

**For direct bilirubin:**

| | Sample Blank | Sample |
|---|---|---|
| Reagent 1 – Sulphanilic acid – 29mmol/l + Hydrochloric Acid 0.17N) | 200µl | 200µl |
| Reagent 2 – (Sodium Nitrate – 38.5mmol/l) | | 1 drop(50µl) |
| 0.9% Sodium Chloride | 2000µl | 2000µl |
| Sample | 200µl | 200µl |

Mix, incubate for 10 min at 20 – 25 ˚C
Insert the curvette into the $R_x$ Monza analyzer

### 3.2.9 Assay of antioxidant enzymes

#### 3.2.9.1Assay of Glutathione peroxidase Activity

This was determined according to the method of King and Wootton (1959).A volume of the sample (0.1 ml) was mixed with 0.9 ml of distilled water in a beaker. Sodium sulphate (0.02 ml) was also added, shaken and allowed to stand for 2mins at room temperature. A volume, 0.02 ml of Lithium Sulphate (20%), 0.2 ml of 20% $NaCO_3$ and 0.2 ml of phosphor- 18-tungstic acid were also added to the beaker; it was shaken and allowed to stand for 4mins while observing for maximum colour development. A volume, 2.5 ml of 2% sodium sulphite was also added and the absorbance was taken at 680nm within 10mins. A blank was also set up.

#### 3.2.9.2 Assay of Superoxide dismutase Activity

The activity of superoxide dismutase (SOD) was assayed using the method of Xin *et al.* (1991).

**3.2.9.2.1 Principle:** Superoxide dismutases are enzymes that catalyses the conversion of two superoxides into hydrogen peroxide and oxygen.


The benefit here is that hydrogen peroxide is substantially less toxic than superoxide. Erythrocyte superoxide dismutase (SOD) activities serve as antioxidant enzymes. The principle of SOD activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction.

**3.2.9.2.2 Procedure:** Adrenalin (0.01 g) was dissolved in 17 ml of distilled water and 0.1ml of serum and 0.9 ml of phosphate buffer (pH 7.8) were taken in triplicates in 2.5 ml buffer. A volume, 0.3 ml adrenaline solution was added and mixed inside the cuvette. The absorbance was taken at 480nm at 30 seconds interval for five (5) times. The changing rate of absorbance was used to determine superoxide dismutase activity.

**3.2.9.3 Assay of Malondialdehyde (MDA)**

Malondialdehyde (MDA), an end product of lipid peroxidation, was estimated by the method of Jacobson *et al* with minor modification. A 10% of tissue homogenate was prepared in Tris–HCl buffer (20 mM, pH 7.4). Prior to homogenization, 10 mL 0.5MBHT in acetonitrile should be added per 1 mL of tissue homogenate. After homogenization, the homogenate was centrifuged at 3000 g at 4°C for 10 minutes, and the clear supernatant was used for the assay. Briefly, 200 mL of supernatant was transferred to 650 mL of 10.3 mM 1-methyl-2-phenylindole in acetonitrite and vortex mixed. To assay MDA alone, 150 mL of 37% HCl was added instead of MSA, vortexed, and incubated at 45°C for 60 minutes. After incubation the sample were kept on ice, centrifuged at 9500 g for 5 minutes, and absorbance was measured at 586 nm. The levels of MDA is expressed as nmol $g^{-1}$ tissue using extinction coefficient 1.1 – 105 M–1 cm–1 (Sharma *et.al.*, 2015).

### 3.2.9.4 Determination of Vitamin C

An amount of 1.5 ml of filtered supernatant was placed into a centrifuge tube, 1.5ml of PTR was added with stirring and after 30 min the fluid from above the sediment called "tested sample" was centrifuged (3500 X g, 10 min). Absorbance *A*700 of this sample and of standard vitamin C solution subjected to the same procedure (without centrifuging) was measured at 700 nm, using as a reference, the mixture 1:1 (/v) of PTR and the solvent applied to prepare the standard solution (Rutkowski *et. al.*, 2002).

**Calculations**

The vitamin C calculation in tissue homogenate was calculated according to the formular (Rutkowski, *et. al.*, 2002):

$$C_{vit\ C}[\mu mol/l] = \frac{A_{test.}}{A_{stand.}} \cdot c_{stand.} \qquad (1)$$

**Where:** $C_{vit\ C}$ - concentration of vitamin C in homogenate, *A* test. – absorbance of the tested sample, *A* stand. – absorbance of standard solution, c stand. – concentration of standard solution. The obtained result was used to calculate vitamin C contents in the investigated tissue according to the formular (Rutkowski *et. al.*, 2002).

$$\text{Contentsof vitamin C } [\mu g/g] = \frac{c_{vit.C}}{m} \cdot 4.4 \qquad (2)$$

Where: m – weight massof the investigated tissue [g], 4.4 – coefficient resulting from the mathmathecal relation between constant elements of the calculations explained below. Since 1 mg AA = 5.68 µmol (MAA – 176.12 g/mol), then dividing *c*vit. C by 5.68 µmol/l changes into mg/l, which divided by 40 (quotient of definition volume 1000ml for molar concentration and the volume of 25 ml homogenates) gives the amount of vitamin in 25ml of tissue homogenate of mass m. Dividing the result by *m*, the contents of vitamin C in the investigated tissue is obtained in mg/g,

which multiplied by 1000 changes into µg/g. a sequence of operations results from that: *c*vit.C. 1000/5.68 . 40 . *m*, in which the quantity 1000/ 5.56. Where 40 is constant and equals 4.4 being the coefficient in formular (Rutkowski *et. al.,* 2002).

### 3.2.9.5 Procedure of α-tocopherol concentration determination

### 3.2.9.6 Standard Preparations

The retinol stock standard solution of 1,000µg/ml was prepared by weighing 25mg into a 25mL amber volumetric flask and bringing up to volume with 1% BHT in methanol. All intermediate standards of retinol were made by serial dilutions in 1% BHT in methanol. The α-tocopherol stock standard 1,000µg/ml was nade by weighing 50mg into a 50mL volumetric flask and bringing up volume with methanol. All intermediate standards were made by serial dilutions in methanol. Stock standard solutions and intermediate solutions for both vitamins were stored -20C in amber˚ vials to protect from light-induced oxidation (Diao *et. al.,* 2020).

### 3.2.9.7 Chromatographic Conditions

An Agilent 1200 high-performance liquid chromatography (HPLC) system eqqipped with 120 Infinity FLD fluorescence detector (Agilent, Santa Clara, CA) and ChemStation software to control the system were used for the quantitative analysis of both vitamins. For α-tocopherol analysis, a spherisorb OD 4.6 x 150mm, 5µm particles (Waters Corporation, Milford, MA) reverse phase column was used. A single mobile phase consisting of 100% methanol was used (isocratic) at a flow rate of 1 mL/min. The total time was 9 min. The fluorescence detector was set to excitation and emission wavelengths of 295 and 340 nm, respectively. The column temperature for both analyses was ambient (Diao *et.al.,* 2020).

### 3.2.9.8 Sample preparation of α-tocopherol analysis

Vitamin E was analyzed as α-tocopherol. For liver tissues, sample preparation began by weighing each tissue (wet mass) into individual 50 mL disposable, plastic test tubes. 0.5mL of % BHT in ethanol was added to the sample and vortexed. After the addition of 5 mL of petroleum ether, each sample was homogenized using a GenoGrinder and 2 steel grinding balls at 750 rpm for 5 min. The samples were then centrifuged at 1,300 x g for 3 mins and the supernatant collected and filtered through Whatman 1PS filter paper. A 2.5mL aliquot was transferred into a clean test tube and evaporated to dryness under a gentle flow of nitrogen at 35-45°C. the extract was then reconstituted in 0.2mL of methanol, which was then sonicated, vortexed, filtered through a 0.45µm Millipore syringe filter into an autosampler vial, and submitted for HPLC analysis (Diao *et.al.*, 2020).

**Histolµogical examination of tissues**

Samples of the liver and kidney of an animal per group were fixed in buffered 10% formal saline The fixed liver and kidney tissue samples were embedded after dehydration in paraffin wax, sectioned at 5-6µm and stained with hematoxylin and Eosin (HandE) for general histopathological examination using the light microscope for any changes in the tissues due to consumption of the five different polar solvent (A, B, C, D, E) leaf extract of *V. amygdalina.*

**Statistical Analysis**

The results were expressed as mean ± SD and test of statistical significance was employed using one-way analysis of variance (ANOVA). The data obtained were analyzed using Statistical

Product and Service Solutions (SPSS), version 20 and p values $< 0.05$ were considered significant.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

## 4.1 RESULTS

### 4.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract on Fasting blood glucose concentration of alloxan-induced diabetic rats

Table 4.1 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on Fasting blood glucose concentration of alloxan-induced diabetic rats. Result shows that alloxan-induced diabetes resulted in a significant ($p<0.05$) increase in blood glucose in exposed rats as compared to normal control animals. After the administration of the *V. amygdalina* extract to diabetic rats during a 7days period, it resulted in varying effect on blood glucose concentration. The extracts demonstrated glucose reducing effect, the blood glucose monitored over a 7 days period showed a 16.40%, 33.19%, 43.66%, 12.65%, 17.12%, 60.17% decrease in blood glucose relative to baseline values in the groups treated with *V. amygdalina* aqueous, ethanol, methanol, toluene, benzene extracts and metformin respectively. The glucose reducing effect of the extract was in the order Methanol > Ethanol>Benzene> Aqueous> toluene. The ethanol and methanol extract yielded 33.19% and 43.66% of baseline blood glucose reduction; compared favourably with the standard drug metformin. .

**Table 4.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on Fasting blood glucose concentration in alloxan-induced diabetic rats**

| | **Glucose Concentration (mg/dl)** | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 3 | Day 5 | Day 7 | Glucose Reduction(%) |
| **NC** | 104.40 ± 10.62 | 98.80 ± 8.41 | 112.80 ± 16.63 | 119.20 ± 3.42 | 103.60 ± 13.83 | 0.77 |
| **DC** | 333.00 ± 38.42 | 371.75 ± 50.86 | 402.25 ± 74.50 | 427.75 ± 59.29 | 349.50 ± 23.30 | -4.95 |
| **AQE** | 338.20 ± 29.76 | 427.00 ± 49.00 | 387.75 ± 12.26 | 321.25 ± 10.28 | 282.75 ± 30.74 | 16.40 |
| **ETH** | 374.20 ± 43.46 | 329.00 ± 56.83 | 314.80 ± 37.19 | 280.20 ± 32.67 | 250.00 ± 40.98 | 33.19 |
| **METH** | 361.00 ± 53.92 | 306.80 ± 55.65 | 270.40 ± 43.37 | 225.20 ± 38.98 | 203.40 ± 26.08 | 43.66 |
| **TOL** | 371.50 ± 39.95 | 398.75 ± 29.55 | 422.25 ± 26.96 | 391.75 ± 59.43 | 324.50 ± 20.82 | 12.65 |
| **BENZ** | 366.50 ± 15.72 | 397.25 ± 53.77 | 335.00 ± 41.00 | 327.00 ± 31.21 | 303.75 ± 21.91 | 17.12 |
| **STD** | 287.25 ± 42.86 | 242.50 ± 35.54 | 228.00 ± 29.74 | 197.80 ± 9.15 | 114.40 ± 13.91 | 60.17 |

Values are Mean ± standard deviation of 4 determinations.

**Table 4.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on body weight in alloxan-induced diabetic rats**

Table 4.2 shows effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on body weight in alloxan-induced diabetic rats.Results presented in table 4.2 shows that diabetic rats showed a gradual steady increase in body weight during the study period with the exception of the diabetic control and benzene extract treated groups. The diabetic control group showed increase in body weight which reduced at the end of the study period. However, group treated with benzene extract showed a drastic decrease in body weight after 3 days of treatment. Thereafter, the body weight was gradually being restored but was not normalized. Body weights of the groups at two days interval are presented in table 4.2 for the 7 days study duration.

**Table 4.2 Effect of *V. amygdalina*leave aqueous, ethanol, methanol, toluene and benzene extract administration on body weight in alloxan-induced diabetic rats**

| | Weight Changes (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 | Day 14 | **Weight gain (%)** |
| NC | 113.60 ± 9.18 | 122.30 ± 10.91 | 130.10 ± 9.66 | 134.00 ± 8.58 | 139.45 ± 6.96 | 144.21 ± 15.88 | 148.1 ± 17.75 | 30.28 [a] |
| DC | 86.40 ± 10.06 | 89.50 ± 6.30 | 100.90 ± 13.56 | 99.80 ± 11.45 | 102.43 ± 8.09 | 108.89 ± 5.44 | 115.23 ± 9.22 | 33.36 [a,b] |
| AQE | 54.75 ± 3.59 | 62.30 ± 22.11 | 74.25 ± 28.17 | 103.30 ± 11.15 | 81.67 ± 7.35 | 83.6 ± 6.69 | 84.55 ± 8.0 | 34.74 [a,b] |
| ETH | 88.80 ± 3.27 | 89.90 ± 3.21 | 102.50 ± 3.67 | 107.80 ± 5.52 | 110.34 ± 5.52 | 118.02 ± 7.08 | 124.12 ± 18.65 | 39.64 [b] |
| METH | 92.40 ± 10.53 | 97.70 ± 10.60 | 97.90 ± 9.76 | 112.20 ± 12.40 | 118.23 ± 8.28 | 121.56 ± 8.51 | 125.48 ± 11.20 | 35.80 [a,b] |
| TOL | 116.45 ± 8.73 | 119.20 ± 9.95 | 135.45 ± 12.69 | 140.45 ± 13.89 | 134.65 ± 6.73 | 139.89 ± 11.19 | 143.67 ± 15.25 | 23.37 [c] |
| BENZ | 151.45 ± 10.40 | 128.78 ± 13.19 | 148.20 ± 10.41 | 149.45 ± 7.93 | 129.33 ± 6.47 | 118.33 ± 7.10 | 116.54 ± 10.14 | -4.04 [d] |
| STD | 169.84 ± 13.57 | 173.10 ± 19.81 | 179.70 ± 17.42 | 190.90 ± 19.12 | 131.33 ± 5.25 | 136.67 ± 8.20 | 141.49 ± 9.48 | 28.81 [c] |

Values are Mean ± standard deviation of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different

### 4.3 Liver function parameters

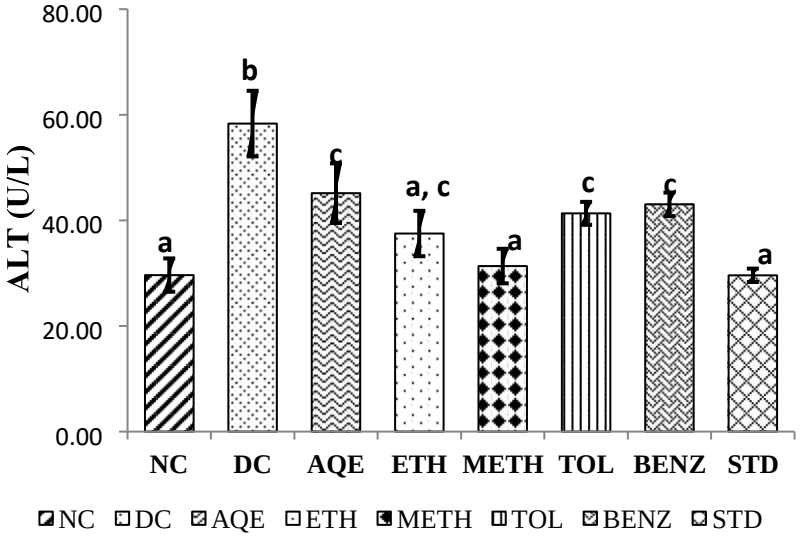
#### 4.3.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts of serum alanine aminotransferase (ALT) activity.

Figure 4.1 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract on serum ALT activity of alloxan-induced diabetic rats.Results obtained shows that diabetes induction was accompanied by significant ($p<0.05$) elevation of ALT activity as seen in alloxan administered rats compared to normal control. However, the administration of methanol extract of *V. amygdalina* and the standard drugs (metformin) resulted in a normalization of ALT activity. Also, ALT activity in the groups receiving aqueous ethanol, toluene and benzene extractswere significantly ($p<0.05$) reduced as compared to diabetic control group. Result presented in figure 4.1 shows that the ALT activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STDwere 29.64 ± 3.16, 58.33 ± 6.21, 45.15 ± 5.65, 37.51 ± 4.30, 31.35 ± 3.27, 41.33 ± 2.22, 43.04 ± 2.18 and 29.59 ± 1.27U/L respectively.

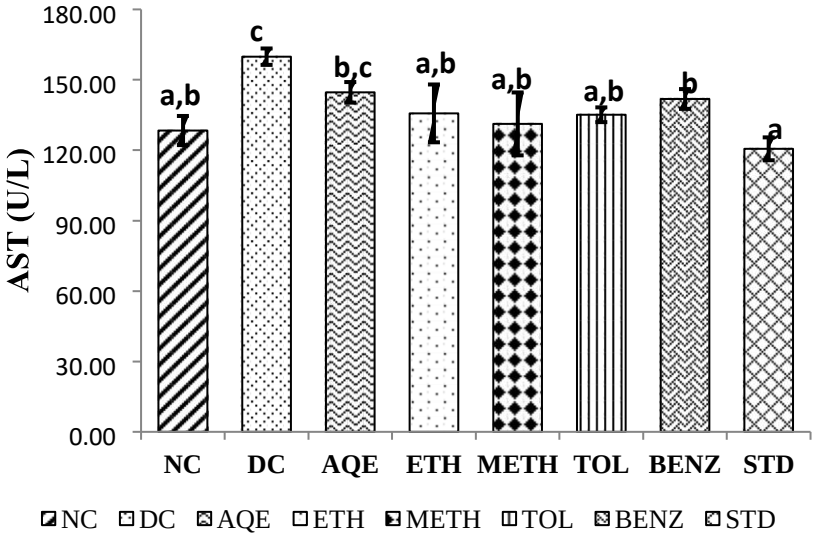
#### 4.3.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum aspartate aminotransferase (AST) activity.

Figure 4.3 shows the effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum AST activityin alloxan-induced diabetic rats.Results obtained showed that diabetes induction resulted in significant ($p<0.05$) elevation of AST activity as seen in alloxan exposed rats compared to normal control. AST activity in the groups receiving ethanol, methanol, toluene and Benzene extract were significantly ($p<0.05$) reduced when compared to diabetic control group. Result presented in figure 4.3 shows the AST activity of the groups NC, DC, AQE,

ETH, METH, TOL, BENZ and STD were 128.40 ± 6.21, 159.86 ± 3.49, 144.66 ± 60 ± 4.39, 135.70 ± 12.32, 131.22 ± 13.40, 135.12 ± 3.14, 141.84 ± 4.21 and 120 ± 4.87U/L respectively.




4.1 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts administration of serum ALT activity in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.




4.2 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts of serum AST activity of alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript across groups are significantly different ($p<0.05$).

### 4.3.3 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extractsof serum alkaline phosphatase (ALP) activity of alloxan-induced diabetic rats.

Figure 4.3 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extractsof serum ALP activityin alloxan-induced diabetic rats.Results obtained showed that diabetes induction was accompanied by significant ($p<0.05$) elevation of ALP activity. However, the different solvent extracts of *V. amygdalina* significantly ($p<0.05$) reduced ALP activity to level comparable to standard with the exception of the benzene extract. Result presented in figure 4.3 shows the ALP activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were 226.78 ± 33.60, 359.26 ± 23.16, 269.79 ± 17.40, 287.96 ± 17.82, 272.32 ± 48.98, 265.42 ± 35.79, 298.54 ± 18.44 and 255.30 ± 8.58U/L respectively.

### 4.3.4 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extractsof total bilirubin concentration of alloxan-induced diabetic rats.

