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RESEARCH ARTICLE

ACUTE TOXICITY AND HYPOGLYCEMIC PROPERTIES OF ETHANOLIC ROOT EXTRACT OF *VERNONIA AMYGDALINA* (BITTER LEAF) IN ALLOXAN- INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a group of metabolic disease characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting to impaired carbohydrate, lipid, and protein metabolism. The qualitative analyses of *Vernonia amygdalina* root extract were carried out using standard methods. Acute toxicity of the extract was determined. Adult male albino rats were induced intraperitoneally with alloxan. The rats were grouped into six groups of five animals per group: Group A rats are not induced with alloxan, Group B animals serve as the negative control, Group C animals serve as positive control and were treated with glibenclaimide, Group D, E and F animals were treated with 200, 400 and 600 mg/kg body weight of ethanolic root extract of *V. amygdalina* respectively. The extracts were administered to the animals orally for 14 days. The animal's blood sugar levels were assayed using blood glucose test strips and Accu-chek active glucometer. The lipid profiles and oxidative stress parameters were assayed using standard methods. The phytochemical analysis of *Vernonia amygdalina* root extract shows the presence of secondary metabolites like saponin, tannin, anthraquinone, reducing sugar, phenol, anthocyanine, steroid and terpenoid. The histopathological studies show that the extract is not toxic and safe for human consumption. The animals administered with 200, 400 and 600 mg/Kg B.W of extract showed significant decrease ($P<0.05$) in blood sugar level compared to the untreated animals. The decrease in the blood glucose level of the animals following the administration of the plant extract suggested that the plant extract possesses hypoglycemic effects in alloxan- induced diabetic rats. The extract of *V. amygdalina* produces hypolipidaemic effect and this is evident as there are significant decrease in plasma total cholesterol,triglycerides, low density lipoprotein-cholesterol and an increase in plasma high density lipoprotein-cholesterol in the treated groups compared to the untreated group. The extract significantly increases ($P<0.005$) superoxide dismutase, catalase, reduced glutathione and also reduces malondialdehyde values in the liver homogenate, an indication that the extract possess antioxidant properties.

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INTRODUCTION

Diabetes mellitus (DM) is a common disorder associated with increased morbidity and mortality and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid, and protein metabolism (Lebovitz, 1994, Bhatena and Velasquez 2002). World Health Organization (WHO) has defined Diabetes mellitus based on laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrates meal or two hours after an oral ingestion of the equivalent of 75 g glucose. According to the World health organization (WHO), there are over 150 million diabetics worldwide and this is likely to increase to about 300 million by the year 2023, in spite of major in roads in understanding the pathophysiology and treatment of the disease (WHO, 1999). Diabetes is generally characterized by hyperglycemia, glucosuria, polyuria, body weight loss, disability, coma and even death. Diabetes mellitus is a global metabolic epidemic affecting essential biochemical activities in almost every age group (Gupta *et al* 2008). Different studies have shown that people with diabetes are prone to liver and kidney abnormalities, they develop resistance to insulin, in-vivo they produce free radicals due to increased lipid peroxidation, glucose oxidation, non-enzymatic glycosylation

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of proteins and subsequent oxidative degradation of glycated proteins, leading to a reduce in antioxidant defence mechanisms and damage of cellular organelles and enzymes (Arora, 2010; Kangralkar *et al*., 2010). Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depend on it as primary health care. Over 800 plants have been reported to possess antidiabetics properties (Preethi 2013). *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. The taxonomic classification of *Vernonia amygdalina* is as follows: Kingdom: plantae, Division: Angiosperms, Order : Asterales, Family: Asteraceae, Genius: Vernonia, Species*: V. amygdalina.* It has a variety of names in various languages. It is commonly called "Bitter leaf" in English language, "Shuwaka" in Hausa language, "Onugbu" in Igbo language, it is called "Etidot"*,* in Efik, Ijaw and Ibibio, "Ewuro" in Yoruba language, "Oriwo" in Edo and "Chusa-doki" in Hausa (Egedigwe 2010). The WHO has recommended the use of alternative therapy, especially in countries or nations where accesses to conventional management procedures are inadequate. This has lead to the search for more effective hypoglycemic and antihyperglycemic agents. The objective of this study is to demonstrate experimentally, the antidiabetic, antihyperglycemic, and effects on some biochemical parameters of *Vernonia amygdalina* root extract in alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Collection and identification of plant material

