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Hypoglycemic and Hepatoprotective Effects of *Vernonia Amygdalina* (Bitter Leaf) and Its Effect on Some Biochemical Parameters in Alloxan-induced Diabetic Male Albino Rats

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ABSTRACT

The effects of methanolic leaf extract of *Vernonia amygdalina* (bitter leaf) on some biochemical parameters on alloxan-induced diabetic male albino rats (Wistar strain) were investigated for a period of 14 days. Adult male albino rats weighing between 140-160 g were induced intraperitoneally with alloxan. The male albino rats were grouped into five groups: Group A are not induced with alloxan, Group B serves as the negative control, Group C serves as positive control and was treated with glibenclaimide, Group D and E were treated with 100 and 200 mg/kg body weight of methanolic leaf extract of *V. amygdalina* respectively. The extracts were given to the animals orally for 14 days. The phytochemical analysis of *Vernonia amygdalina* extract indicates the presence of secondary metabolites like tannins, saponins, alkaloids, flavonoids, anthraquinones, Glycosides, Cyanogenic glycosides, polyphenols and Terpenoids. The weight of diabetic untreated rats (Group B) were significantly ($P<0.005$) reduced when compared to other groups. The group of rats given 100 and 200mg/Kg B.W of extract showed significant decrease ($P<0.05$) of blood sugar level compared to the untreated rats. The decrease in the blood glucose level of the rats following the administration of the plant extract suggests that the plant extract possesses anti-diabetic, anti-hyperglycemic and hypoglycemic effects on alloxan- induced diabetic rats. The extract of *V. amygdalina* produces hypolipidaemic effect and this is evident as there is a significant decrease in plasma TC, TG, LDC-Cholesterol, atherogenic index and an increase HDL-Cholesterol in the treated groups compared to the negative control group. The extracts significantly increase HGB, RBC, HCT and the WBC is significantly reduced in the treated group compared to the untreated group. This is an indication that the extract does possess hematopoietic activities and is not hematotoxic. The extract significantly reduced ($P<0.005$) liver biomarker enzymes (AST, ALT, ALP and GGT), an indication that it has hepatoprotective effect.

KEYWORDS: Alloxan-induced diabetic rats, biochemical parameters, hepatoprotective effect, phytochemical analysis and *vernonia amygdalina.*

INTRODUCTION

Diabetes mellitus (DM) is one of the major complex and chronic disorders of carbohydrate, protein and lipid metabolism characterized by persistent elevation of blood glucose, resulting from a partial or complex cessation of insulin secretion or synthesis, or peripheral resistance to insulin action (Murray and Pizzomoj,(1997)).

Medicinal plants have the basis of health care throughout the world and remain relevant both in the developing and developed nations of the world for various chemotherapeutic purposes. Several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb (Tiwari and Rao (2002)). Baynes (1991) showed that diabetic nephropathy is one of the major causes of morbidity and premature mortality in patients with insulin-dependent DM. Medicinal plants contain potentially useful chemicals that serve as basis for the manufacturing of modern medicines (Okigbo et al., 2009). *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,* Botanical Name: *Vernonia 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).

Pharmacological studies have shown that the leaf extract of *V. amygdalina* has both hypoglycemic and hypolipidemic properties in experimental animals and so could be used in the management of diabetes, hypertension etc (Akah and Okafor, 1992).

METHODOLOGY

Collection and identification of plant material

The leaves of *Vernonia amygdalina* were collected from a farm in Ikorodu in Lagos State, Nigeria and authenticated by Mr Godonu Kolawole Gbemavo, a Chief Lecturer from the Department of Crop Production and Horticulture, School of Agriculture, Lagos State Polytechnic, Ikorodu, Lagos- Nigeria.

Preparation of methanolic leaf extract of *Vernonia amygdalina*

The leaves were air dried under shade in the Biochemistry laboratory. The dried leaves were pounded to coarse powder in a mortar and then to fine powder with a blender. Extraction was carried out by dispersing 200g of the grounded plant material in 1L of 80% Methanol and shaking was done with GFL shaker for 72 hours.This was followed with vacuum filtration and evaporation at a temperature not exceeding 40°C. The concentrate was dried to complete

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dryness in an aerated oven at 40°C for 48 hours. The extract was stored in a refrigerator at 4°C.

Phytochemical analysis of methanolic leaf extract of *Vernonia amygdalina*

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures (Harborne (1993), Trease and Evans (1995) and Sofowora (1993).