Figure 4.4 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extractsof serum total bilirubin concentration of alloxan-induced diabetic rats. Total bilirubin concentration significantly ($p<0.05$) increased with alloxan induction when compared to normal control group. However, administration of the different solvent extracts of *V. amygdalina* did not result in a significant ($p<0.05$) normalization of serum bilirubin concentration. Result presented in figure 4.4 Total bilirubin concentration was 0.28 ±0.020, 0.34 ±0.026, 0.313±0.023, 0.32 ±0.018,0.33 ±0.038, 0.31 ±0.010, 0.262±0.028, and 0.28 ±0.044 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

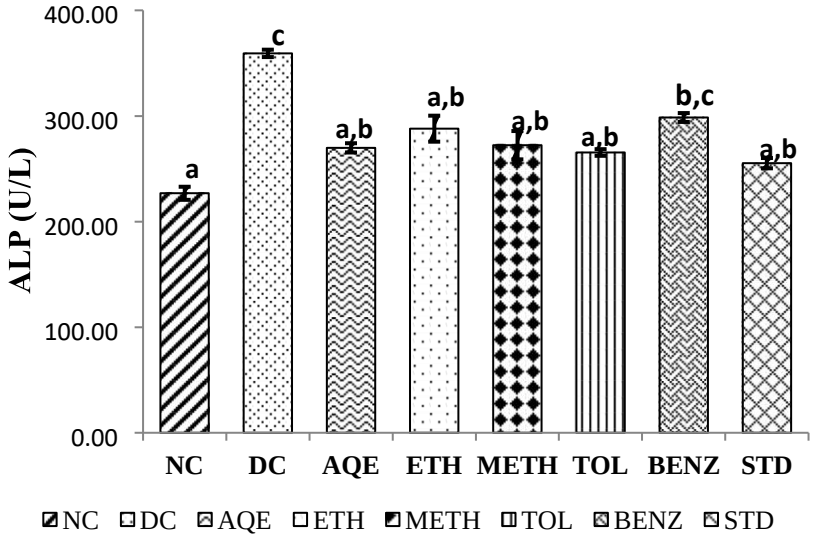



Figure 4.3 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts of serum ALP activity in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

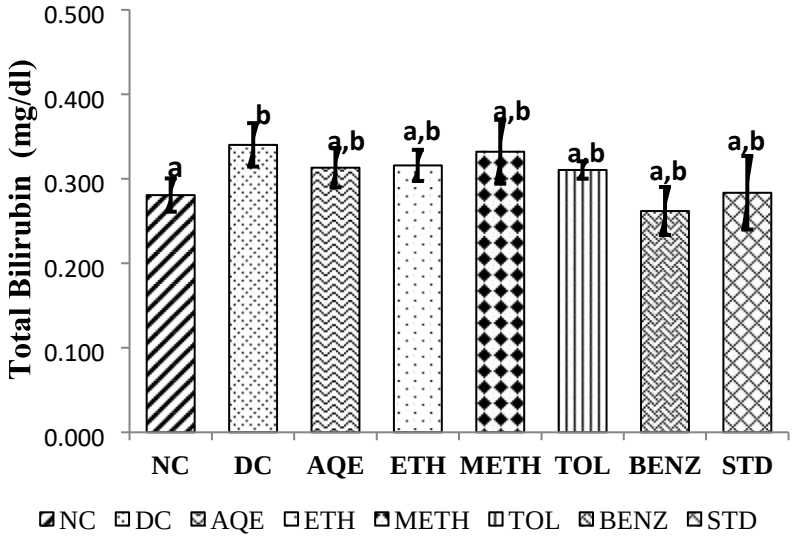



Figure 4.4 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extractsof serum total bilirubin concentration of alloxan-induced diabetic rats. Results are Mean ±

SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

### 4.4 Lipid Profile Parameters

#### 4.4.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum total cholesterol concentration of alloxan-induced diabetic rat.

Figure 4.5 shows the effect of *V. amygdalina* aqueous, ethanol; methanol, toluene and benzene leaf extractson serum total cholesterol concentration of alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) elevation of total cholesterol concentration. However, the ethanol and methanol extracts caused a significant ($p<0.05$) reduction of total cholesterol concentration when compared to the diabetic control. Furthermore, result presented in figure 4.5, serum total cholesterol concentration was 61.82±3.41, 91.74±3.34, 83.68 ±6.29, 75.94±5.13, 69.87±2.51, 80.96±6.42, 80.75±4.95 and 87.66±6.46 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

#### 4.4.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum triacylglycerol concentration of alloxan-induced diabetic rats.

Figure 4.6 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum triacylglycerol concentration of alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) elevation of triacylglycerol concentration. Administration of the extracts produced varying degree of triacylglycerol reduction. The methanol extracts of *V. amygdalina* caused a normalization of TG similar to those of normal control and the standard. The extracts of ethanol, toluene, benzene and aqueous also caused a

significant ($p<0.05$) reduction of TG but did not normalize it. Result presented in figure 4.6 serum triacylglycerol concentration was 51.85±4.61, 84.36±7.41, 68.93±5.80, 70.58±8.75, 50.82±4.67, 65.84±3.98, 63.79±3.19 and 39.92±7.13 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respective ely.

### 4.4.3 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum HDL-cholesterol concentration of alloxan-induced diabetic rats..

Figure 4.7 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum HDL-cholsterol concentration alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) decrease in HDLcholesterol concentration. However, administration of the aqueous and methanol extract resulted in a significant ($p<0.05$) decrease of HDL-cholesterol concentration. Result presented in figure 4.7, serum HDL-cholesterol concentration was 45.09±1.30,26.25±1.92, 41.71 ±2.89, 35.09±3.22, 44.18±3.00, 30.93 ±4.02, 27.68±2.66 and 47.69±2.89 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

### 4.4.4 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL-cholesterol concentration of alloxan-induced diabetic rats.

Figure 4.8 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL-cholesterol concentration of alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) increase in LDLcholesterol concentration. However, administration of the ethanol, methanol toluene and benzene extract resulted in a significant ($p<0.05$) decrease of LDL-cholesterol concentration. Result presented in figure 4.8, serum LDL-cholesterol concentration was 40.95±4.37, 64.31± 3.45,

56.39±5.03, 46.31±2.92, 36.55±6.42, 35.44±8.02, 46.97±7.48 and 41.69±4.36 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

### 4.4.5 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum VLDL-cholesterol concentration of alloxan-induced diabetic rats..

Figure 4.9 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum VLDL-cholesterol concentration of alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) elevation of VLDLcholesterol concentration. However, only the methanol extract resulted in a normalization of VLDL-cholesterol concentration. Furthermore, result presented in figure 4.9 serum VLDL-cholesterol concentration was 10.37±0.92,16.87±1.48, 13.79±1.16,14.12±1.75,10.16±0.93,13.17±0.80,12.76±0.64 and 7.98±1.43 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

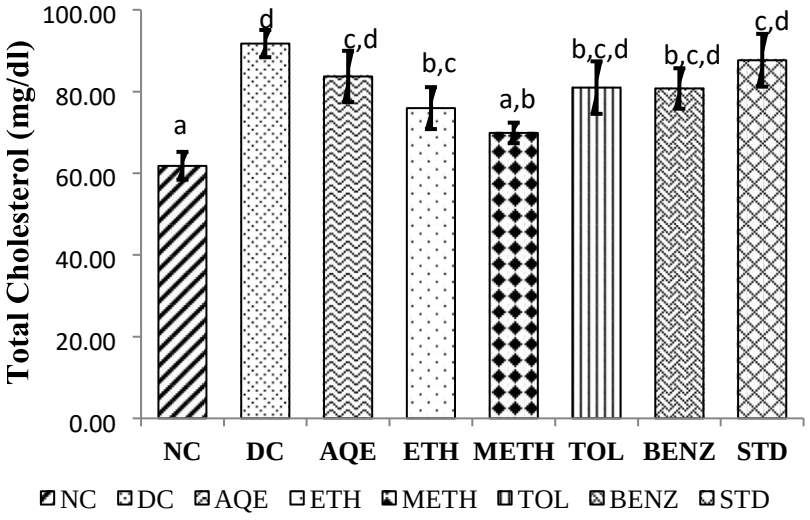



Figure 4.5: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum total cholesterol concentration of alloxan-induced diabetic rats. Results are Mean

± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

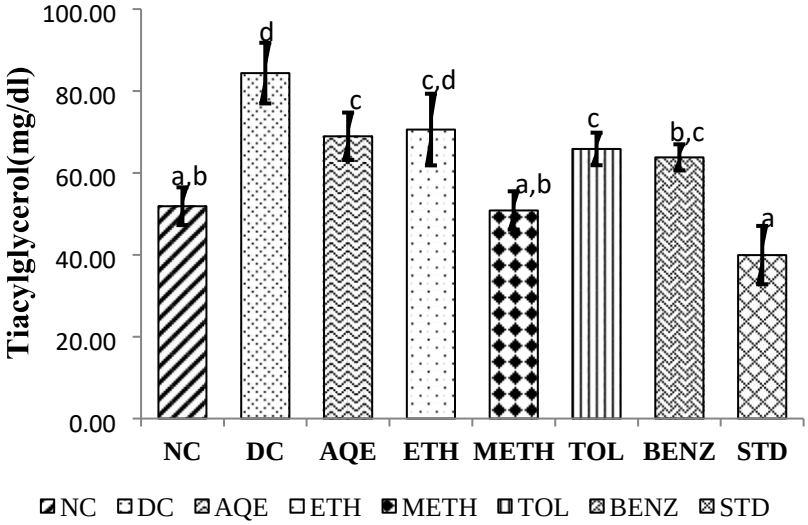



Figure 4.6: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts administration on serum triacylglycerol concentration of alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

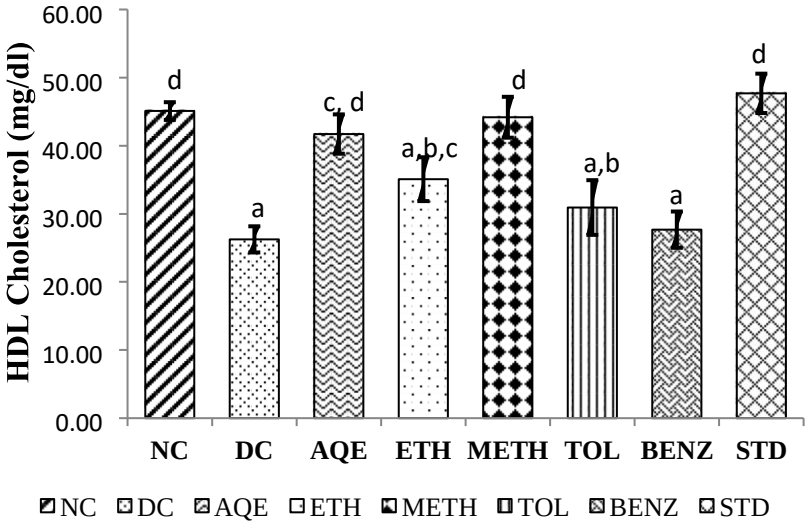



Figure 4.7: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum HDL-cholesterol concentration of alloxan-induced diabetic rats. Results are

Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

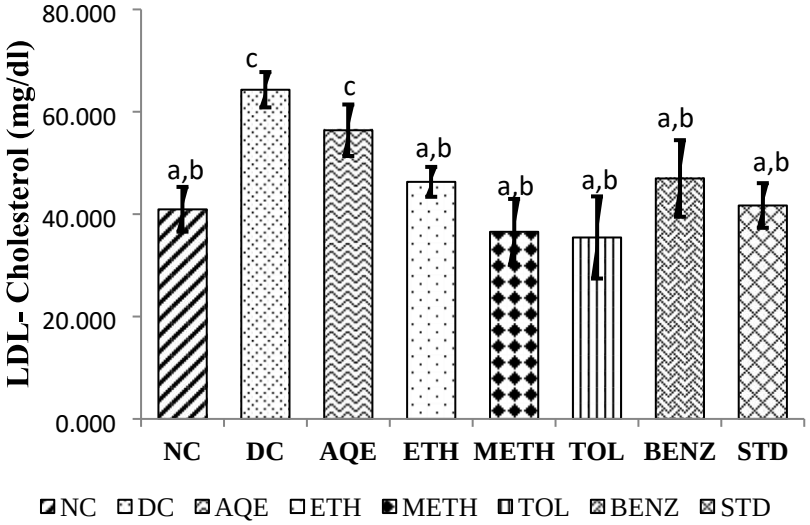



Figure 4.8: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL-cholesterol concentration of alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

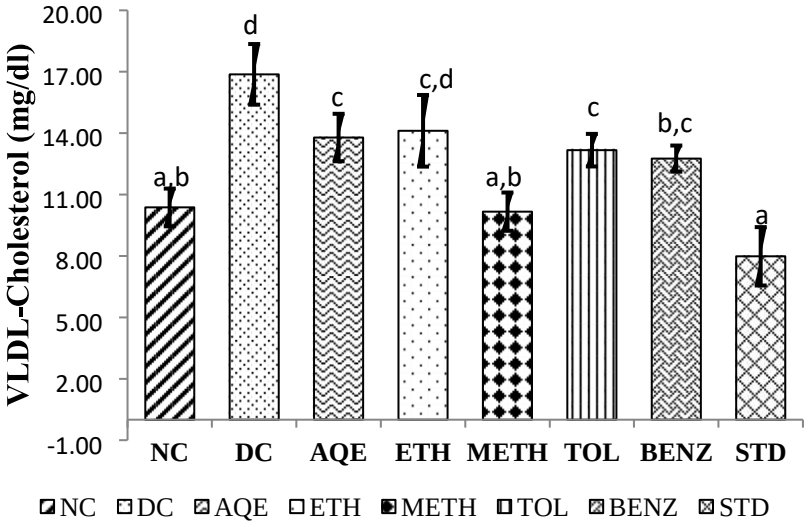



Figure 4.9: Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum VLDL-cholesterol concentration of alloxan-induced diabetic rats. Results are Mean ±

SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

### 4.4.6 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL/HDL-cholesterol ratio of alloxan-induced diabetic rats.

Figure 4.10 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract administration on serum LDL/HDL-cholesterolratio of alloxan-induced diabetic rats. The different solvent extracts of *V. amygdalina* resulted in a significant ($p<0.05$) reduction of LDL/HDL ratio. This reduction in the groups that received mSethanol and toluene extracts was comparable to those of normal control and standard. In the result presented in figure 4.10, serum LDL/HDL ratio was 0.910±0.12, 2.462±0.26, 1.350±0.03, 1.330±0.16, 0.834±0.19, 1.135±0.11, 1.690±0.15 and 0.878±0.12 in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

### 4.4.7 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on cardiovascular risk ratio (CRR) of alloxan-induced diabetic rats.

Figure 4.11 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on CRR in alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) increase in cardiovascular risk. The different solvent extracts of *V. amygdalina* resulted in a significant ($p<0.05$) reduction of CRR. This reduction was significant in the groups receiving ethanol, Methanol and aqueous extracts was comparable to those of normal control and standard. Furthermore, result presented in figure 4.11, cardiovascular risk ratio was 1.37±0.11, 3.51±0.25, 2.01±0.10, 2.19±0.31, 1.58±0.06, 2.63±0.21, 2.93±0.14 and 1.84±0.17 in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.

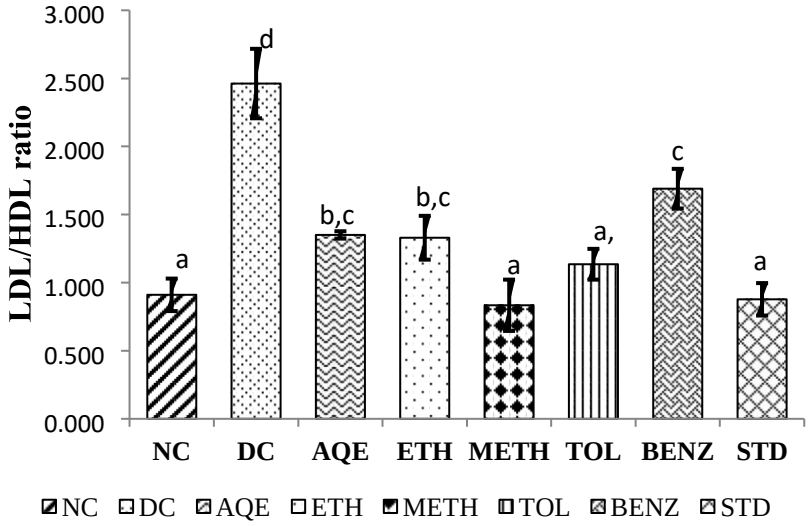



Figure 4.10: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on LDL/HDL ratio in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

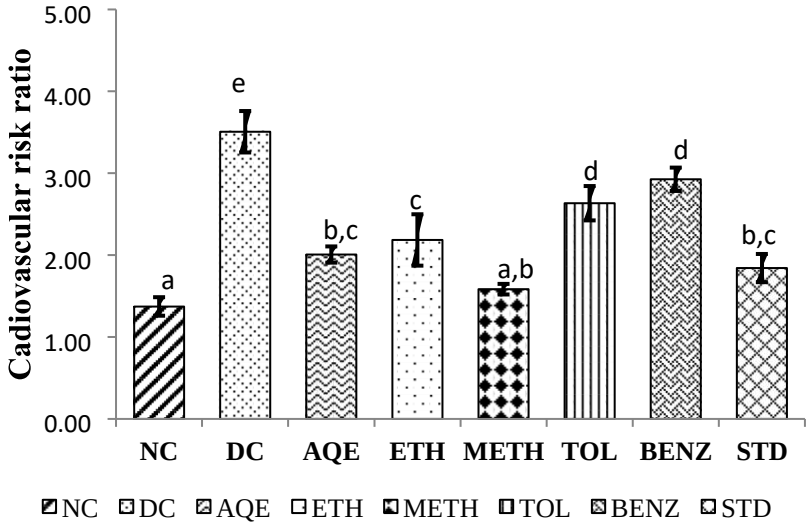



Figure 4.11: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on cardiovascular risk ratio in alloxan-induced diabetic rats. Results are Mean ± SD of 4

determinations. Values with different superscript are significantly different ($p<0.05$) while values with same superscripts are not significantly different.

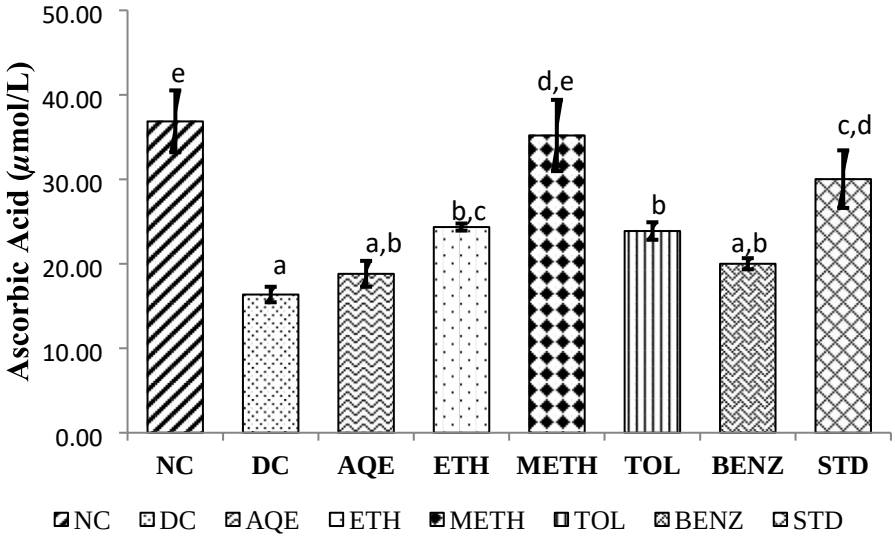
## 4.5 Antioxidant enzymes

### 4.5.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate ascorbic acid concentration of alloxan induced diabetic rats.

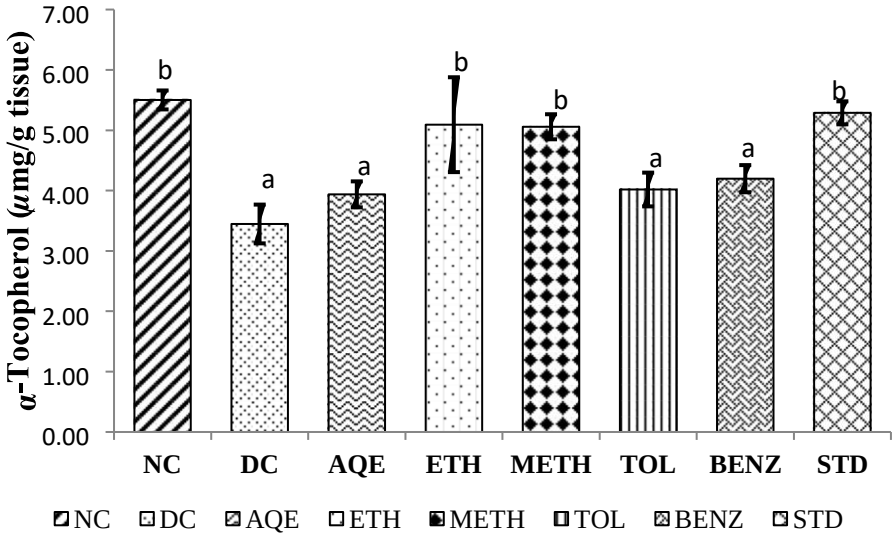
Figure 4.12 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate ascorbic acid concentration of alloxan-induced diabetic rats. The result presented in figure 4.12 showed that the liver homogenate ascorbic acid concentration were significantly ($p<0.05$) reduced by diabetes induction in the groups administered aqueous, ethanol, toluene and benzene extracts of *V. amygdalina*. The methanol extracts effectively increased ascorbic acid concentration. Furthermore, The ascorbic acid concentration were 36.86±3.66, 16.36±0.92, 18.81±1.53, 24.35±0.42,35.18±4.22, 23.88±1.04, 20.00±0.66 and 30.02±3.41 µmol/L in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STDwere respectively.