The roots of *Vernonia amygdalina* were obtained from Ikorodu in Lagos State, Nigeria. The plant was authenticated by a botanist from Science Laboratory Technology Department (Environmental Biology Unit), Lagos State Polytechnic Ikorodu, Lagos-Nigeria.

Preparation of ethanolic root extract of *Vernonia amygdalina*

The roots of *Vernonia amygdalina* were washed, air dried under shade in the Biochemistry Laboratory, pulverised to coarse power using blender. Extraction was carried out by dispersing 200g of the grounded *Vernonia amygdalina* root material in 1L of 80% ethanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40^{o}C. The concentrated extract was dried to complete dryness in an aerated oven at 40^{o}C for 48 hours. The extract was later stored in a refrigerator at 4^{o}C.

Qualitative phytochemical analysis of *Vernonia amygdalina* root

Phytochemical analysis for phytochemical constituents were carried out on the ethanolic root extract of *Vernonia amygdalina* using standard phytochemical procedures described by Sofowora (1993), Harborne (1973), Trease and Evans (1986).

Acute toxicity test

The acute toxicity test of ethanolic root extract of *Vernonia amygdalina* were carried out using modified Lorkes method (1993). In the first phase, nine mice were randomized into three groups of three rats each and were given 10, 100 and 1000 mg/Kg body weight of the extract orally for one day and later observed for signs of toxicity. In the second phase, twelve mice were randomized into four groups of three mice each. The first group animals were control and the remaining groups animals were given 1400, 1800 and 2600 mg/Kg body weight of the extract respectively for one week and later observed for signs of toxicity and mortality. The oral median dose LD_{50} was calculated. The histopathological study of the mice liver was carried out in the Department of Anatomy, college of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria

Experimental animals

A total of 30 male albino rats with body weight ranging from 160 to 180g were obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. They were acclimatized for three week to Laboratory condition of 23 ±2ºC. They were kept in plastic cages and fed with commercial rat chow and supply with water *adlibitum.* The rats were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

Administration of alloxan

The male albino rats (Sprague Dawley) were made diabetic by injecting them with alloxan monohydrate intraperitoneally. With dosage of 150mg/kg body weight. Development of diabetes was confirmed after 72 hours of alloxanisation by using "Accuchek Active Glucometer" (Roche Diagnostics) and blood glucose test strips.

Grouping of animals

The animals were grouped into six groups. Each group contain five animals.

The animals were grouped as follows:

Group A- Normal control (non-diabetic rats)
Group B- Negative control (diabetic without treatment)
Group C- Positive control (diabetic + glibenclamide)
Group D- Diabetic + 200mg/Kg B.WT of *Vernonia amygdalina root*
Group E- Diabetic + 400mg/Kg B.WT of *Vernonia amygdalina root*
Group F- Diabetic + 600mg/Kg B.WT of *Vernonia amygdalina root*

Collection of blood samples

The albino rats were sacrificed by cervical decapitation after 24 hours fasting on the fourteen day. Blood were collected from the male albino rats by ocular puncture into EDTA tubes for hematological analysis and the remaining blood were collected into an heparinised tubes and centrifuge at 3000 rpm for 20 minutes using a centrifuge and the plasma stored at -20°C.

Measurement of plasma lipid profiles

The plasma Total cholesterol (TC), Triglyceride (TG) and HDL-Cholesterol (HDL-Chol) were determined using Randox diagnostic kits.