Administration of alloxan

Male albino rats (Wistar strain) of about eleven weeks old with average weight of 149 g were made diabetic by injecting them with alloxan monohydrate intraperitoneally with dosage of 100mg/kg of body weight (Pari and Venkateswaran, 2002). Development of diabetes was confirmed after 72 h of alloxanisation by using "Accuchek Active Glucometer" (Roche Diagnostics) and blood glucose test strips.

Grouping of animals

The animals were grouped as follows:

Group A - Nornal control (non-diabetic rats)
Group B- Negative control (diabetic without treatment)
Group C- Positive control (diabetic + glibenclamide)
Group D- Diabetic + 100mg/Kg B.WT of *V. amygdalina*
Group E- Diabetic + 200mg/Kg B.WT of *V. amygdalina*

Determination of hematological parameters.

The total red blood cell (RBC), hemoglobin concentration (HGB) ,white blood cell count (WBC), platelet count and other hematological parameters were determined using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching

Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

Collection of blood samples for plasma preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. The blood was later centrifuged for 10mins at 3000rpm using a centrifuge. The clear supernatant was used for the estimation of lipid profiles and liver function tests.

Determination of plasma lipid profiles

The plasma total cholesterol, triglyceride and HDL-Cholesterol were determined using Randox diagnostic kit [Trinder,1969 and Tietze, 1990]. Low density Lipoprotein-Cholesterol (LDL-C) was calculated using formula from [Friedwald, et al 1972]. The atherogenic index was also calculated.

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Determination of liver function tests

Plasma enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyltranferase (GGT) were determined using Randox diagnostic kits.

Data Analysis

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

RESULTS

Phytochemical screening of methanolic leaf extract of *Vernonia amygdalina* shows the present of secondary metabolite like tannins, flavonoids, alkaloids, glycosides, cyanogenic glycosides, anthraquinones, terpenoids and saponin (Tabble 1). The presence of these secondary metabolites in *Vernonia amygdalina* may be responsible for the anti-diabetic and hepatoprotective effect of the plant.

Table II above shows that ***Vernonia amygdalina*** extract and the standard drug have hypoglycaemic effects on alloxan-induced diabetic rats.

As expected, alloxan-induced diabetic rats showed all the characteristic of diabetes such as polyuria, polyphagia, polydipsia, loss of body weight, hyperglycemia and hyperlipidemia. This is evident from the above figure (fig 1). Group B (diabetes untreated animals) albino rats have significant weight lost compared to other groups.

Table I: Phytochemical screening of methanolic leaf extract of *Vernonia amygdalina*

Phytoconstituent	Qualitative abundance
Tannins	++
Flavonoids	++
Alkaloids	++
Glycosides	++
Cyanogenic glycosides	++
Anthraquinones	+++
Terpenoids	++
Saponin	+++
Polyphenols	++

(+) present at low levels, (++) present at moderate levels, (+++) present at high levels.

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Table II. Effect of standard drug (glibenclamide) and methanolic leaf extract of *Vernonia amygdalina* on blood glucose level of male albino rats induced with alloxan.

Groups	Initial glucose concentration (mg/dl)	Glucose conc. after Alloxan induction (mg/dl)	Glucose conc. after 3 days of treatment (mg/dl)	Glucose conc after 7 days of treatment (mg/dl)	Glucose conc. after 14 days of treatment (mg/dl)
Group A	100 ±10	99 ±11	105 ±10	102 ±11	107 ±13
Group B	108 ±11	Very high	Very high	Very high	Very high
Group C	101 ±10	Very high	385 ±115	220 ±150	111 ±8
Group D	103 ±13	Very high	430 ±110	260 ±95	123 ±11
Group E	106 ±7	Very high	397 ±121	216 ±110	115 ±10

Very high means the blood sugar level is above 600 mg/dl. Group A animals were not induced with Alloxan while Group B animals were not treated.