### 4.5.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate vitamin E concentration.

Figure 4.13 shows the effect of *V. amygdalina* leaf aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate Vitamin E concentrationin alloxan-induced diabetic rats. The Result presented in figure 4.13 showed that the liver homogenate vitamin E concentration were significantly ($p<0.05$) reduced by diabetes induction in the groups administered aqueous, toluene and benzene extracts of *V. amygdalina*. However, the ethanol, methanol extracts effectively increased vitamin E concentration.Furthermore, The ascorbic acid concentration were 5.50±0.16, 3.45±0.32, 3.94±0.22, 5.09±0.79, 5.06±0.21, 4.02±0.28, 4.20±0.23 and 5.29±0.19 µg/g tissue in the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD respectively.




4.12 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on Liver homogenate ascorbic acid concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.




4.13 The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on Liver homogenate α-tocopherol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

### 4.5.6 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract on liver GSH concentration

Result presented in figure 4.14 shows the serumliver GSH concentration of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were20.80±0.57, 10.05±0.48, 14.93±0.90, 15.42±0.74, 16.01±0.20, 14.86±0.24, 12.78±0.93 and 17.85±1.56mg/g tissue respectively. The results showed that the liver homogenate GSH concentration was significantly ($p<0.05$) reduced across diabetic groups, except in the standard group when compared to normal control group. The extracts did not normalize GSH concentration but offered a significant protection against GSH depletion when compared to diabetic control group.

### 4.5.7 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate superoxide dismutase activity.

Figure 4.15 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate superoxide dismutase activity in alloxan-induced diabetic rats. The results showed that superoxide dismutase activity was significantly ($p<0.05$) reduced by diabetes induction. However, the extracts offered significant ($p<0.05$) protection against SOD depletion when compared to diabetic control group. Result presented in figure 4.15 showed that the liver homogenate SOD activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were 1.80 ±0.016, 0.55 ±0.060, 1.56±0.029, 1.65 ±0.013, 1.69 ±0.016, 1.61 ± 0.028, 1.60 ±0.027, 1.73 ±0.020 IU respectively.

### 4.5.8 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract on serum superoxide dismutase activity.

Figure 4.16 shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum superoxide dismutase activity in alloxan-induced diabetic rats. The results showed that serum superoxide dismutase activity was significantly ($p<0.05$) reduced by diabetes induction. However, the extracts offered significant ($p<0.05$) protection against SOD depletion when compared to diabetic control. Result presented in figure 4.16 Showed that the serumSOD activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were 1.72±0.02, 0.55±0.06, 1.26±0.05, 1.38±0.04, 1.54±0.02, 1.44±0.10, 1.35±0.03 and 1.61±0.03 IU respectively.

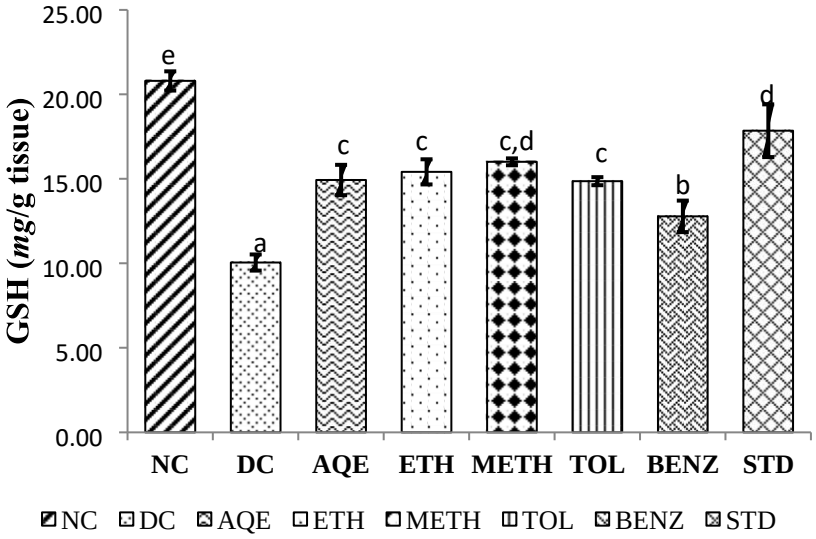



Figure 4.14: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on Liver homogenate Glutathione (GSH) concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

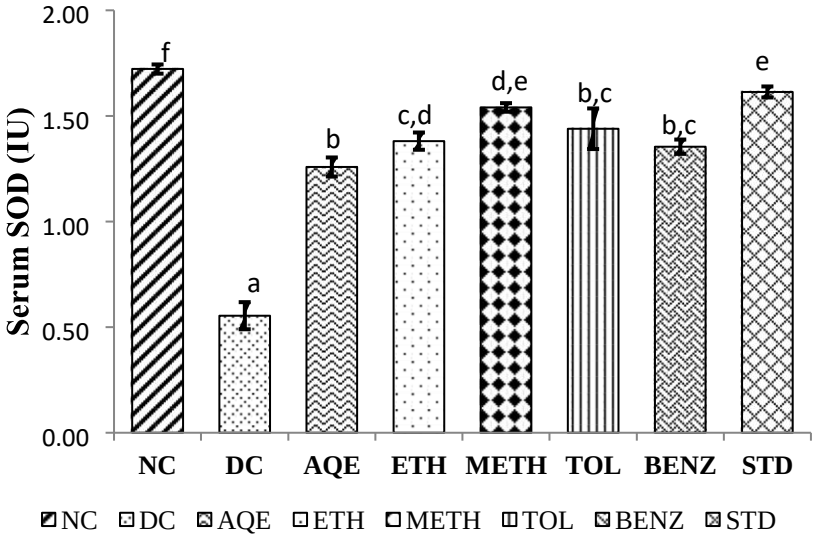



Figure 4.15: Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extract on serum SOD activity in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

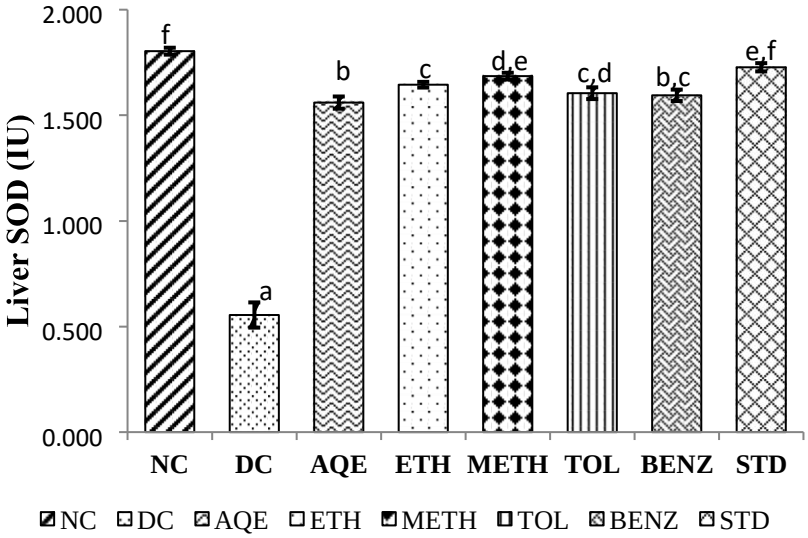



Figure 4.16: The effect of *V. amygdalina* leave aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate SOD activity in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

### 4.5.9 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate glutathione peroxidase activity of alloxan-induced rats.

Result presented in figure 4.17 Shows the liver homogenate glutathione peroxidase activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were9.22±0.89, 4.71±0.20, 6.61±1.02, 7.00±0.40, 7.98±0.45, 6.69±0.60, 6.65±0.78 and 8.44±0.41mgGSH/min/gtissue respectively. The results showed that liver homogenate glutathione peroxidase activitywas significantly ($p<0.05$) reduced by diabetes induction as seen in all alloxan exposed groups with exception of methanol and standard group. However, the administration of *V. amygdalina* extracts offered significant ($p<0.05$) protection against GPx depletion when compared to diabetic control group.

### 4.5.10 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extract on serum glutathione peroxidase activity of alloxan-induced diabetic rats.

Result presented in figure 4.18 shows the serum glutathione peroxidase activity of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were 7.51±0.72, 3.00± 0.46, 5.25±0.54, 5.45± 0.54, 5.41±0.53, 5.29±0.76, 3.89±0.40 and 7.12±0.53mgGSH/min/gtissue respectively. The results showed that serum glutathione peroxidase activitywas significantly ($p<0.05$) reduced by diabetes induction with exception of the Standard group. However, the administration of *V. amygdalina* extracts except Benzene extract offered significant ($p<0.05$) protection against GPx depletion when compared to diabetic control.

### 4.5.11 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate MDA concentration of alloxan-induced diabetic rats.

Result presented in figure 4.19 shows the liver homogenate MDA concentration of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were 2.51 ± 0.07, 4.61 ± 0.36, 3.95± 0.28, 2.89 ±

0.19, 2.63 ± 0.17, 3.68 ± 0.30, 3.91 ± 0.2 and 2.17 ± 0.05 nmol/L respectively. Malondialdehyde (MDA) concentration was observed to be significantly ($p<0.05$) elevated in the groups DC, aqueous, toluene and benzene extracts of *V. amygdalina*. The ethanol and methanol extracts offered significant reduction in MDA concentration when compared to standard.

### 4.5.12 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum MDA concentration of alloxan-induced diabetic rats.

Figure 4.20 shows the effect of *V. amygdalin a*queous, ethanol, methanol, toluene and benzene leaf extracts on serum Malondialdehyde (MDA) concentrationin alloxan-induced diabetic rats. Malondialdehyde (MDA) concentration was observed to be significantly ($p<0.05$) elevated in the groups DC, aqueous, toluene and benzene extracts of *V. amygdalina*. The ethanol and methanol extracts significantly reduced MDA concentration which compared favourably to standard. Result presented in figure 4.20 showed that the serum MDA concentration of the groups NC, DC, AQE, ETH, METH, TOL, BENZ and STD were2.51±0.07, 4.61±0.36, 3.95±0.29, 2.89±0.19, 2.63±0.17, 3.68±0.30, 3.91±0.20 and 2.17±0.05 nmol/L respectively.

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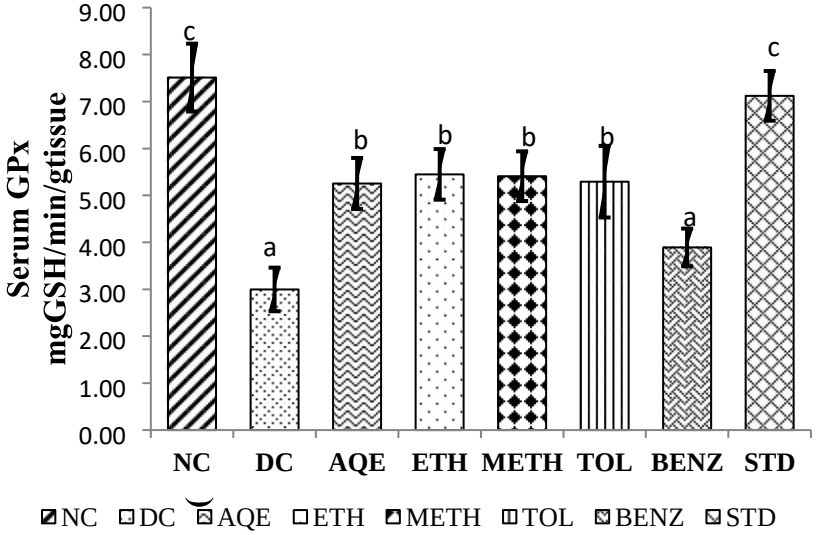

Figure 4.17: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum GPx activity in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

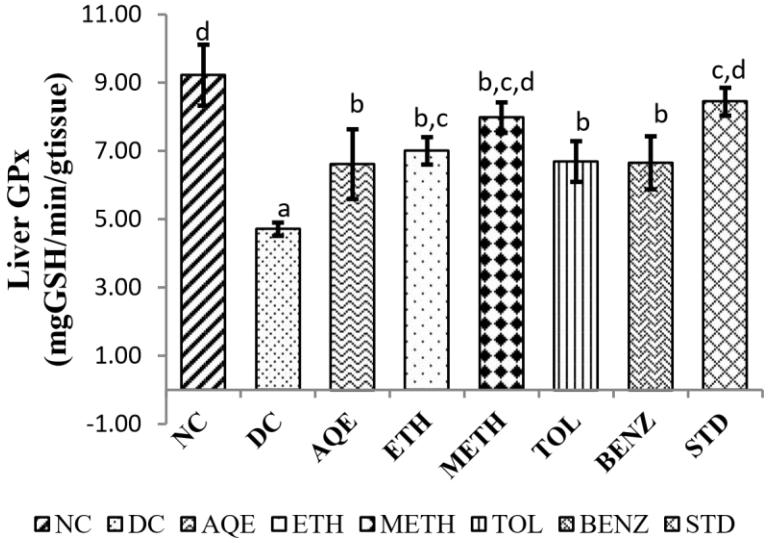


Figure 4.18: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate GPx activity of alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

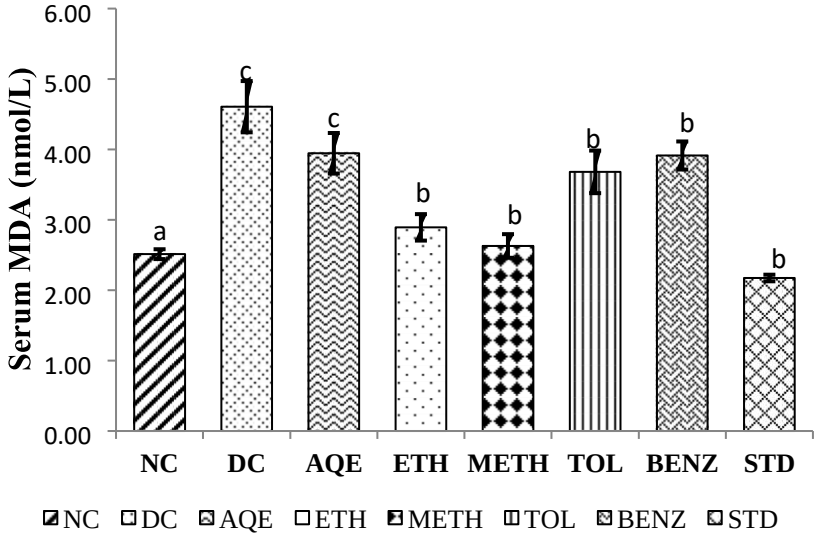

Figure 4.19: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum MDA concentration of alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

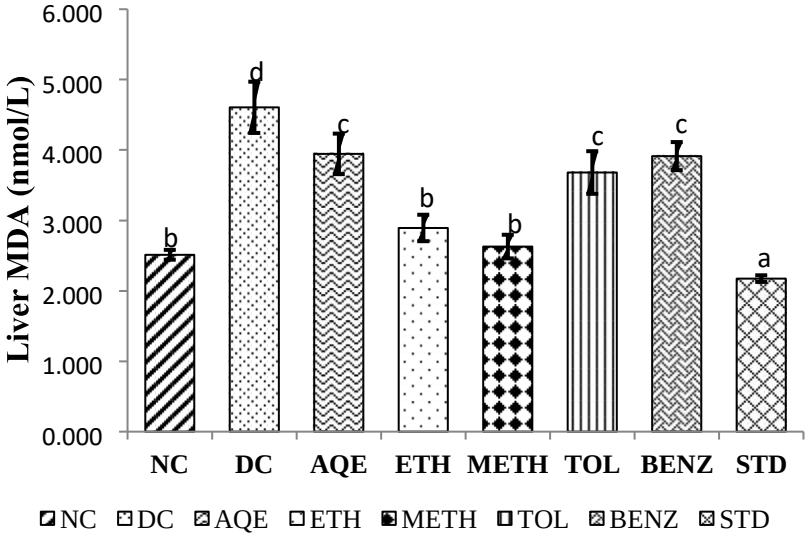


Figure 4.20: The effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate MDA concentration ofalloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript are significantly different ($p<0.05$) while values with the same superscripts are not significantly different.

## 4.6 HISTOLOGICAL EVALUATION

### 4.6.1 Histological examinations of liver sections

Histological sections (x400) of liver tissues of normal control, diabetic control, groups treated with *V. amygdalina leaves* extracts, aqueous (200mg/Kgb.wt), ethanol (200mg/Kgb.wt) , methanol (200mg/Kgb.wt) , toluene (200mg/Kgb.wt) and benzene (200mg/Kgb.wt); and Metformin (200mg/kgb.wt).

The histological results obtained are shown in plates 1- 8.

**Plate 1 (Normal control group):** Shows a typical normal architecture of the liver. This photomicrograph shows a central vein with numerous binucleated hepatocytes which are uniformly distributed in the cytoplasmic space. The nuclei are rounded and eccentrically positioned. Morphological features are in line with that of a normal liver; sinusoids are intact and no pathological lesion seen.

**Plate 2 (Diabetic Control group):** The group was induced with diabetes using 120mg/Kgb.wt and remained untreated for 7 days. The photomicrograph shows a section of the liver with enlarged central veins, infiltration of inflammatory cells around the central vein, enlarged sinusoids and necrosis of the hepatocytes (NH).

**Plate 3 (Aqueous extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200/Kgb.wt *V. amygdalina leaves* aqueous extract orally for 7 days. The photomicrograph shows a section of the liver with mild degenerative changes evidenced in enlarge central veins. There was also regeneration of hepatocytes with intact sinusoids compared to diabetic control.

**Plate 4 (Ethanol extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves,* ethanol extract orally given for 7 days. The photomicrograph shows a section of the liver with mild degenerative changes evidenced in congested central veins, areas with mild necrosis of the hepatocytes (NH) with intact sinusoids.

**Plate 5 (Methanol extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves,* methanol extract orally given for 7 days. The photomicrograph shows a section of the liver with congested central veins, areas with intact sinusoids. The liver tissue shows regeneration of hepatocytes when compared to diabetic control.

**Plate 6 (Toluene extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves,* toluene extract orally given for 7 days. The photomicrograph shows a section of the liver with mild degenerative changes, congested central veins, areas with mild necrosis of the hepatocytes (NH) and enlarged sinusoids.

**Plate 7 (Benzene extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves,* benzene extract orally for 7 days. The photomicrograph shows a section of the liver with regenerative changes when compared to diabetic control, central vein, portal sinusoid, and hepatocytes were evident.

**Plate 8 (Metformin200mg/Kgb.wt):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 150mg/Kgb.wt metformin orally for 7 days. The photomicrograph shows a section of the liver with apparently normal architecture. The morphological features seen are in line with that of a normal liver, central vein, portal sinusoid, and hepatocytes were evident.