Low density Lipoprotein-Cholesterol (LDL-Cholesterol) was calculated using formula from Friedwald *et al*. 1972.
LDL-Cholesterol in mg/dl:

LDL-Chol. = Total cholesterol - Triglycerids/5 – HDL-Chol.

Estimation of Oxidative Stress Parameters

Preparation of Liver Homogenate

The Liver tissues of some of the sacrificed albino rats were excised and the liver samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 5,000 rpm for 50 minutes. The supernatant obtained was later used for assay of thiobarbituric acid reactive substances (TBARS) content, superoxide dismutase, catalase and reduced glutathione.

Estimation of Lipid peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Niechaus and Sameulsson.

Estimation of superoxide dismutase (SOD)

The liver homogenate was assayed for the presence of SOD by utilizing the technique of Magwere *et al* 1997 with slight modification by Zou *et al.* (1986).

Estimation of catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein as described by sinha.

Estimation of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellma.

Statistical Analysis

Data analysis was done using the Graph Pad prism computer software version 5. Students't'-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant.

RESULTS

Table 1. The phytochemical constituents of methanolic root extract of *V.amygdalina*

Phytochemical components	Inference
Tannins	+
Saponins	+
Anthraquinone	+
Steroids	+
Terpenoids	+
Phenol	+
Anthocyanine	+
Reducing sugar	+

(+) indicate present

Phytochemical screening of the ethanolic root extract of *Vernonia amygdalina* showed the present of secondary metabolite like saponin, tannins, anthocyanine, steroid, anthraquinones, phenol, reducing sugar, terpenoids (Table 1). Figure 1 to 4 shows the histopathology of the liver of the mice after one week of toxicity test.

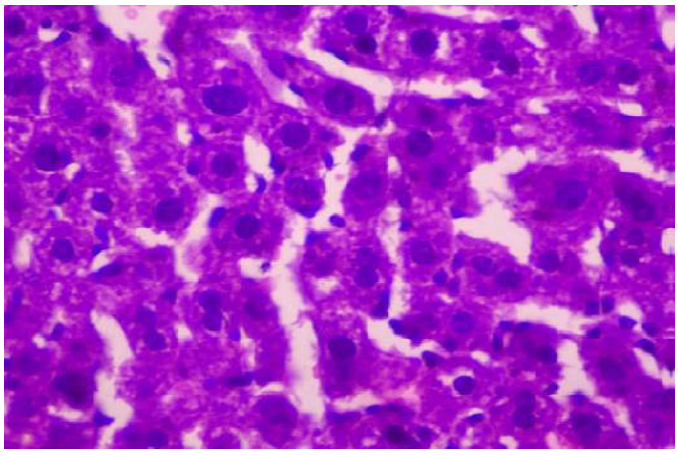

Figure 1. Photomicrographs of control animal liver sections stained with hematoxylin and eosin (H&E). The slide was examined at a magnification of × 40 under a light microscope

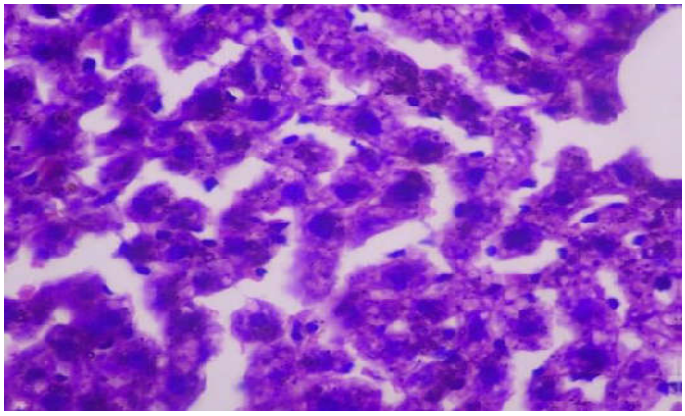

Figure 2. Photomicrograph of liver sections stained with hematoxylin and eosin (H&E). The slide was examined at a magnification of × 40 under a light microscope. Hepatic tissue of mouse administered with 1400 ml/kg body weight of *Vernonia amygdalina root* extract.