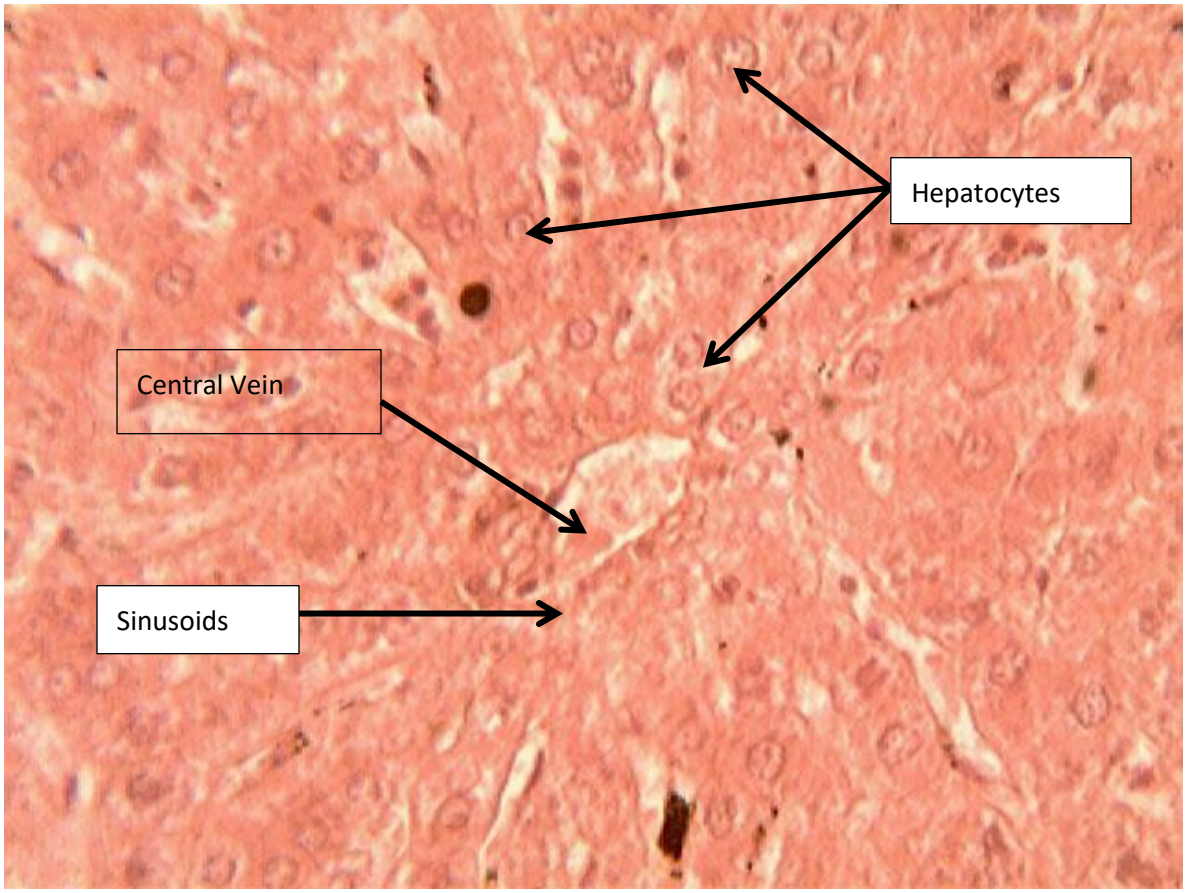


Plate 1: Photomicrograph of section of the liver tissue from normal control (NC). Group served as normal control group received standard rat diet and water *ad libitum* for 7 days. On the 7th day, the animals were given vehicle. H&E X400

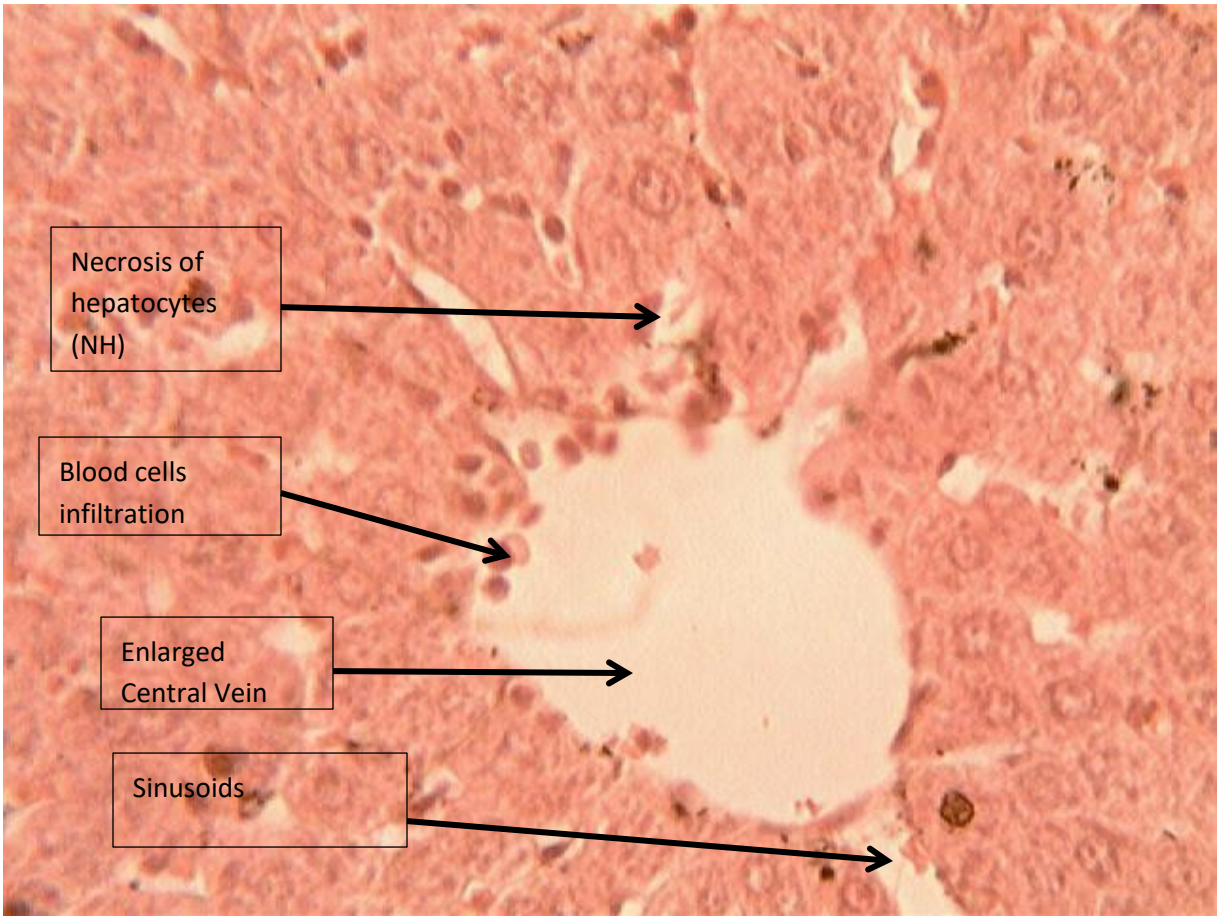


Plate 2: Photomicrograph of section of the liver tissue from diabetic control (DC), received standard rat diet and *ad libitum* for 7 days. The animals in the group were induced with diabetes using 120mg/Kgb.wt alloxan.

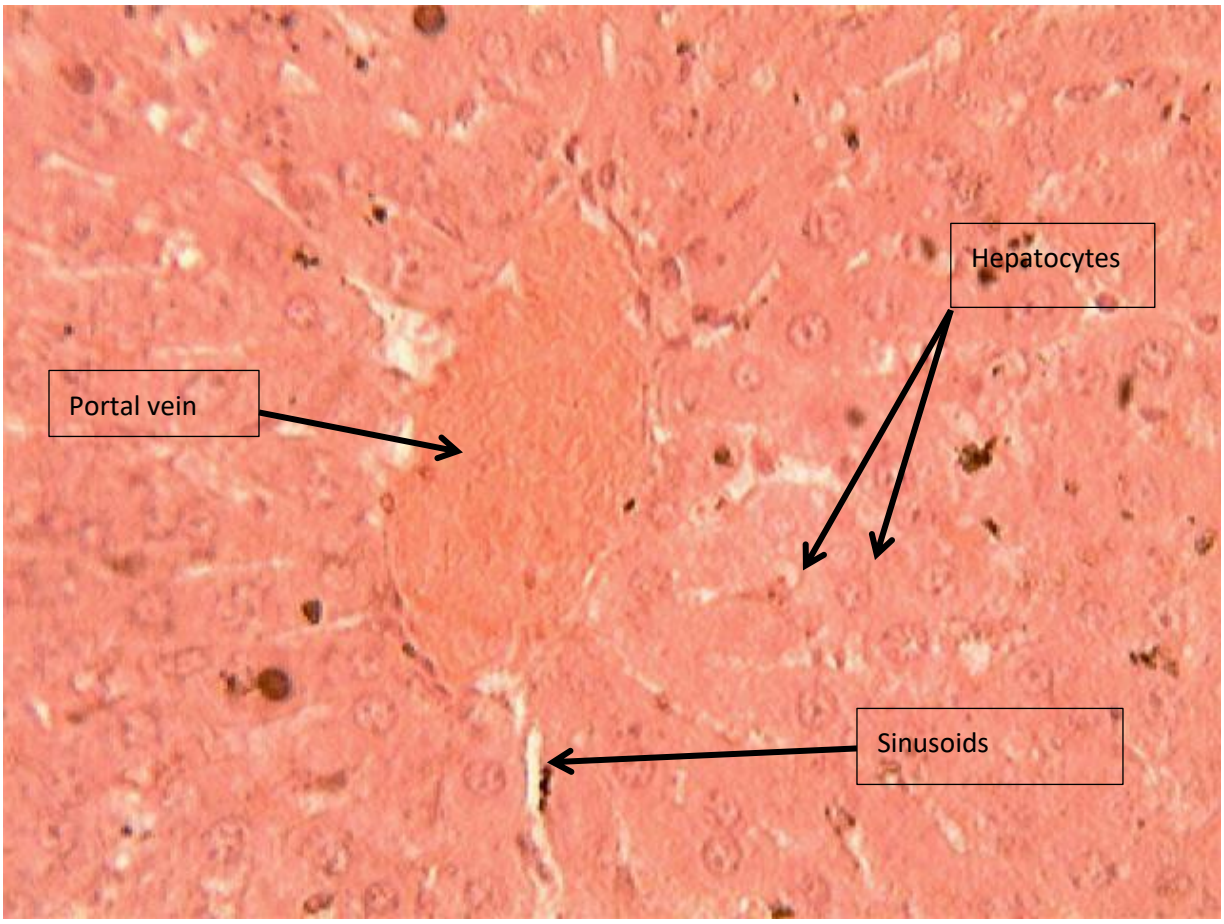


Plate 3: Photomicrograph of section of the liver tissue from aqueous extract group (AQE). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt *V. amygdalina leaves* aqueous extract for seven days.

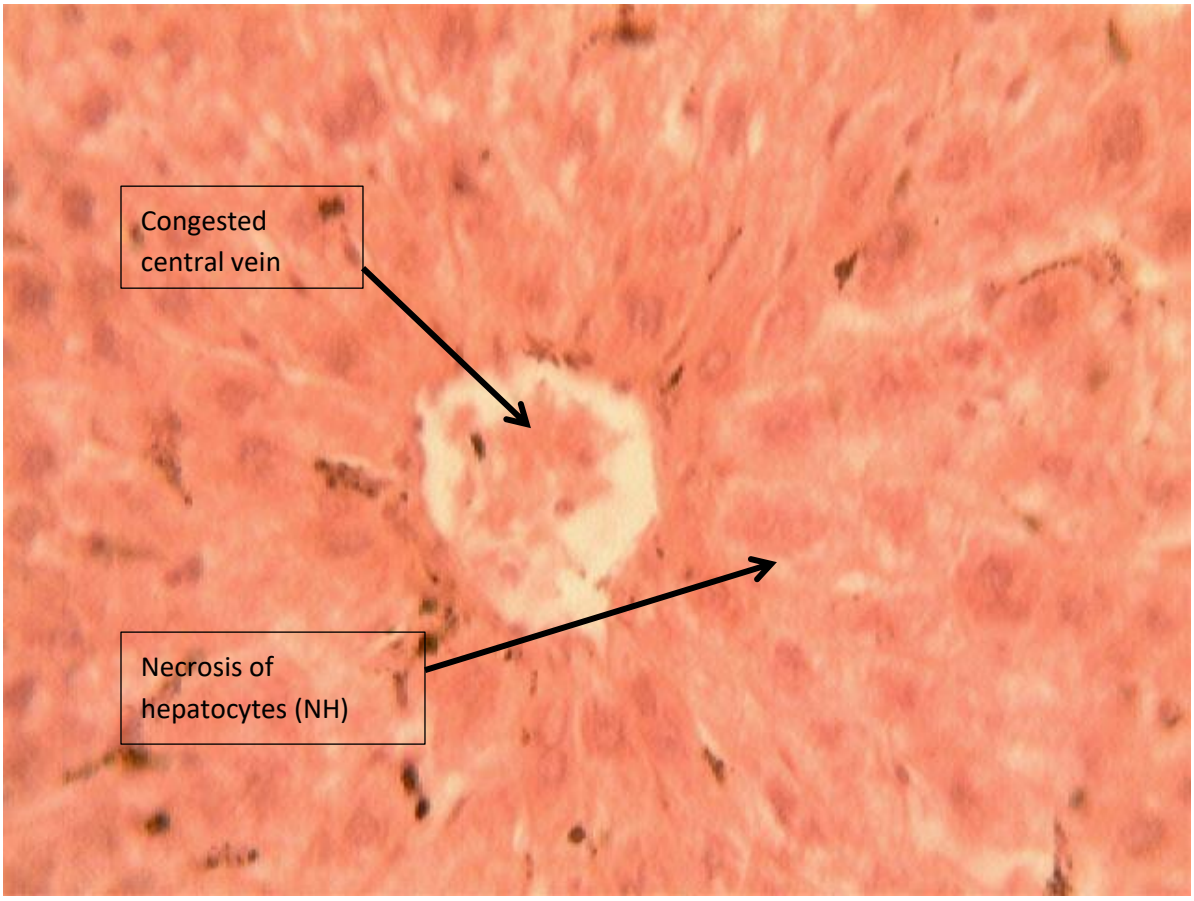


Plate 4: Photomicrograph of section of the liver tissue from ethanol extract group (Eth). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt *V. amygdalina leaves* ethanol extract for seven days.

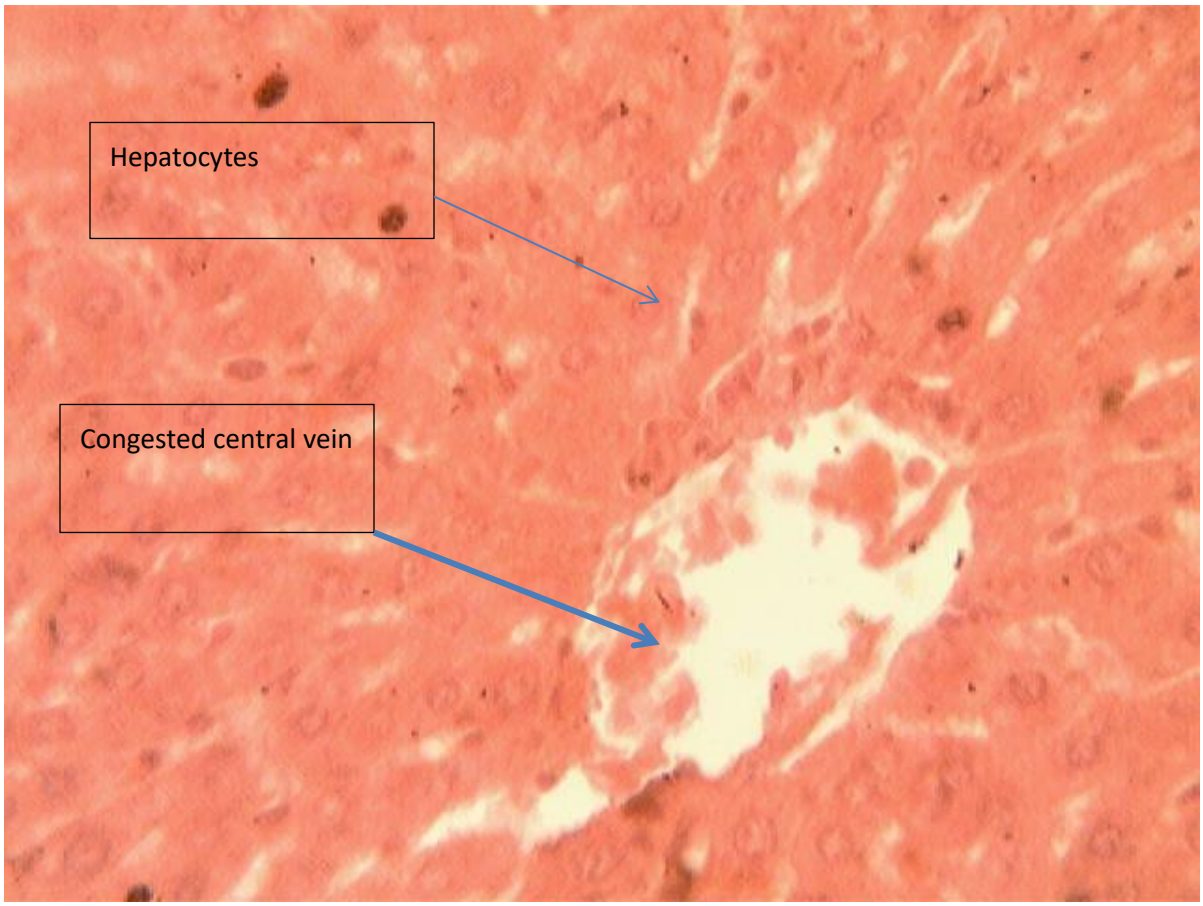


Plate 5: Photomicrograph of section of the liver tissue from methanol extract group (METH). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt *V. amygdalina leaves* methanol extract for seven days.

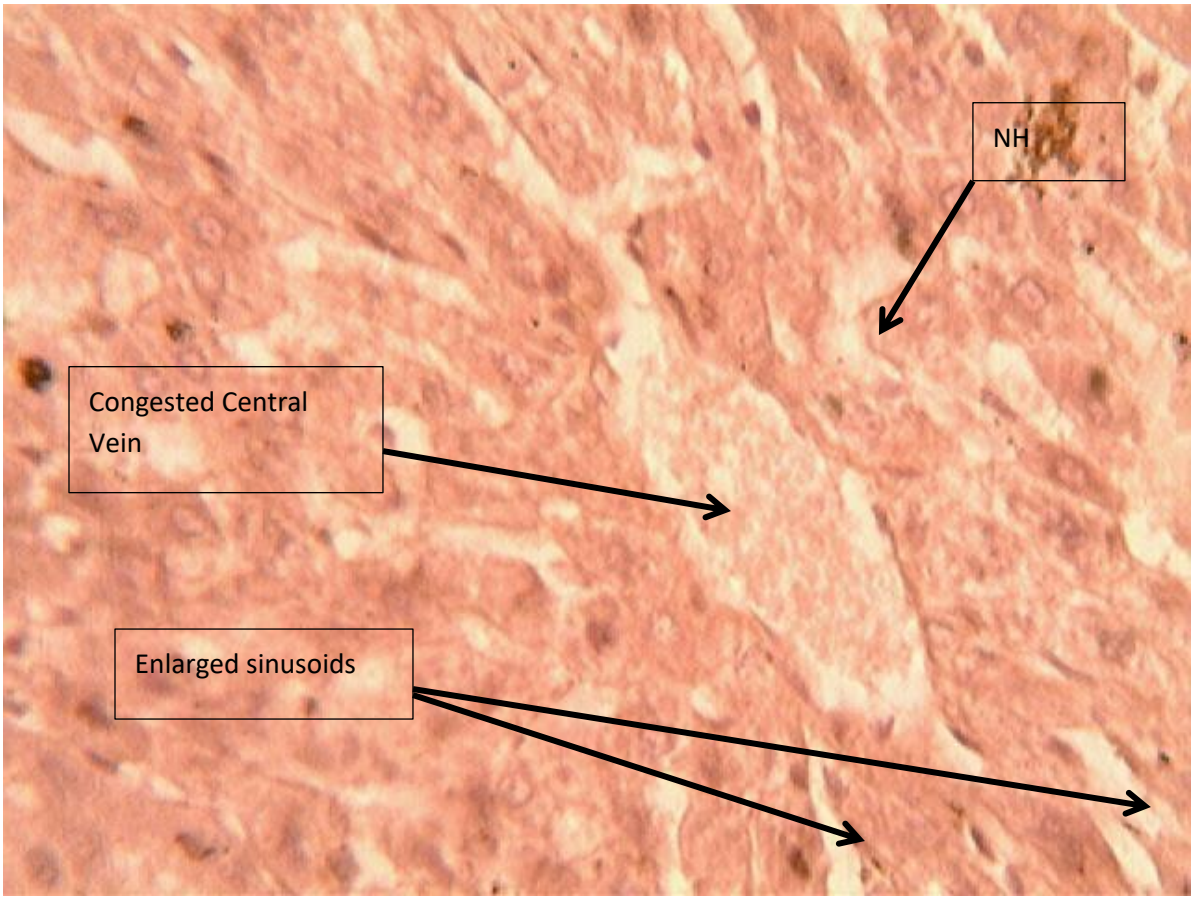


Plate 6: Photomicrograph of section of the liver tissue from toluene extract group (Tol). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt *V. amygdalina leaves* toluene extract for seven days.

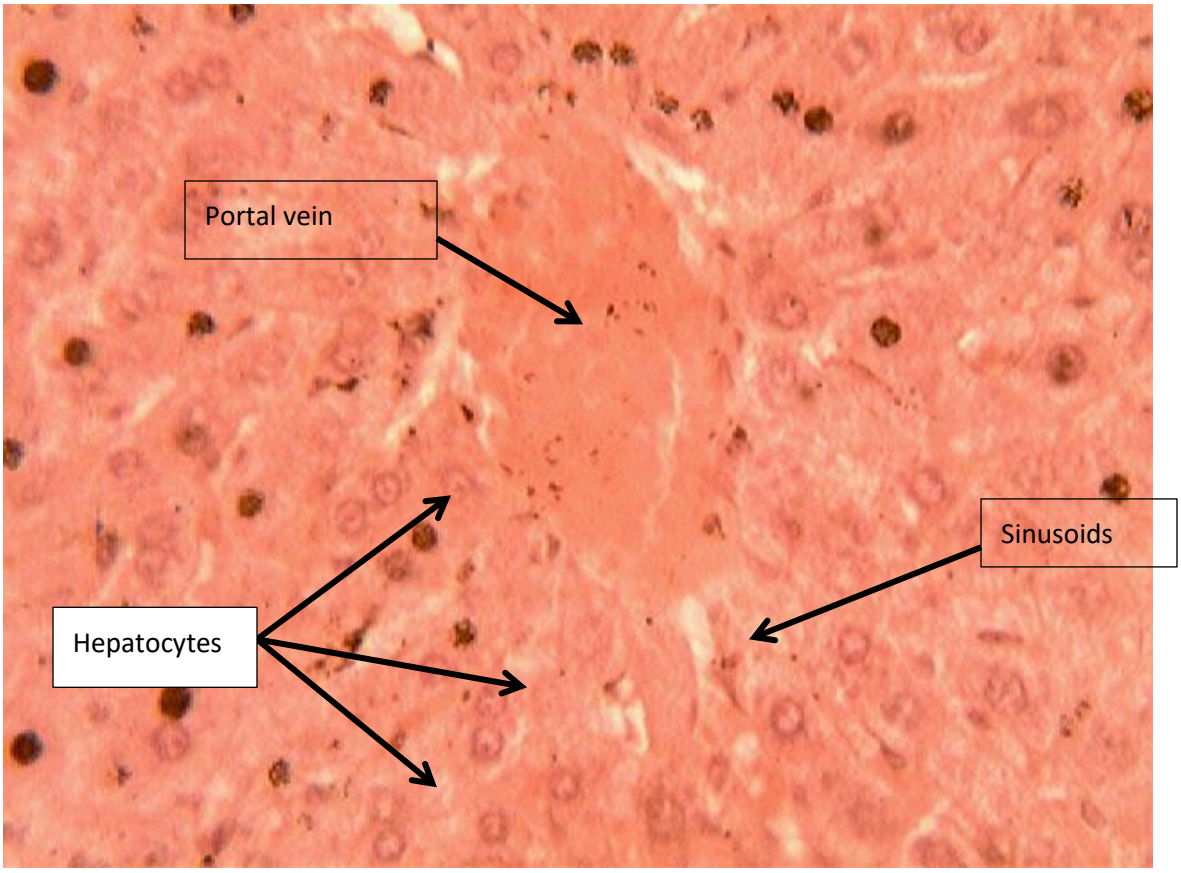


Plate 7: Photomicrograph of section of the liver tissue from benzene extract group (BENZ). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt*V. amygdalina leaves* benzene extract for seven days.

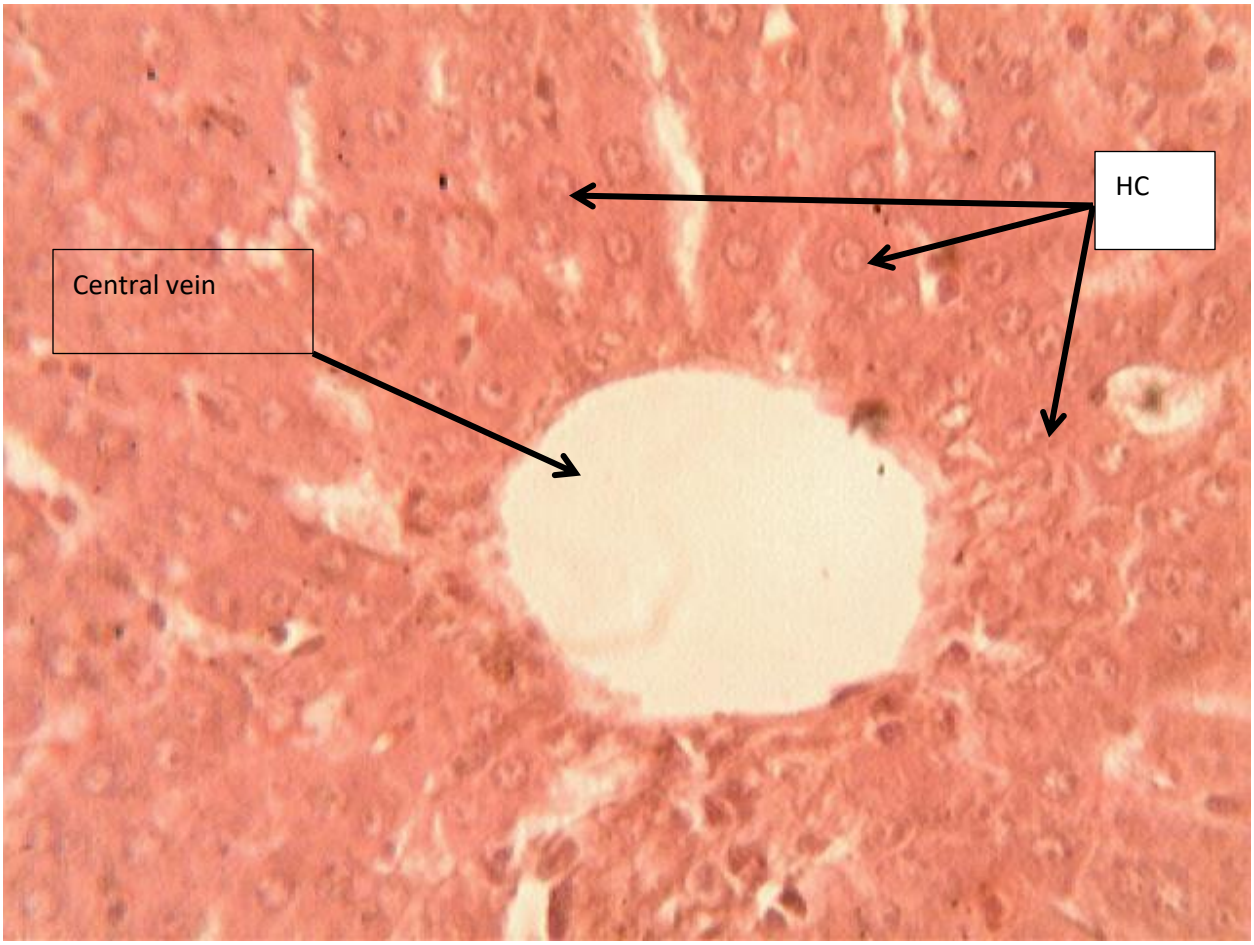


Plate 8: Photomicrograph of section of the liver tissue from Standard group (STD). Group received standard rat diet and drinking water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt Metformin for seven days.

### 4.6.2**Histological examinations of Kidney tissue sections**

Histological sections (x400) of liver tissues of normal control, diabetic control, groups treated with *V. amygdalina leaves* extracts, aqueous (200mg/Kgb.wt), ethanol (200mg/Kgb.wt) , methanol

(200mg/Kgb.wt) , toluene (200mg/Kgb.wt) and benzene (200mg/Kgb.wt); and Metformin (200mg/kgb.wt).

The histological results obtained are shown in plates 9- 16.

**Plate 9 (Normal control group):** Showing a section of typical normal architecture of the kidney. The cortex is seen to be housing the tuft of the glomerulus with slightly loose basement membrane. The distal and proximalconvoluted tubules appear normal; no histopathological lesion was seen.

**Plate 10 (Diabetic Control group):** The group was induced with diabetes using 120mg/Kgb.wt and remained untreated for 7 days. In DC the photomicrograph shows a section of the kidney with degeneration of urinary spaces and necrosis (N), these features are in line with inflammatory response.

**Plate 11 (Aqueous extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves* aqueous extract orally given for 7 days. The photomicrograph shows a section of the kidney with mild degeneration and necrosis of renal tubule and enlargement of glomerular space in line with inflammatory response

**Plate 12 (Ethanol extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves* ethanol extract orally given for 7 days. The photomicrograph shows a section of the kidney with mild degeneration of renal tubule and cellular necrosis.

**Plate 13 (Methanol extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves* methanol extract orally given for 7 days.

The photomicrograph shows a section of the kidney with organized cellular architecture than that of diabetic control with enlarged glomerular space.

**Plate 14 (Benzene extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 250mg/Kgb.wt *V. amygdalina leaves* benzene extract orally given for 7 days. The photomicrograph shows a section of the kidney with slight necrosis of renal tubule and regenerative changes, organized cellular architecture than that of diabetic control

**Plate 15 (Toluene extract):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt *V. amygdalina leaves* toluene extract orally given for 7 days. The photomicrograph shows a section of the kidney withmild degeneration of renal tubules, organized cellular architecture than that of diabetic control.

**Plate 16 (Metformin200mg/Kgb.wt):** the group was induced with diabetes using 120mg/Kgb.wt and treated daily with 200mg/Kgb.wt metformin orally for 7 days. The photomicrograph shows a section of the kidney with apparently normal architecture. The group shows mild cell necrosis and organized cellular architecture than that of diabetic control.

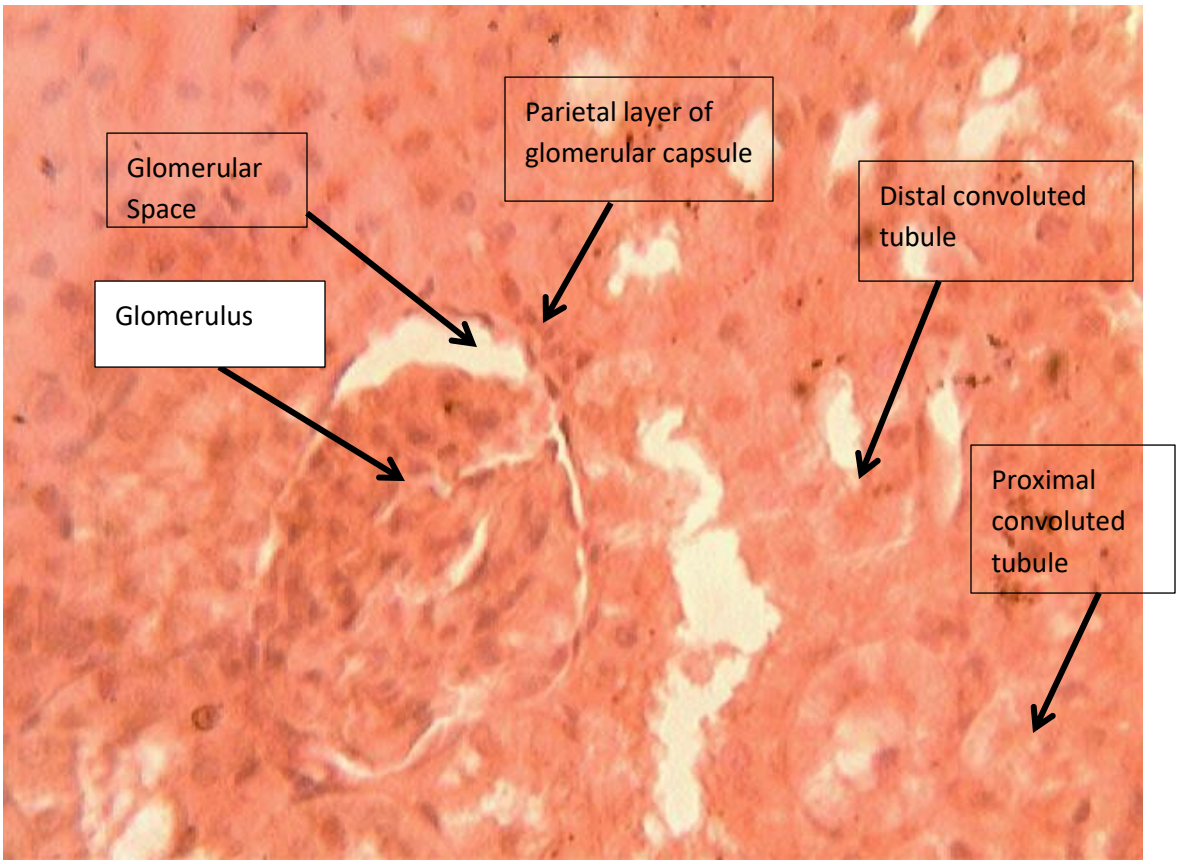


Plate 9: Photomicrograph of section of the kidney tissue from normal control (NC). Group served as normal control group received standard rat diet and water *ad libitum* for 7 days. On the 7th day, the animals were given vehicle. H&E X400

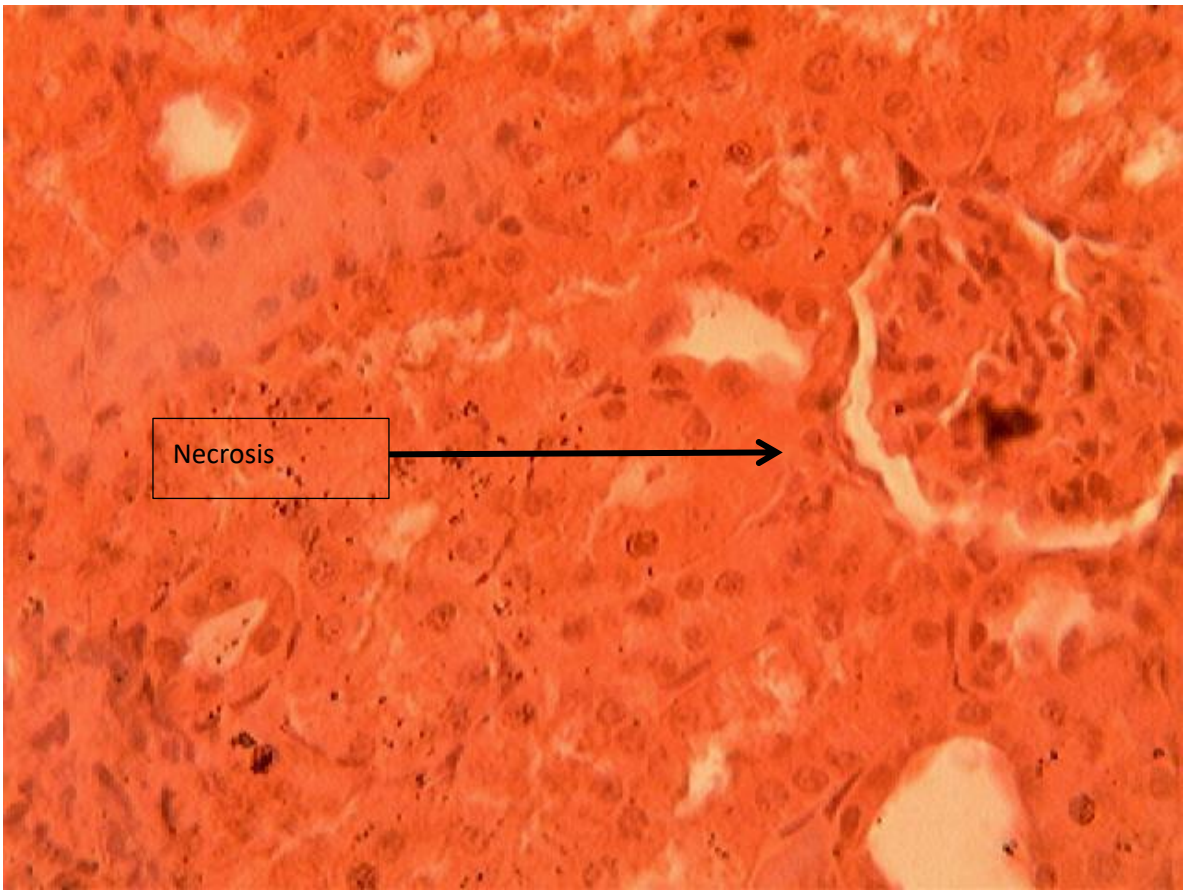


Plate 10: : Photomicrograph of section of the kidney tissue from diabetic control (DC), received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan.

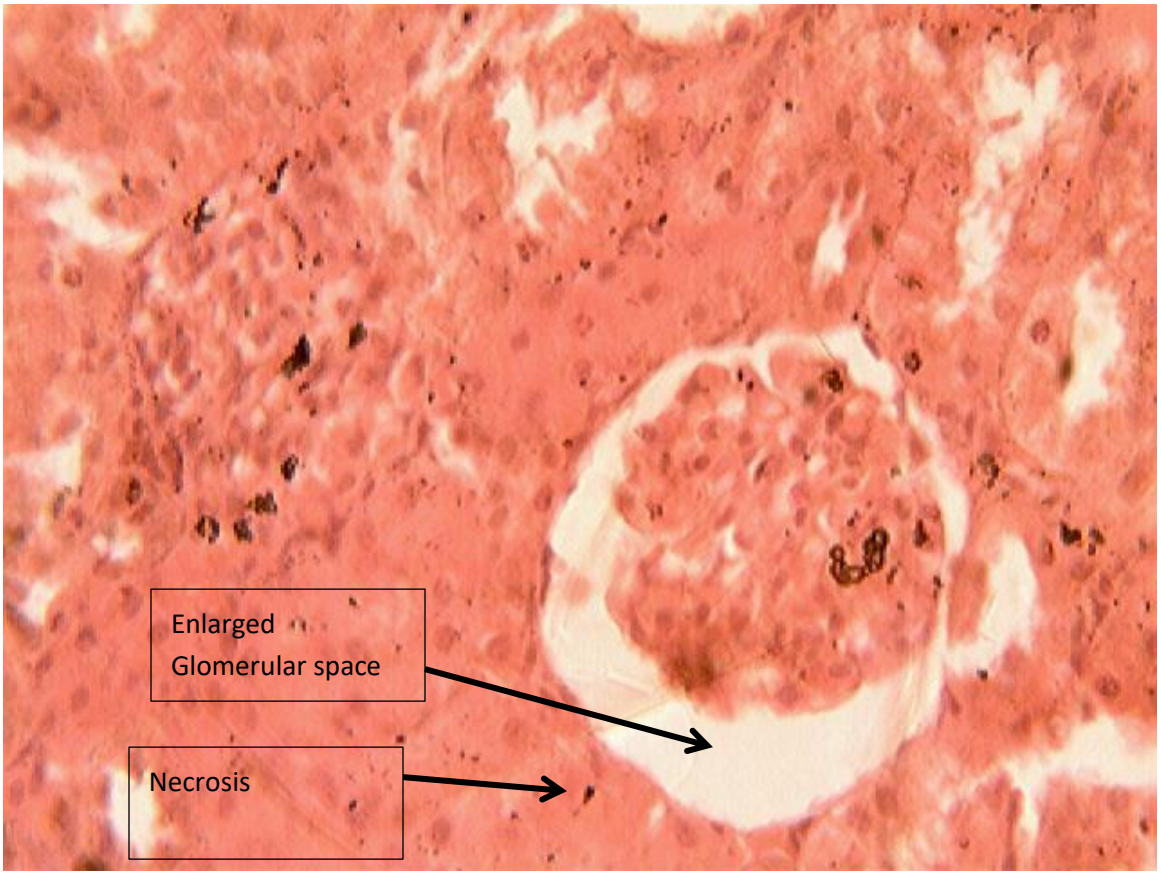


Plate 11: Photomicrograph of section of the kidney tissue from aqueous extract group (AQE). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt*V. amygdalina leaves* aqueous extract for seven days.