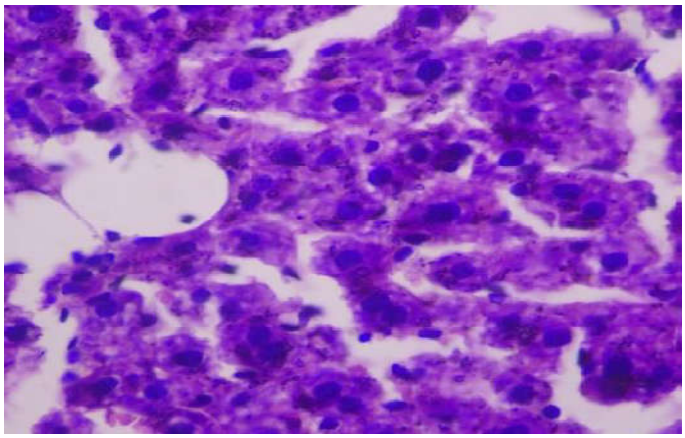

Figure 3. Photomicrograph of liver sections stained with hematoxylin and eosin (H&E). The slide was examined at a magnification of × 40 under a light microscope. Hepatic tissue of mouse administered with 1800 ml/kg body weight of *Vernonia amygdalina root* extract

Table 2. The effect of *Vernonia amygdalina* on lipid profile in alloxan–induced diabetic albino rats

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
TotalCholesterol (mg/dl)	76.23±2.43 [a]	132.63±3.37	89.45±2.59 [c]	85.37± 1.94 [b]	80.42 ±2.29 [b]	79.84±2.87 [b]
Triglyceride (mg/dl)	88.55±3.64 [a]	129.22±4.77	109.71±3.74 [c]	94.81±3.35 [b]	104.43±2.15 [b]	89.14±2.54 [b]
Low-density Lipoprotein (mg/dl)	23.74±0.47 [a]	89.25±11.03	37.68±0..76 [c]	30..84±0.66 [b]	20.91±0.47 [b]	20.83±0.83 [b]
High-density Lipoprotein (mg/dl)	34.78±0.86 [a]	17.54 ±0.94	29.83±0.7.6 [c]	35.57±0.45 [b]	38.62±0.38 [b]	41.18±1.17 [b]

[a] $p < 0.05$ Between diabetic group and control group; [b] $p < 0.05$ Between diabetic groups treated with *V.A* extract and diabetic group;
[c] $p < 0.05$ Between diabetic groups treated with standard drug and diabetic group

Table 3. The effect of *Vernonia amygdalina* on oxidative stress parameter of liver homogenate in alloxan–induced Diabetic male albino rats

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
MDA (nmol/l)	4.13±0.45 [a]	11.36±0.97	5.32±0.63 [c]	6.07±0.73 [b]	5.74±0.82 [b]	4.86±0.79 [b]
Catalase (µmol/min/mg protein)	19.39±1.08 [a]	7.03±0.79	11.83±0.98 [c]	12.16±1.15 [b]	11.78±1.25 [b]	14.36±1.64 [b]
SOD (units/ mg protein)	9.18±0.89 [a]	3.29±0.31	7.86 ±0.67 [c]	7.43 ±0.38 [b]	8.35 ±0.44 [b]	8.14±0.87 [b]
GSH (mg/mg protein)	0.56±0.02 [a]	0.13 ±0.01	0.38 ±0.02 [c]	0.35 ±0.01 [b]	0.41±0.02 [b]	0.59±0.02 [b]

[a] $p < 0.05$ Between diabetic group and control group; [b] $p < 0.05$ Between diabetic groups treated with *V.A* extract and diabetic group;
[c] $p < 0.05$ Between diabetic groups treated with standard drug and diabetic group