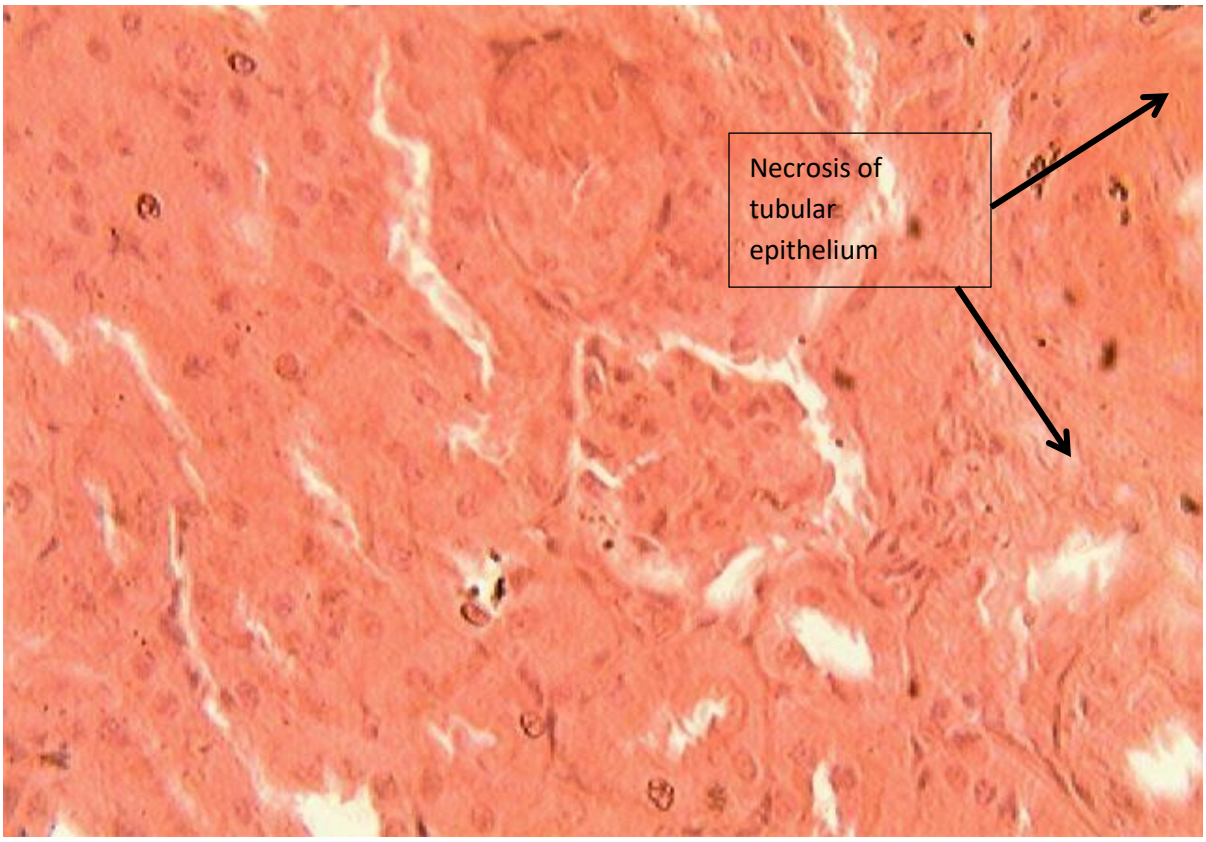


Plate 12: Photomicrograph of section of the kidney tissue from ethanol extract group (Eth). Group received standard rat diet and drinking water ad libitum for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt*V. amygdalina leaves* ethanol extract for seven days.

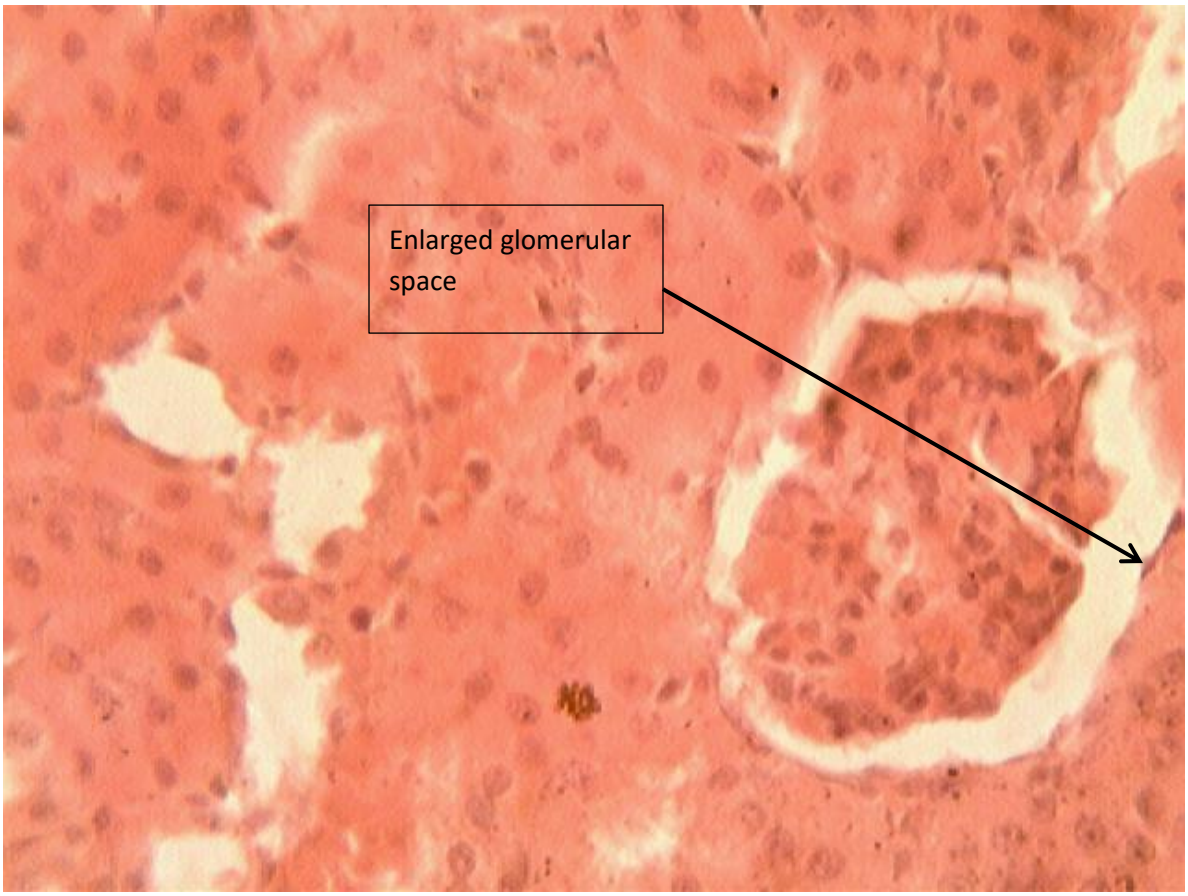


Plate 13: Photomicrograph of section of the kidney tissue from methanol extract group (METH). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt*V. amygdalina leaves* methanol extract for seven days.

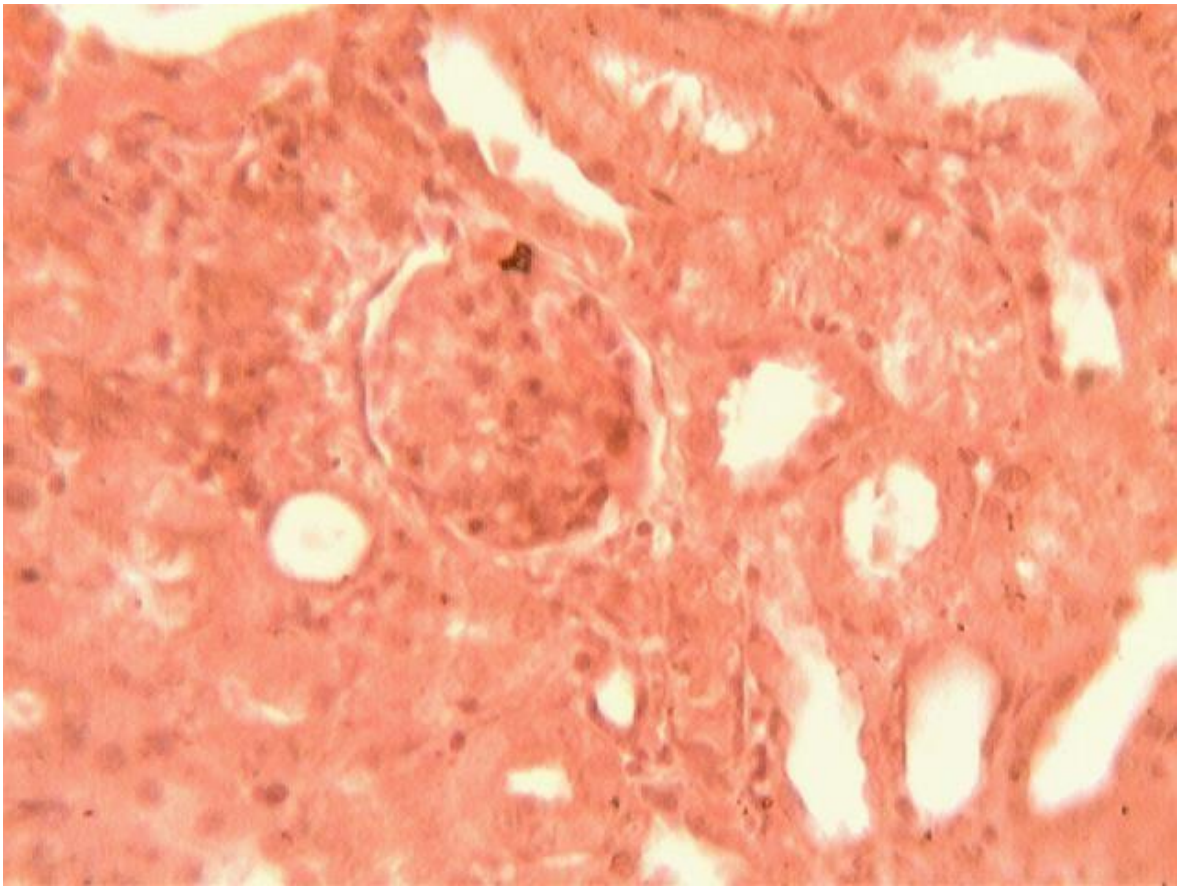

Plate 14: Photomicrograph of section of the kidney tissue from benzene extract group (BENZ). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wtalloxan, and treated with 200mg/Kgb.wt *V. amygdalina leaves* benzene extract for seven days.

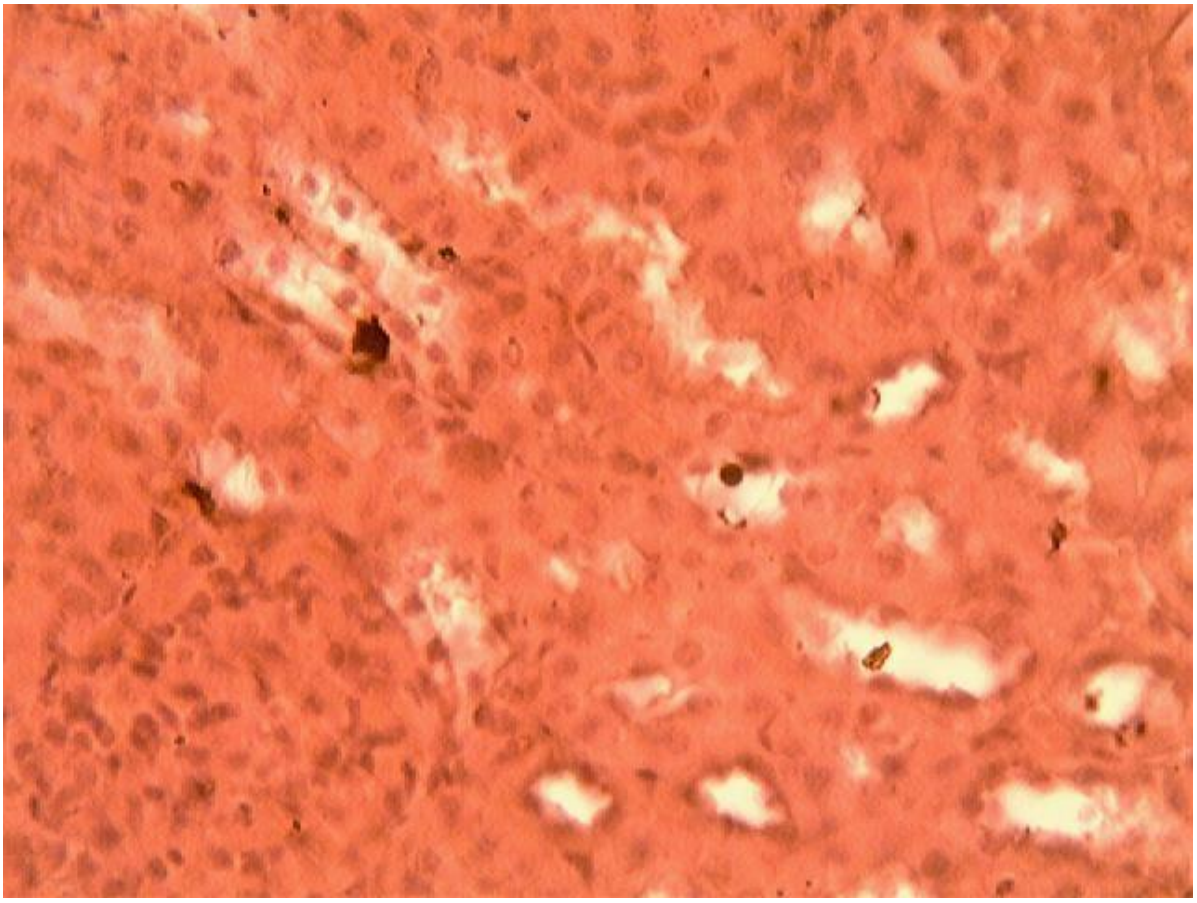

Plate 15: Photomicrograph of section of the kidney tissue from toluene extract group (TOL). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt*V. amygdalina leaves* toluene extract for seven days.

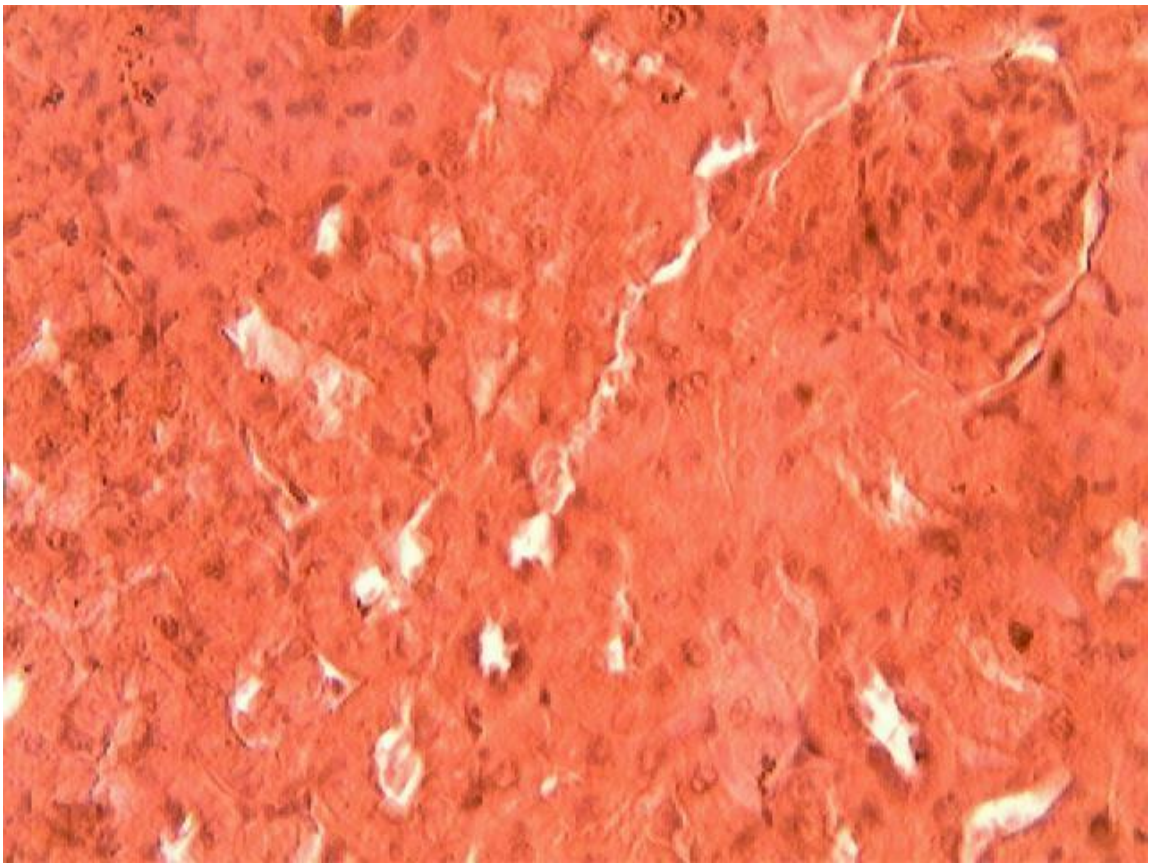

Plate 16: Photomicrograph of section of the kidney tissue from Standard group (STD). Group received standard rat diet and water *ad libitum* for 7 days. The animals in the group were induced for diabetes using 120mg/Kgb.wt alloxan, and treated with 200mg/Kgb.wt Metformin for seven days.

## 4.1DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder that affects carborhydrate, protein, and fat metabolism; marked with chronic hyperglycemia from insulin deficiency or insulin inaction (Porth, 1998; Dilworth *et.al.,* 2021)

From a very long time in the past, nature has been a source of healing agents. A good number of recent drugs have been isolated and characterized from natural source (Atangwho *et al.,* 2013). In Africa, for economic and cultural reasons, 80% of the populace uses curative plants from traditional medication to take care of their body when they get ill. When it comes to the issue of diabetes mellitus, about 800 plants have been known and used alone or in combination in ethnomedicine as an antidiabetic treatment worldwide. In African countries such as Côte d'Ivoire, Nigeria, Kenya, and Egypt, numerous ethnobotanical surveys established that a lot of of these medicinal plants are used against diabetes mellitus (N'Goran *et al.,* 2019). Green plants are known to hold in them a reservoir of effective chemotherapeutic agents with more systemic and easily biodegradable potentials (Atangwho *et al.,* 2013).

The promotion of traditional African diets still remains a powerful approach when it comes to the fight against diabetes. Various antidiabetic drugs such as biguanide, sulphonylurea, along with insulin have been in use for the treatment of diabetes. Still none of these drugs have been able to cure the diseases without adverse effects (Luke *et. al.,* 2013).

Although, the usefulness of this is well known as anti-diabetic, the comparism of a selective range of polar solvent extractions of VA are not studied so vastly with reference to anti-diabetic property. Therefore, the present study was carefully designed to evaluate the antidiabetic effect of polar and non-polar extracts and drug of VA. Five different solvents (having different

polarity) were used for the extraction purpose. These solvents include toluene, benzene, methanol, ethanol and water. With respect to some biochemical parameters, the aim of this study was to determine the antidiabetic effect of VA extracts on adult male albino rats and to compare the relative antidiabetic effect of polar and non-polar extracts of these plant.

### 4.1.1 Fasting Blood Glucose

Aside other hormones, insulin remain the most important hormone that regulates the amount of glucose in the blood. It also regulates the rate at which glucose is taken up by the tissues and the conversion of glucose to glycogen. A healthy control of the blood glucose levels in diabetics helps to avert or delay the progression of complications which may lead to premature disability or death from blindness, kidney failure, cardiovascular disease, stroke etc (Kalsbeek *et al.*, 2014). The deficiency of insulin or the insensitivity of its receptor plays a vital role in all forms of diabetes mellitus.

The most routine and biochemical marker that is used in the diagnosis and progress monitoring during management of diabetes mellitus in both clinical and experimental settings is blood and/or serum glucose concentration (Luka, *et.al.*, 2013).

Alloxan is a urea derivative that causes selective necrosis of the β (Beta)-cells of pancreatic islets. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Shafe *et.al.*, 2014).

In this study, the blood glucose reducing effects of ethanol and methanol extractyielded 33.19% and 43.66% of baseline blood glucose reduction; compared favourably with the standard drug

Metformin, of which is similar to other studies on the antihyperglycaemic effects of Vernonia amygdalina (Atangwho *et al.* 2007; Ebong *et al.* 2008; Momoh, Akoro & Godonu 2014). In some previous studies, administration of Vernonia amygdalina extract was shown to regenerate the β-cells of the pancreas, thereby contributing in part to its anti-diabetic effect (Item, et. al., 2014).

Nutritionally, it has been established that the plant contains moisture and fiber which makes available less sugar to the blood sugar content (Okolie *et.al.*, 2008) as plants rich in non starch polysaccharides were reported to cause a decrease of postprandial blood glucose concentrations in humans (Manach et.al., 2004). Other findings have revealed that fiber rich food does not increase blood glucose (Ylonen *et.al.*,2003) rather it improves insulin sensitivity and may have a role to play in the prevention and management of type 2 diabetes (Henry, 2004).

The *Vernonia amygdalina* extract alone lowered the glycemia significantly in the alloxan treated rats and this serves as proof that it has some peripheral action which is similar to metformin. Other research fellows in this area have also noted that Vernonia amygdalina extract could have a direct insulin-like effect on glucose metabolism (Taiwo, 2009; Sepici, 2004).The issue of drug-drug interaction should be properly addressed especially, when a patient is combining a potent antidiabetic agent and herb remedies (Micheal *et.al.*, 2010).

Metformin is known as one of the most widely prescribed drug for the treatment of Type 2 Diabetes Mellitus because aside its role in lowering blood glucose concentrations without causing overt hypoglycemia, it also leads to a significant reduction in plasma fasting insulin levels (Viollet, *et. al.*, 2012). Owen et al. also reported that metformin exercised its anti-diabetic effects via the inhibition of complex 1 of the mitochondrial respiratory chain.

One of the most significant factors affecting the extraction efficiency of bioactive compounds from plant materials and their resultant health benefits is the extraction solvent. The differences can be made clear by the variation in polarities of the solvents, which selectively extract different hydrophobic or hydrophilic phenolic compounds in the sample (Thanh *et.al.*, 2017).