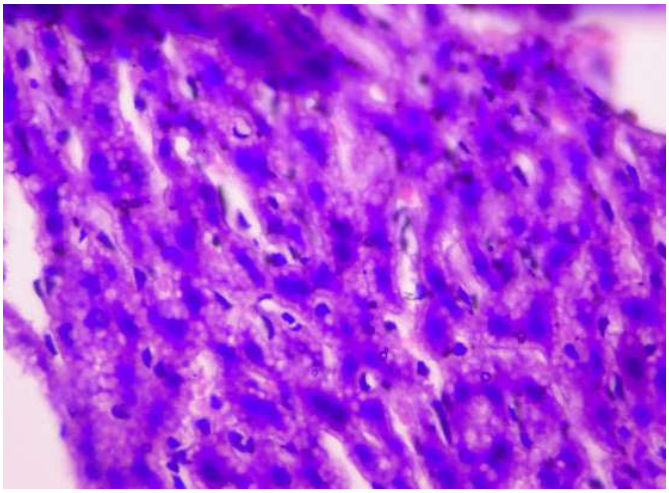

Figure 4. Photomicrograph of liver sections stained with hematoxylin and eosin (H&E). The slide was examined at a magnification of × 40 under a light microscope. Hepatic tissue of mice administered with 2600 ml/kg body weight of *Vernonia amygdalina root* extract

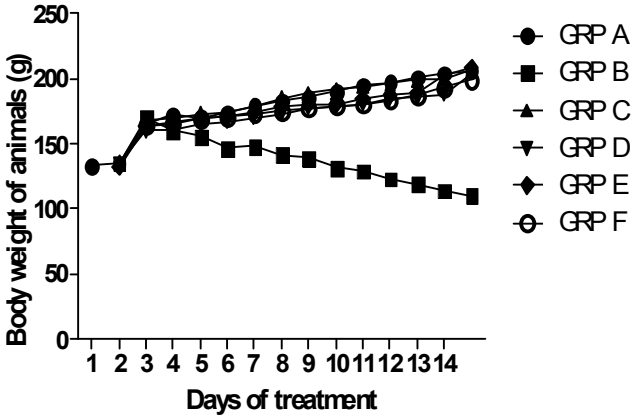

Figure 5. Body weight of animals after treatment with glibenclamide, 200, 400 and 600 mg/kg BW of *Vernonia amygdalina*

Figure 5 shows the effect of alloxan on the body weight of the animals after 14 days of treatment.

Figure 6 below shows the initial blood sugar level before induction. This indicates that all the animals used for the experiments were healthy.

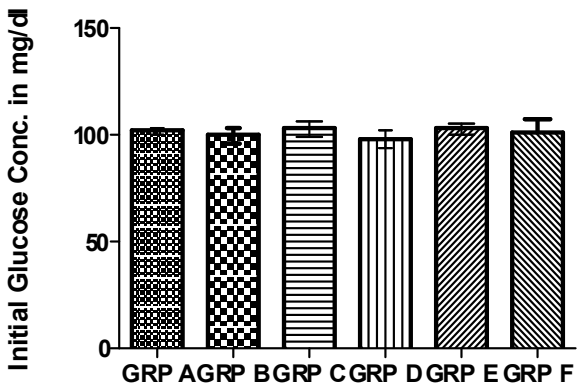

Figure 6. Initial blood glucose concentration values in mg/dl for normal, diabetic untreated, diabetic animals treated with gliben

Figure 7 shows the blood sugar concentration after induction. Only group A animals were not induced with alloxan. This study shows that all the animals in group B to F were diabetic after induction with alloxan with a dosage of 150mg/kg body weight of the extract.

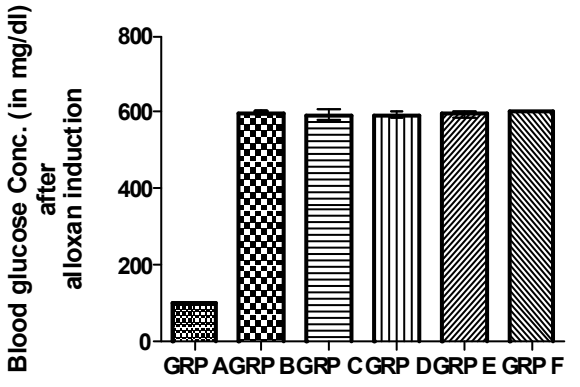

Figure 7. Blood glucose concentration values in mg/dl after alloxan induction for all the rats in group B to group F. Group A animals were not induced

Figure 8 below shows the blood glucose concentration values in mg/dl after 3 days of treatment. Group C to F rats were treated with glibenclamide, 200, 400 and 600 mg/kg B.W of *Vernonia amygdalina* root extract respectively.

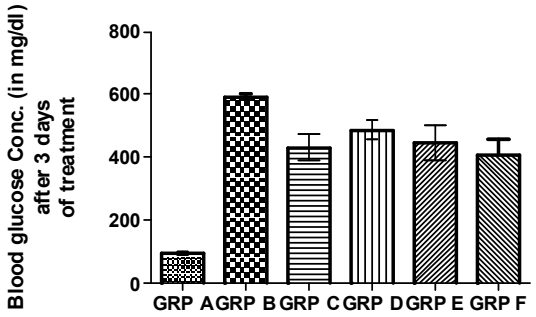

Figure 8. Blood glucose concentration after 3 days of treatment with standard drug and the different concentration of the extract

Figure 9 below shows the blood glucose concentration values in mg/dl after 14 days of treatment. Group C to F animals were treated with glibenclamide, 200, 400 and 60 0 mg/kg B.W of *Vernonia amygdalina* root extract respectively.

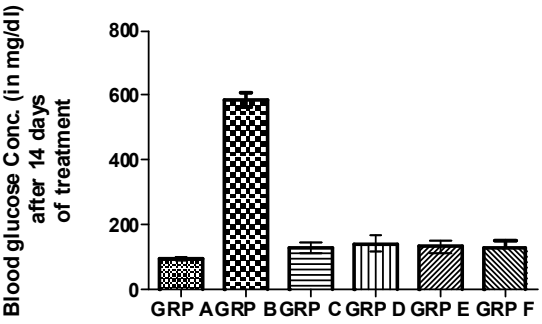

Figure 9. Blood glucose concentration after 14 days of treatment for all the experimental groups except group A

DISCUSSION

The results obtained from the phytochemicals analysis of *Vernonia amygdalina* root extract showed the presence of some secondary metabolite like saponin, tannins, reducing sugar, anthocyanine, steroid, anthraquinones, phenol and terpenoids (Table 1). The presence of these secondary metabolites in *Vernonia amygdalina* root extract may be responsible for the hypoglycemic properties of the plant and the use of the plant has a medicinal plant. Tiwari and Rao 2002 suggested that secondary metabolites of plants such as some of the once listed above possess some alpha-glycosidase inhibitors and competitively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut-postprandial hyperglycemia, hence resulting in an effective glucose control. Winlenam 1989 reported that a positive correlation indicated between the presence of flavonoids, glycosides and phytosterols in plants with hypoglycemic and anti-hyperglycemic actions. Histopathological studies also provided important evidence supporting the biochemical analysis. The median lethal dose LD_{50} was estimated to be >2400mg/Kg body weight of the extract and no death was recored. Behavioural signs of toxicity like restlessness, paw licking and stretching were observed. Figure 1 shows the liver section of healthy mice stained with hematoxylin and eosin with normal liver architecture, normal hepatic cells with well preserved cytoplasm. Figure 2 and 3 show that the livers were not affected by the administraction of the extract (1400 and 1800mg/kg body weight). The histological sections of these liver tissues show radial plates of hepatocytes and no cytoplasmic fat vacuoles. Figure 4 shows the histological sections of the liver tissue of mouse administered with 2400mg/kg body weight of V.A extract showing radial plates of hepatocytes. There is infiltration of parenchyma by diffuse infiltrates of inflammatory cells.