This significant effect could be attributed to the synergism that is usually associated with bioactive compounds from medicinal plants and other agents over a prolonged period of time (Njoku *et. al.*, 2017).

### 4.1.2 Body Weight

Weight loss is a sign of tissue wasting that happens as a result of poor glycaemic control in diabetes mellitus and this usually leads to an increase in protein and fat mobilisation, resulting in eventual weight loss. Alloxan induced diabetes significantly reduces body weight of the diabetic untreated rat as the study duration progresses compared with the diabetic treated and normal control rats. Alloxan induced diabetes usually results to a significant loss in body weight while treatment with Venonia amygdalina extract restores the body weight. Diabetes is also generally followed up with increased glycogenolysis, lipolysis, gluconeogenesis and these biochemical activities leads to muscles wasting and loss of tissue protein. Vernonia amygdalina is seen to prevent these changes and thus brings back the body weight of the diabetic treated rats to normalcy (Chinwe *et.al*, 2015).

One week after confirmation of diabetes, before the commencement of treatment, a significant ($P < 0.05$) increase in fluid intake and decrease in feed intake were noted. This may most likely be because of the obligatory renal water loss combined with hyperosmolarity in diabetes which tends to decrease intracellular water, triggering the osmoreceptor of the thirst centre of the brain

and polydipsia occurrence, which leads to water intake. At this stage, there is a decrease in appetite and thus catabolic effect prevails leads to weight loss which was obvious in the diabetic control group in this study

This observation agrees with what was reported by Ekam et al. and Goje et al. in their studies of 3 days post-induction of diabetes with alloxan. They observed a reduction in body weight (b.w) in all the groups administered with alloxan though not significant ($P > 0.05$), but that was not the case with their non diabetic control (NC) group which did not show any decrease in body weight (b.w.). This observation was also consistent with that of Russell et al. who also proved that there is decreased appetite in diabetes.

The impact of the extract on body weight tends to suggest that whole extract from VA leaves might be safer as a medicine compared to its fractions (Atangwho, *et. al.*, 2014). Tiwari and Rao have in their review indicated and advocated the use of whole plant extracts in preference to isolated and pure compounds from plants articulating several draw backs of pure compounds, prominent amongst which is increased toxicity.

The effect of the extracts on body weight may be as a result of the interference with metabolic processes and absorption or inflammatory effects on hepatocytes, which may have led to the decrease in weight observed especially at higher concentration (Njoku *et al.*, 2017)

In the course of treating diabetic rats with extracts of Vernonia amygdalina and metformin, a significant increase in body weight was observed. This means that treatment with experimental drugs helps to increase availability of glucose to the tissues, both for supply of energy and to build tissue materials required for growth (Iwara *et. al.*, 2017)

### 4.1.3 LIVER FUNCTION TESTS

**Alanine aminotransferase** (EC 2.6.1.2) is reffered to as a tissue enzyme that catalyzes the transfer of an amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. Pyridoxal phosphate serves as a coenzyme in this reaction (Nelson & Cox, 2000).

Alanine aminotransferase is produced mainly in the liver with only little amount being present in other regions. When there is hepatic disease, the serum or plasma level of alanine aminotransferase is improved. Measurement of alanine aminotransferase activity is carried out alongside aspartate aminotransferase and alkaline phosphatase to examine for liver disease. It is also measured to monitor patients that are being treated with antiretroviral drugs associated with hepatotoxicity such as nevirapine (Wang *et al.*, 2012).

Administration of alloxan might have caused a leakage of these enzymes from the liver and some other tissues into circulation, signifying the injury of these.

In this study the administration of methanol extract of *V. amygdalina* and the standard drugs (metformin) resulted in a normalization of ALT activity. Vernonia amygdalina leaf-extract in being a reason for the reduction in the elevated levels of the serum enzymes may have reversed totally or partially reactive oxygen species (ROS) generation process or mopped them up by its antioxidant action (Atangwho *et.al.*, 2007).

**Aspartate aminotransferase** (EC 2.6.1.1) is refferred to as a tissue enzyme that catalyzes the exchange of amino acid groups between aspartate and α-keto acids. Oxaloacetate and glutamate

are product of a transamination reaction between L-aspartate and α-ketoglutarate that is catalyzed by aspartate aminotransferase (Stryer, 2006).

Our findings showed that AST activity in the groups receiving ethanol, methanol, toluene and Benzene extract were significantly ($p<0.05$) reduced when compared to diabetic control group. This may be due to the hepatoprotective ability of the bitter leaf extract due to its antioxidant property. This property is attributable to flavonoids are in the extract. Flavonoids are reported to have antioxidant activity and are effective scavengers of superoxide anions (Ulicna *et. al.*, 2003)

**Alkaline phosphatase** (EC 3.1.3.1) is reffered to as a homodimeric protein enzyme of 86kilodaltons. It plays the physiological role of dephosphorylating compounds, and has significance in medicine as a diagnostic marker. It also plays an integral part in metabolism within the liver and development within the skeleton. Due to its extensive prevalence in these areas, its concentration in the bloodstream is used by medical laboratory scientists as a biomarker in diagnosing hepatitis or osteomalacia (Foucault *et al.*, 1991).

Our findings showed that diabetes induction was accompanied by significant ($p<0.05$) elevation of ALP activity.Increased level of ALP has been attributed to the damaged structural integrity of hepatic cells because the enzyme alkaline phosphatase is situated in the cytoplasm and is allowed to freely move into the circulation after cellular damage. ALP levels in plasma will increase with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver (Momoh *et. al.*, 2014).

However, treatment for 28days with leaf extracts of Vernonia amygdalina and Metformin drug decreases the activity of this enzyme in a dose related manner compared with the diabetic control thus improving renal and hepatic functions. This observation concurred with report of Atangwho *et al.,* who reported the hepatoprotective effect of leaves extract of Vernonia amygdalina in diabetic rat (Luke *et.al,* 2013).

**Bilirubin** is reffered to as a catabolic intermediate of haem. High concentration of this molecule leads to jaundice. However, the liver plays a vital role in mopping bilirubin from the plasma. In the presence of liver disease or damage, the level of bilirubin is improved (Spencer *et. al.,* 2011)

In this study the total bilirubin concentration significantly ($p<0.05$) increased by alloxan induction when compared to normal control group. However, administration of the different solvent extracts of *V. amygdalina* did not result in a significant ($p<0.05$) normalization of altered serum bilirubin concentration.

Emerging evidence has shown that the serum bilirubin concentrations are associated to the development of Type 2 Diabetes Mellitus and its complications (You-Fan *et. al.,* 2017). Authors proposed that higher serum concentration of bilirubin decreases the development of Type 2 Diabetes Mellitus via its anti-oxidative effects (Deetman *et al.,* 2013). In addition, lower serum bilirubin has been reported to be linked with other cardiovascular complications in patients with Type 2 Diabetes Mellitus, such as autonomic neuropathy, arterial stiffness, peripheral neuropathy, retinopathy and carotid atherosclerosis (You-Fan *et.al.*,2017).

### 4.1.4 LIPID PROFILE TEST

#### 4.1.4.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum total cholesterol concentration.

Cholesterol is primarily a structural component of membranes and of the outer layer of plasma lipoproteins. The disorders that are associated with lipid and lipoprotein metabolism occur with hepatocellular injury (Sadava *et. al.,* 2011).

Factors such as age, sex, and genetics can also affect lipid profile. Some aspects of lifestyle, such as diet, level of physical activity, level of diabetes control, and smoking status, also influence lipid profile. Certain medical conditions can increase or lower cholesterol and triglyceride levels. Cholesterol is a key substance that the body makes us of to make such things as digestion-aiding material, hormones, and cell membranes. It is both manufactured by the body and absorbed from some of the foods we eat. While cholesterol is needed for different bodily functions, too much cholesterol is damaging, since excess cholesterol can be deposited in blood vessel walls. These fat deposits can result to atherosclerosis, or hardening of the arteries, and cardiovascular disease (Shafe *et. al.,* 2014).

The estimation of serum cholesterol concentration is a major aid in the diagnosis and classification of lipaemias and in the screening for atherogenic risk. Other conditions such as hepatic and thyroid disease affect cholesterol concentration (Robinson *et al.,* 2012).

The elevated values for lipid profile parameters such as cholesterol, LDL and TG observed in the alloxan-induced diabetic control group could be partly as a result of an increased intestinal cholesterogenesis, which is due to high activity of 3- hydroxyl-3-methylglutamyl-CoA reductase in the intestine of the alloxan-induced diabetic rats, the rate limiting enzyme in the

biosynthesis of cholesterol and also increased availability of acetyl-CoA resulting from progression of fat oxidation in diabetes mellitus (Luka *et.al,* 2013).

In a study conducted by Omede *et.al,* the result conforms to the increase in triglycerides and cholesterol and was found to be markedly suppressed in animals treated with Vernonia amygdalina. This is also similar to another study, where Vernonia amydalina blunted the provoked levels of cholesterol and triglycerides (Saghir *et.al,* 2014).

The mortality rate seen to take place in alloxan untreated rat group can be caused by a high increase in blood glucose which was left untreated.

#### 4.1.4.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serumtriacylglycerol concentration.

Triglycerides determinations when performed in conjunction with other lipid assays are useful in the diagnosis of primary and secondary hyperlipoproteinaemia, artherosclerosis and the risk of heart disease and stroke (Drummond & Brefere, 2014).

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases and is often linked with diseases such as hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Ikewuchi, 2012).

Due to the fact that triglyceride is the source of energy that is next in line when glucose is depleted, it is mobilized back to the stored depot (adipose tissue), thereby accounting for the reduction in triglyceride concentration in the blood as well as significant difference between the blood glucose level and serum triglyceride concentration (Shafe *et.al.*,2014).

In this study an altered serum lipid profile was, nonetheless, reversed significantly ($p < 0.05$) following treatment with the methanol leaf extract of Vernonia amygdalina (MLVA).

According to research, aqueous leaf extract of Vernonia amygdalina has been reported to have anti–hypertriglyceridemic and hypolipidemic effects in alloxan induced diabetic model

**4.1.4.3 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serumHDL-cholesterol concentration.**

An inverse relationship is known to exist between serum HDL-cholesterol and the risk of coronary heart disease. The measurement of total HDL and triglycerides supplies valuable information for the prediction of coronary heart disease and for lipoprotein phenotype (Betteridge, 2008).

In this study, administration of the aqueous and methanol extract resulted in a significant ($p<0.05$) restoration of altered HDL-cholesterol concentration. The concentration of high density lipoprotein (HDL) increased in the group treated with aqueous and methanol extract as compared to control may be as result of the presence of phytochemicals in the extracts as well as the improvement in the antioxidant status.

An elevation in HDL levels perhaps was directly provoked by the plant. This could be through the mobilization of cholesterol from extra hepatic tissue, where it is catabolised. Hepatic HMGCoA reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway and its inhibitors are very effective in reduction of plasma cholesterol. With the significant decline in cholesterol, Vernonia amygdalina could probably be an inhibitor of HMGCoA reductase in rats. Speculating in great detail on the mechanism involved is far away from the scope of this research. Moreover, the hypolipidemic effect of Vernonia amygdalina administration could be

linked from the activation of the rate limiting step in cholesterol catabolism, which is the conversion of cholesterol 7-α-hydroxylase to bile acid (Omede *et. al.*, 2018).

HDL applies part of its anti–atherogenic effect by counteracting LDL oxidation and also of late studies also have shown that HDL improves the functioning the reverse cholesterol transport pathway by inducing an efflux of excess build up of cellular cholesterol and puts a stop to the generation of an oxidatively modified LDL (Yokozawa *et al.,* 2006). In addition, the aqueous and methanol leaf extract of Vernonia amygdalina may have most likely played the anti–atherogenic role through the elevation of HDL cholesterol (Ibegbu *et. al.,* 2018).

Several studies have shown that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also increases HDLcholesterol (Luka &Tijjani, 2013).

#### 4.1.4.4 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL-cholesterol concentration.

LDL is the vehicle that carries cholesterol and cholesteryl esters into various tissues. LDL measurement is of diagnostic importance in both primary and secondary hyperlipoproteinaemia. They are also an indictive parameter that is used to diagonize for atherosclerosis and coronary heart disease risk (Ivanova *et. al.*, 2017).

Our research showed the effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL-cholesterol concentrationin alloxan-induced diabetic rats. It further showed that diabetes induction resulted in a significant ($p<0.05$) increase in LDLcholesterol concentration. However, administration of the ethanol, methanol toluene and

benzene extract resulted in a significant ($p<0.05$) restoration of altered LDL-cholesterol concentration.

In medical practice, the use of LDL cholesterol has taken over total cholesterol as a risk marker and the primary treatment target for hyperlipidemias (Stone et. al., 2014). Reduction in LDL cholesterol has been experimentally proven to decrease cardiovascular risk and mortality in a continuous and graded manner over a wide range of LDL cholesterol levels. (Wadhera *et.al.*, 2016).

Research shows that insulin raises the number of LDL receptor, so chronic insulin deficiency might be linked with a diminished level of LDL receptor which results to their high levels in serum or plasma thereby increasing LDL particles and result in the elevated levels of LDLcholesterol value in diabetes mellitus. Elevated levels triglycerides, LDL and total cholesterols also serve as an indicator for a high risk of atherosclerosis. They are used to determine the risk of heart disease. Virtually all the extracts used in this study could have a positive effect on insulin levels as they significantly lower serum LDL cholesterol (Luka *et. al.*, 2013).

**4.1.4.5 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serumVLDL-cholesterol concentration.**

Very-low-density lipoprotein is a type of lipoprotein produced by the liver. VLDL is one among the five main groups of lipoproteins (chylomicrons, VLDL, intermediate-density lipoprotein, low-density lipoprotein, high-density lipoprotein) that allows the mobility of fats and cholesterol within the water-based solution of the blood stream (Gibbons *et al.*, 2004)

The presence of high plasma levels of VLDL cholesterol serves as a risk factor for cardiovascular disease and often accompanies diabetes mellitus and obesity(Ikewuchi, 2012). Our research showed the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum VLDL-cholesterol concentrationin alloxan-induced diabetic rats. Results obtained shows that diabetes induction resulted in a significant ($p<0.05$) elevation of VLDL-cholesterol concentration.

Alloxan-induced diabetes is linked with decreased insulin level due to the destruction of β-cells following alloxan administration. This decreased level of insulin progresses into hypertriglyceridemia and leads to the secretion of VLDL cholesterol. Increased levels of TGs turn out to subsequently displace protein content of VLDL and LDL. This form of imbalance of protein and triglyceride content of lipoproteins leads to a decreased uptake of these lipoproteins by lipoprotein receptors. The buildup of these lipoproteins and TGs is related to a lot of vascular disorders**.**

As addition to the roles mentioned above VLDL-cholesterol serve as an antihyperglycemic agent while metformin has an important property of its ability to modestly reduce hyperlipidemia (Richard & Pamela 2009).

**4.1.4.6 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum LDL/HDL-cholesterol ratio.**

It has been suggested that an LDL-c/ HDL-c ratio is a more worthy risk indicator for CVD than individual parameters. It is well established that the oxidative modification of LDL-c plays an important role in the pathogenesis of atherosclerosis. Very much elevated levels of HDL-c have also been experimentally proven not to be linked with the risk of vascular events. Indeed, the

evidence suggests that improving the function of HDL-c rather than increasing its levels, is connected with clinical benefit. Based on data from clinical trials, a high LDL-c/HDL-c ratio is linked with coronary plaque progression, whereas a lower LDL-c/HDL-c ratio that is achieved by pharmacological interventions may be linked with coronary plaque regression. Some studies have suggested that with a high ratio of LDL-c/HDL-c in certain individuals treatment should commence because of abnormal cholesterol levels. In a certain study, a collective analysis of data from four prospective randomized trials showed a positive linear correlation between an index of LDL-c/HDL-c ratio and changes in coronary plaque volume. In addition, a high LDLc/HDL-c ratio has been recommended to be a indicator to reveal the presence of coronary lipidrich plaques and plaque vulnerability leading to an elevated SCD risk. Rupture of high-risk vulnerable plaques is reffered to be the major pathway in the progression of coronary thrombosis, which ultimately results to acute MI and SCD. Coronary heart disease is the generally accepted pathology underlying SCD (sudden cardiac death) (Kunutsoret. *et. al.*, 2017).

#### 4.1.4.7 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on cardiovascular risk ratio.

Our research showed the effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on cardiovascular risk ratio **(**CRR) in alloxan-induced diabetic rats. It further showed that diabetes induction resulted in a significant ($p<0.05$) increase in cardiovascular risk. The different solvent extracts of *V. amygdalina* resulted in a significant ($p<0.05$) reduction of CRR. This reduction was significant in the groups receiving ethanol, Methanol and aqueous extracts was comparable to those of normal control and standard.

According to American Heart Association, a value of (>3.5) reveals a higer propensity towards cardiovascular diseases. A good number of epidemiological studies have discovered that in type 2 diabetic patients, metformin improves vascular function and decreases cardiovascular events and mortality by mechanisms that are not totally attributed to its antihyperglycemic effects (Manjusha *et.al,* 2016)

The results in this study has proven that metformin have a positive healing effect in type 2 diabetes, such as improved lipid profiles, and enhanced endothelial function. This would in due course improve atherogenic risk in diabetic subjects. A number of studies have pointed out that metformin may enhance some of the features of the metabolic syndrome as it not only improves insulin sensitivity in the liver and muscle, as its main anti-hyperglycemic mechanism of action, but also induces additional useful effects on several metabolic abnormalities linked with the metabolic syndrome (Hundal & Inzucchi 2003; Viollet *et.al.*, 2009; Hardie *et.al.,* 2012). A study also reported that metformin induced enhancement of metabolic disorders that is linked with the energy state of the body, decrease of macrovascular morbidity and mortality, anti-atherogenic, anti-inflammatory and antioxidant effects (Foretz *et al.,* 2010)

The treatment dose actively lowered (though not significantly) the atherogenic indices of the animals used in this study. Atherogenic indices part of the strongest indicators of the risk of heart disease: the higher the value, the higher the risk of coming down with cardiovascular disease and vice versa. Low atherogenic indices are a good protective indicator against coronary heart disease (Ikewuchi, 2011).

### 4.1.5 ENDOGENOUS ANTIOXIDANT TEST

#### 4.1.5.1 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate ascorbic acid concentration.

Vitamin C is also essential for use by connective tissues and also promotes the progressive healing of fracture and wounds. This reasonable proves the use of this extract in solving malnutrition problems and also the healing of bone fracture (Ojogbane *et.al.,* 2015).

Vitamin C is a key non-enzymatic antioxidant in *in vivo* and *in vitro*. Its pimary roles, are to perform as free radicals scavenger and prevention of oxidative damage under virtually all types of oxidative stress (Prakasam *et al.,* 2005**).**

Our research showed the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate ascorbic acid concentrationin alloxan-induced diabetic rats. It further showed that the liver homogenate ascorbic acid concentration were significantly ($p<0.05$) reduced by diabetes induction in the groups administered aqueous, ethanol, toluene and benzene extracts of *V. amygdalina*. The methanol extracts effectively increased depleted ascorbic acid concentration.

Such vitamin C (ascorbic acid) from the Vernonia amygdalina extracts is said to enhance the lipid profile in alloxan-induced diabetic mellitus in rats by functioning via the enzyme, cholesterol 7α-hydroxylase to move cholesterol into bile synthesis. In addition, by scavenging free radicals it reduces oxidative damage to oxidized LDL-cholesterol. There was also a decrease in blood glucose in diabetic rats when compared to untreated diabetic rats (Owu *et.al*, 2006).

#### 4.1.5.2 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate vitamin E concentration.

Vitamin E is manufactured by plants and is an antioxidant that shields all membranes and other fat- soluble parts of the body, such as low-density lipoprotein cholesterol, from damage. It is from the intestine that Vitamin E is absorbed through lymph. It circulates through the body plasma in conjunction with Beta-lipoprotein (George & Adegoke, 2011).

Our research showed the effect of V. amygdalinaleave aqueous, ethanol, methanol, toluene and benzene extract administration on liver homogenate Vitamin E concentrationin alloxan-induced diabetic rats. It further showed that the liver homogenate vitamin E concentration were significantly ($p<0.05$) reduced by diabetes induction in the groups administered aqueous, toluene and benzene extracts of V. amygdalina. However, the ethanol, methanol extracts effectively increased depleted vitamin E concentration.

The reduced plasma α-Tocopherol levels in many experimental rats may be as a result of the improved lipid level pattern. In addition to the enhanced utilization of α-Tocopherol in lowering the elevated oxidative stress as evidenced by decrease in the hepatic MDA marker in such groups (Johar *et.al.,* 2018).

#### 4.1.5.3 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver GSH concentration

Our research showed that the liver homogenate GSH concentration was significantly ($p<0.05$) reduced across diabetic groups, except in the standard group when compared to normal control

group. The extracts did not normalize GSH concentration but offered a significant protection against GSH depletion when compared to diabetic control group.

It has been reported for those diabetic problems showed in alloxan-affected animals are premepted by free radical. Decreasing pancreatic antioxidant enzyme's action in diabetic animals was as a result of the explanation that pancreas being reduced in antioxidant enzymes in essence is helpless to ROS assault. Since antioxidant enzymes are low, alloxan intervened ROS resulted due to decreased antioxidant enzymes action. Treatment of diabetic rats with Vernonia amygdalina extracts increased the GSH levels and restored the activity of glutathione reductase in diabetic rats to its normal levels (Sheweita *et.al*, 2016).

### 4.1.5.5Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate superoxide dismutase activity.

SOD is an enzyme that performs the role of protection by repairing the cells and reducing the damage done to them by superoxide, by also acting as catalyst when it comes to the dismutation of the superoxide radical ($O_2$) into hydrogen peroxide ($H_2O_2$) and elemental ($O_2$). Our research showed that superoxide dismutase activity was significantly ($p<0.05$) reduced by diabetes induction. However, the extracts offered significant ($p<0.05$) protection against SOD depletion when compared to diabetic control group.

Studies carried out by Muth (2004) have revealed that SOD performs the function as both an antioxidant and an anti inflammatory agent in the body by neutralizing the free radicals that can result to wrinkles and pre cancerous cell changes. It also assists the body to make use of zinc, copper and manganese and a likely essential in the production of healthy fibroblast; one of the deficiencies in protein energy malnutrition. The extract induced a rise in SOD indicating the

antioxidant and anti inflammatory potentials of Vernonia amygdalina leaves (Ojogbane *et.al*, 2015).

This discovery has already been documented in diabetic animals (Pavana *et.al,* 2009, Onyeka *et.al,* 2013).

#### 4.1.5.6 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum superoxide dismutase activity.

Results from this study shows that serum superoxide dismutase activity being significantly ($p<0.05$) reduced by diabetes induction. However, the extracts offered significant ($p<0.05$) protection against SOD depletion when compared to diabetic control group. SOD is another indicator of oxidative stress injury, which mopps up reactive oxygen species, a representative of the body's antioxidant system An increase in the levels of MDA and the lowering in the levels of SOD in liver homogenate were higher than those in the serum, which shows that the liver in alloxan induced rats had severe oxidative stress damage.

#### 4.1.5.7 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate glutathione peroxidase activity.

The results from this study showed that liver homogenate glutathione peroxidase activity was significantly ($p<0.05$) reduced by diabetes induction as seen in all alloxan exposed groups with exception of methanol and standard group. However, the administration of *V. amygdalina* extracts offered significant ($p<0.05$) protection against GPx depletion when compared to diabetic control group.

Hence, it is obvious that extract was able to improve the activity of GPx which could explain the effectiveness in treating various ailments which was also reported by (Hans *et al.,* 2004).

**4.1.5.8 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum glutathione peroxidase activity.**

Results from this study showed that serum glutathione peroxidase activity was significantly ($p<0.05$) reduced by diabetes induction as seen in all alloxan exposed groups with exception of Standard group. However, the administration of *V. amygdalina* extracts except Benzene extract offered significant ($p<0.05$) protection against GPx depletion when compared to diabetic control group.

CAT, SOD and GPx activities were elevated significantly at 95% confidence level by the actions of the leaf extracts. Catalase, Superoxide Dismutase and Glutathione Peroxidase are the key intracellular defence mechanism to withstand the increased oxidative stress, doing away with superoxide anion and hydrogen peroxide that may oxidise cellular substrates thereby preventing free radical chain reactions. The aqueous leaf extract of Vernonia amygdalina resulted to the highest increase in GPx activity. All of these protections may be as a result of the antioxidant properties of the plants, which stems from their phytochemical components. It has been discovered that phytochemicals possess the potential to lower oxidative damage to cells (Enemali & Udedi, 2018).

**4.1.5.9 Effect of *V. amygdalina*aqueous, ethanol, methanol, toluene and benzene leaf extracts on liver homogenate MDA concentration**

MDA is a widely used marker that serves as clear indicator to check for lipid peroxidation (Johar *et.al.,* 2018). MDA concentration was observed to be significantly ($p<0.05$) elevated in the

groups DC, Aqueous, toluene and benzene extracts of *V. amygdalina*. The ethanol and methanol extracts offered significant reduction in MDA concentration when compared to standard.

A profound alteration in the concentration of lipid peroxidation end product (MDA) and antioxidant enzyme status in the liver was revealed in the diabetic control rats (Akpan & Ekpo, 2015). The level of MDA was significantly ($P<0.05$) elevated while the level of the enzymes were significantly lowered ($P<0.05$) (Akpan & Ekpo, 2015). Increased lipid peroxidation and the drastic alterations in the antioxidant enzyme status is a sign of oxidative stress in the diabetic control rats. An increase in the level of MDA in the liver of diabetic control rats and that of rats treated with aqueous, toluene and benzene extracts of *V. amygdalina* was an indication of hyperglycemia caused lipid peroxidation.

According to a study, GPx, SOD and CAT level were significantly increased ($P<0.05$) while MDA was significantly reduced ($P<0.05$)in the diabetic treated groups compared to the diabetic control. This explains the reason to the protective effects of the Vernonia amygdalina extracts on the antioxidant enzyme status of diabetic rats.

#### 4.1.5.10 Effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum MDA concentration

Results from this study shows the effect of *V. amygdalina* aqueous, ethanol, methanol, toluene and benzene leaf extracts on serum MDA concentrationin alloxan-induced diabetic rats. MDA concentration was observed to be significantly ($p<0.05$) elevated in the groups DC, Aqueous, toluene and benzene extracts of *V. amygdalina*. The ethanol and methanol extracts significantly reduced MDA concentration which compared favourably to standard.

In the experimental group of the present study, MDA levels is enhanced first and then decreased, which may be as a result of the direct oxidation of unsaturated fatty acids in the cell membrane which is used to generate MDA during liver congestion and hypoxia, changing the fluidity and permeability of the cell membrane and thus resulting to an acute injury (Sharma *et.al,* 2009). MDA can react and destroy DNA bases. It is documented to be mutagenic in bacterial and mammalian cells and carcinogenic in rats (Valko *et al.,* 2007).

### 4.1.6 HISTOLOGICAL EVALUATION

The liver is frequently damaged during diabetes, as a consequence of increased levels of oxidative stress and dysregulation of immune function (Park, *et.al.,* 2013). Profiles of normal liver histology show hepatocytes with a polyhedral shape and a round nucleus at the center (Kleiner, 2014).

These hepatoprotective potentials of *V.amygdalina* were also corroborated by the histopathological studies(Enemali & Udedi, 2018). All these protections may be due to the antioxidant properties of the plants, which stem from their phytochemical components (Enemali & Udedi, 2018).

*Vernonia amygdalina* may keenly contribute in the elimination of metabolites that are harmful to the kidney (Howard et.al., 2003). This mild protective effect of bitter leaf may be due to the flavonoid as documented. Howard *et. al.,* 2003 suggested that the flavanoids and its saponins are the active principles which are reasons the antioxidant activities of *Vernonia amygdalina* plant. The protective effect of *Vernonia amygdalina* leaf may also come from its free radical scavenging activity or by impeding the generation of reactive oxygen species thereby demonstrating its antioxidant capability (Ehimigbai & Aneke, 2015).

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

The results of the present study indicate that V. amygdalina the glucose reducing effect of the extract was in the order Methanol > Ethanol>Benzene> Aqueous> toluene which could be the result of the bioactive secondary components of the plant. It also increased antioxidant enzymes activities in diabetic rats. In addition, V. amygdalina extracts suppressed the level of free radicals and dyslipidemia in diabetic rats and consequently could alleviate complications of TD.

The result reflected the effect of V. amygdalina leaves on the body weight  an attributed it to the same reason. V. amygdalina leaves extracts positively affected the biochemical indices when compared to the effect of Metformine which served as the standard drug. This justifies the use of V. amygdalina leaves in the treatment and prevention of DM; hence its intake should also be encouraged in homes.

This establishes the fact that V. amygdalina and Metformine provide additional antioxidant protection as antidiabetic remedies, thereby protecting the liver and kidney from oxidative stressinduced damage during diabetic complications.

## 5.2 Recommendation

Further studies should seek to isolate and characterize more phytochemicals in V. amygdalina, and relate its role in controlling diabetes mellitus and its related complications as well as the molecular mechanism of its action. The percentage yield of each of these solvent extracts should be included in the further studies. The latest medically accepted drugs should be used as the standard drug in

subsequent research to ensure that the research matches with the latest trends when it comes to the treatment of diabetes. Further research can be redefined using a novel plant. The model of this research can be tried in female rats so as to compare the result with this research which focused on male albino rats. A new and freshly selected group of polar solvents can be modeled in the next study so as to compare results gotten with this study. Another diabetogenic agent like streptozotocin can be tried under a more controlled environment because of its sensitive nature so as to be able to compare results with this research. Use other more advanced methods such as membrane separation and nanoparticales in subsequent studies. Criticism of the methods used by other researchers who did similar research work should be done in the subsequent research. The use of toxic compounds like benzene should carefully be ascertained before including it in the model of research with similar nature so as not to put the animals under study at risk of toxicity. The smaller the group of polar solvents to be compared in the study the better. Proper connection of any research related to the disease worked on in this research should be done by having contact with patients who are carriers of this disease so that we can better understand the etiology of the disease and its relevance to the body of knowledge. Histopathology of the pancreas should also be included in the further study.

### 5.3. Contribution to knowledge

The analysis in this research has given results that proves that V. amygdalina has curative potentials that can be used for a further production of a highly potent bitter leaf supplement capsule or a therapeutic agent or as preventive medicine or to increase the efficacy of the orthodox drugs so that persons with diabetes mellitus can make good use of it with/without the bitter taste.

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## APPENDIX

**Test of Homogeneity of Variances for Lipid Profile**

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| TotalCholesterol | Based on Mean | .651 | 7 | 24 | .710 |
| | Based on Median | .465 | 7 | 24 | .850 |
| | Based on Median and with adjusted df | .465 | 7 | 16.178 | .846 |
| | Based on trimmed mean | .605 | 7 | 24 | .746 |
| HDL | Based on Mean | 1.299 | 7 | 24 | .293 |
| | Based on Median | 1.117 | 7 | 24 | .385 |
| | Based on Median and with adjusted df | 1.117 | 7 | 15.271 | .401 |
| | Based on trimmed mean | 1.285 | 7 | 24 | .299 |
| TG | Based on Mean | 1.392 | 7 | 24 | .254 |
| | Based on Median | 1.164 | 7 | 24 | .359 |

| | | | | | |
|---|---|---|---|---|---|
| | Based on Median and with adjusted df | 1.164 | 7 | 17.163 | .372 |
| | Based on trimmed mean | 1.371 | 7 | 24 | .262 |
| LDL | Based on Mean | 1.366 | 7 | 24 | .264 |
| | Based on Median | .997 | 7 | 24 | .457 |
| | Based on Median and with adjusted df | .997 | 7 | 9.632 | .487 |
| | Based on trimmed mean | 1.280 | 7 | 24 | .302 |
| VLDL | Based on Mean | 1.401 | 7 | 24 | .251 |
| | Based on Median | 1.170 | 7 | 24 | .356 |
| | Based on Median and with adjusted df | 1.170 | 7 | 17.094 | .369 |
| | Based on trimmed mean | 1.380 | 7 | 24 | .259 |
| LDLHDLRatio | Based on Mean | 2.395 | 7 | 24 | .052 |
| | Based on Median | 1.596 | 7 | 24 | .185 |
| | Based on Median and with adjusted df | 1.596 | 7 | 12.679 | .223 |
| | Based on trimmed mean | 2.254 | 7 | 24 | .065 |
| CRR | Based on Mean | 1.666 | 7 | 24 | .165 |
| | Based on Median | .730 | 7 | 24 | .649 |
| | Based on Median and with adjusted df | .730 | 7 | 11.335 | .652 |
| | Based on trimmed mean | 1.480 | 7 | 24 | .222 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| TotalCholesterol | Between Groups | 2615.082 | 7 | 373.583 | 14.762 | .000 |
| | Within Groups | 607.375 | 24 | 25.307 | | |
| | Total | 3222.456 | 31 | | | |
| HDL | Between Groups | 1982.215 | 7 | 283.174 | 35.033 | .000 |
| | Within Groups | 193.994 | 24 | 8.083 | | |
| | Total | 2176.209 | 31 | | | |
| TG | Between Groups | 5420.553 | 7 | 774.365 | 21.756 | .000 |
| | Within Groups | 854.255 | 24 | 35.594 | | |
| | Total | 6274.808 | 31 | | | |
| LDL | Between Groups | 2756.271 | 7 | 393.753 | 12.842 | .000 |
| | Within Groups | 735.846 | 24 | 30.660 | | |
| | Total | 3492.117 | 31 | | | |
| VLDL | Between Groups | 216.795 | 7 | 30.971 | 21.742 | .000 |
| | Within Groups | 34.187 | 24 | 1.424 | | |
| | Total | 250.981 | 31 | | | |
| LDLHDLRatio | Between Groups | 8.312 | 7 | 1.187 | 50.040 | .000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | .570 | 24 | .024 | | |
| | Total | 8.881 | 31 | | | |
| CRR | Between Groups | 14.512 | 7 | 2.073 | 58.795 | .000 |
| | Within Groups | .846 | 24 | .035 | | |
| | Total | 15.358 | 31 | | | |

**Test of Homogeneity of Variances for Antioxidants**

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| LiverAscorbicacid | Based on Mean | 9.738 | 7 | 24 | .000 |
| | Based on Median | 7.256 | 7 | 24 | .000 |
| | Based on Median and with adjusted df | 7.256 | 7 | 6.038 | .014 |
| | Based on trimmed mean | 9.626 | 7 | 24 | .000 |
| LiverVitaminE | Based on Mean | 2.786 | 7 | 24 | .029 |
| | Based on Median | .621 | 7 | 24 | .733 |
| | Based on Median and with adjusted df | .621 | 7 | 5.149 | .727 |
| | Based on trimmed mean | 2.250 | 7 | 24 | .065 |
| LiverGSH | Based on Mean | 5.191 | 7 | 24 | .001 |
| | Based on Median | 3.526 | 7 | 24 | .010 |
| | Based on Median and with adjusted df | 3.526 | 7 | 9.570 | .038 |

| | | | | | |
|---|---|---|---|---|---|
| | Based on trimmed mean | 4.897 | 7 | 24 | .002 |
| LiverSOD | Based on Mean | 1.716 | 7 | 24 | .153 |
| | Based on Median | .930 | 7 | 24 | .502 |
| | Based on Median and with adjusted df | .930 | 7 | 7.000 | .537 |
| | Based on trimmed mean | 1.479 | 7 | 24 | .222 |
| SerumSOD | Based on Mean | 1.956 | 7 | 24 | .104 |
| | Based on Median | .562 | 7 | 24 | .779 |
| | Based on Median and with adjusted df | .562 | 7 | 7.493 | .768 |
| | Based on trimmed mean | 1.590 | 7 | 24 | .186 |
| LiverMDA | Based on Mean | 2.623 | 7 | 24 | .037 |
| | Based on Median | .697 | 7 | 24 | .674 |
| | Based on Median and with adjusted df | .697 | 7 | 8.380 | .676 |
| | Based on trimmed mean | 2.193 | 7 | 24 | .072 |
| SerumMDA | Based on Mean | .841 | 7 | 24 | .564 |
| | Based on Median | .427 | 7 | 24 | .876 |
| | Based on Median and with adjusted df | .427 | 7 | 13.646 | .869 |
| | Based on trimmed mean | .780 | 7 | 24 | .611 |
| LiverGPX | Based on Mean | 1.561 | 7 | 24 | .195 |

| | | | | | |
|---|---|---|---|---|---|
| | Based on Median | .923 | 7 | 24 | .507 |
| | Based on Median and with adjusted df | .923 | 7 | 8.651 | .533 |
| | Based on trimmed mean | 1.456 | 7 | 24 | .230 |
| SerumGPX | Based on Mean | .516 | 7 | 24 | .813 |
| | Based on Median | .456 | 7 | 24 | .856 |
| | Based on Median and with adjusted df | .456 | 7 | 17.629 | .853 |
| | Based on trimmed mean | .513 | 7 | 24 | .816 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| LiverAscorbic acid | Between Groups | 1622.463 | 7 | 231.780 | 38.904 | .000 |
| | Within Groups | 142.984 | 24 | 5.958 | | |
| | Total | 1765.447 | 31 | | | |
| LiverVitaminE | Between Groups | 16.059 | 7 | 2.294 | 18.454 | .000 |
| | Within Groups | 2.984 | 24 | .124 | | |
| | Total | 19.043 | 31 | | | |
| LiverGSH | Between Groups | 285.651 | 7 | 40.807 | 61.419 | .000 |
| | Within Groups | 15.946 | 24 | .664 | | |
| | Total | 301.597 | 31 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| LiverSOD | Between Groups | 4.453 | 7 | .636 | 733.084 | .000 |
| | Within Groups | .021 | 24 | .001 | | |
| | Total | 4.474 | 31 | | | |
| SerumSOD | Between Groups | 3.601 | 7 | .514 | 211.836 | .000 |
| | Within Groups | .058 | 24 | .002 | | |
| | Total | 3.659 | 31 | | | |
| LiverMDA | Between Groups | 20.569 | 7 | 2.938 | 56.283 | .000 |
| | Within Groups | 1.253 | 24 | .052 | | |
| | Total | 21.822 | 31 | | | |
| SerumMDA | Between Groups | 5.327 | 7 | .761 | 32.590 | .000 |
| | Within Groups | .560 | 24 | .023 | | |
| | Total | 5.887 | 31 | | | |
| LiverGPX | Between Groups | 53.465 | 7 | 7.638 | 18.179 | .000 |
| | Within Groups | 10.084 | 24 | .420 | | |
| | Total | 63.548 | 31 | | | |
| SerumGPX | Between Groups | 61.969 | 7 | 8.853 | 26.962 | .000 |
| | Within Groups | 7.880 | 24 | .328 | | |
| | Total | 69.850 | 31 | | | |

**Test of Homogeneity of Variances for Liver Function test**

| | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Aspartate Transaminase | Based on Mean | 2.193 | 7 | 24 | .072 |
| | Based on Median | .491 | 7 | 24 | .832 |
| | Based on Median and with adjusted df | .491 | 7 | 8.888 | .820 |
| | Based on trimmed mean | 1.790 | 7 | 24 | .136 |
| Alanine Transaminase | Based on Mean | 1.117 | 7 | 24 | .385 |
| | Based on Median | .627 | 7 | 24 | .729 |
| | Based on Median and with adjusted df | .627 | 7 | 13.763 | .726 |
| | Based on trimmed mean | 1.013 | 7 | 24 | .447 |
| Alkaline Phosphatase | Based on Mean | 3.567 | 7 | 24 | .009 |
| | Based on Median | 2.858 | 7 | 24 | .026 |
| | Based on Median and with adjusted df | 2.858 | 7 | 17.581 | .035 |
| | Based on trimmed mean | 3.511 | 7 | 24 | .010 |
| Total Bilirubin | Based on Mean | 2.524 | 7 | 24 | .043 |
| | Based on Median | 1.989 | 7 | 24 | .099 |
| | Based on Median and with adjusted df | 1.989 | 7 | 14.805 | .126 |
| | Based on trimmed mean | 2.435 | 7 | 24 | .049 |

**ANOVA**

| | Sum of Squares | df | Mean Square | F |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Aspartate Transaminase | Between Groups | 3944.739 | 7 | 563.534 | 9.962 |
| | Within Groups | 1357.655 | 24 | 56.569 | |
| | Total | 5302.394 | 31 | | |
| Alanine Transaminase | Between Groups | 2674.308 | 7 | 382.044 | 25.267 |
| | Within Groups | 362.892 | 24 | 15.120 | |
| | Total | 3037.200 | 31 | | |
| Alkaline Phosphatase | Between Groups | 42019.290 | 7 | 6002.756 | 7.528 |
| | Within Groups | 19138.585 | 24 | 797.441 | |
| | Total | 61157.875 | 31 | | |
| Total Bilirubin | Between Groups | .020 | 7 | .003 | 3.695 |
| | Within Groups | .019 | 24 | .001 | |
| | Total | .039 | 31 | | |

**ANOVA**

| | | Sig. |
|---|---|---|
| Aspartate Transaminase | Between Groups | .000 |
| | Within Groups | |
| | Total | |
| Alanine Transaminase | Between Groups | .000 |
| | Within Groups | |
| | Total | |
| Alkaline Phosphatase | Between Groups | .000 |

| | | |
|---|---|---|
| | Within Groups | |
| | Total | |
| Total Bilirubin | Between Groups | .008 |
| | Within Groups | |
| | Total | |