Alloxan induce diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Szkudelski 2001). The Group B diabetic untreated animals, significantly lost body weight (P<0.05) when compared to the control and treated animals (Figure 5). The lost of weight may be due to loss in muscle adipose tissue protein and fatty acids (Granner, 1996). Alberti, and Zimmet reported that factors responsible for weight lost in diabetes include proteolysis, lipolysis and acute fluid loss. Figure 6 shows that all the experimental animals used in the study were all healthy. Figure 7 show that all the animals in group B to F were all diabetic after induction with alloxan after three days. Alloxan induces hyperglycemia by selective cytotoxic effect on pancreatic β-cells (Szkudelski, 2001) causing permanent destruction of β-cells. Moller, 2001 shows that the acute hypoglycemic effect of glibenclamide has stimulatory effect on the production of residual β-cells of the pancrease in addition to enhancement of glucose utilization. Figure 8 and 9 show that the extract and the standard drug have hypoglycemic effect, since there was a significant reduction (P<0.005) of blood sugar level of animals in group C, D, E and F respectively compared to group B untreated animals after periods of 3 and 14 days of treatment. The results showed that the roots extract exhibited a profound reduction (P<0.005) in blood glucose level of the diabetic albino rats. The results of this experiment are in agreement with those obtained by some workers who reported the hypoglycemic effect of *V. amygdalina* when administered to diabetic rats (Mazunder *et al*., 2003 and Reginald Nwazue Nwaoguikpe. 2010). It has been suggested that the antihyperglycemic effects attributed to the plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or by inhibiting the intestinal absorption of glucose. Hence treatment with herbal drugs has an effect on protecting beta-cells and smoothening out fluctuation in glucose level (Elder C 2004).

The result obtained in Table 2 above shows that the root extract of *V. amygdalina* significant reduced (P<0.05) plasma total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-Chol) and increase high density lipoprotein cholesterol (HDL-Chol) concentrations in the treated animals (group C to group F) compared to the untreated animals (group B). Research shows that insulin increases the number of LDL receptor, so chronic insulin deficiency might be associated with a diminished level of LDL receptor which leads to their high levels in serum or plasma thereby increasing LDL particles and result in the increase in LDL-cholesterol value in diabetes mellitus.

The results of this study clearly indicate that the administration of ethanolic root extract of *V. amygdalina* produces hypoglycemic and hypolipidaemic effect and may prevent the risk of cardiovascular diseases. This account for it use in folk medicine for the treatment of diabetes and hypertension. Different studies have *s*hown that increased in the risk factor of cardiovascular disease correlate with increase in plasma TC, TG, LDL-Chol, VLDL and a decrease in HDL-Chol concentrations. The results of this study is in accordance with other experimental result obtained in other studies (Akah *et al.*, 2004, Atangwho *et al* 2007, Luka *et al* 2013). Liver is the

major organ used for removing xenobiotic substances from the body and as such is subjected to many substances causing oxidative stress. In the anti-diabetes assay, there were significant reduction ($P<0.05$) in activities of CAT, GSH and SOD in the liver homogenate of group B animals compared to group A, C, D, E and F animals respectively. This is an indication that the root extract possess antioxidant properties. MDA values significantly increase ($P<0.05$) in the liver homogenate of group B animals compared to group A, C, D, E and F animals. Increase in MDA value indicates increased in lipid peroxidation which could have resulted from the depletion of CAT and SOD concentration.

Conclusion

The findings of this research shows that the ethanolic root extract of *Vernonia amygdalina* possess hypoglycemic and hypolipidemic properties and it reduces oxidative stress.

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