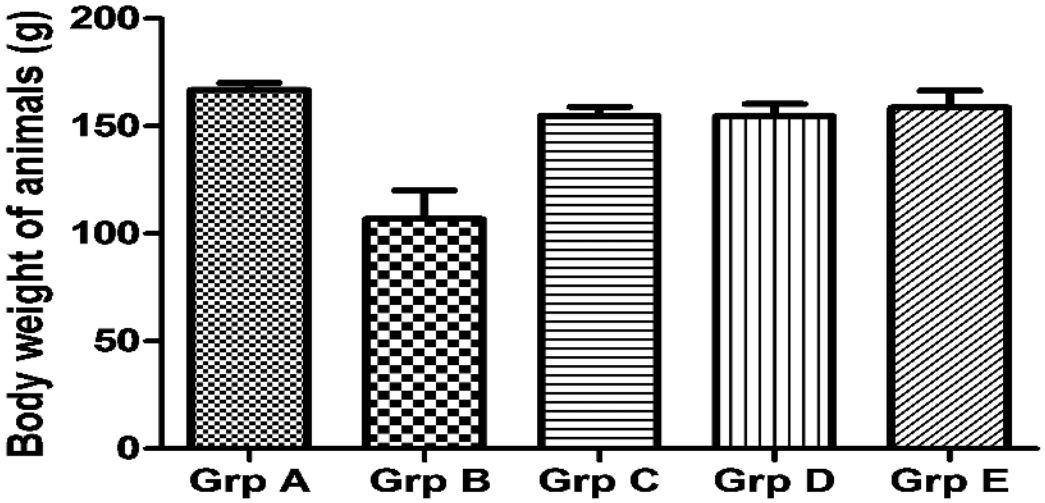

Figure 1: Mean body weight (g) of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 200mg/kg body weight of treated albino rats.

Table III, shows the lipid profile test for all the different experimental groups. The extracts have hypolipidaemic effect on the alloxan-induced diabetic rats.

Table III. The effect of Vernonia amygdalina on lipid profiles of alloxan-induced Diabetic male albino rats.

Parameters	Group A	Group B	Group C	Group D	Group E
Total Cholesterol (mg/dl)	95.3 ±2.1	118.7±6.5	96.1±2.4	91.3 ±1.8	89.5 ±2.6
Triglyceride (mg/dl)	87.4±3.1	128.2±10.1	99.2 ±4.7	86.3 ±2.7	88.4±1.9
Low-density Lipoprotein (mg/dl)	30.9 ±0.71	62.1±0.3	37.1 ±1.1	29.1 ±1.3	23.2 ±1.4
High-density Lipoprotein (mg/dl)	46.9 ±2.3	31.0 ±1.5	39.2 ±1.1	45.0 ±1.8	48.6±1.3
Atherogenic index	2.0 ±0.9	3.8±0.1	2.5 ±0.2	2.04 ±0.4	1.8±0.6

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Table IV. Effect of Methanolic leaf extract of *Vernonia amygdalina* on hematological parameters.

Hematological parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
WBC ($\times 10^9$/L)	8.7 ± 2.2*	14.3±3.1	10.1±2.3*	9.2±1.4*	8.9 ±2.2*
HGB g/dl	12.5 ± 1.2*	7.7 ± 2.2	11.5 ± 2.2*	12.5 ± 2.2*	13.5 ± 2.5*
RBC ($\times 10^{12}$/L)	12.8 ± 2.6*	6.6 ± 1.8	11.1 ± 1.2*	12.1± 1.2*	12.9 ± 1.8*
HCT %	43.5 ± 2.3*	36.1 ± 1.2	42.8 ± 1.2*	47.5 ± 1.5*	48.2 ± 1.7*
MCV fl	56.6 ± 2.4	51.1 ± 0.7	57.3 ± 2.2	61.5 ± 3.2	63.5 ± 1.6
MCH pg	17.5 ± 1.5	16.5 ± 1.2	18.1 ± 0.8	19.1 ± 1.2	19.8 ± 0.9
MCHC g/dl	30.2 ± 1.4	30.5 ± 1.1	31.2 ± 1.4	30.4 ± 1.3	30.1 ± 0.8
RDW-CV %	16.4 ± 0.8	16.8 ± 0.5	16.2 ± 0.5	16.1± 0.9	16.9 ± 1.1
RDW-SD fl	34.1 ± 0.7	34.1 ± 0.2	33.2 ± 1.2	34.1 ± 0.4	34.7 ± 0.5
PLT ($\times 10^9$/L)	520.1 ± 38.9	530.0 ± 41.6	521.1 ± 026.3	510.3 ± 35.1	495.2 ± 41.8
MPV fl	7.4 ± 0.3	7.5 ± 0.2	7.3 ± 0.3	7.5± 0.5	7.8 ± 0.4
PDW	16.7 ± 0.9	16.1 ± 0.7	15.8 ± 0.3	15.6 ± 0.4	15.4 ± 0.5
PCT %	0.453 ± 0.053	0.402 ± 0.072	0.440 ± 0.061	0.456 ± 0.057	0.473 ± 0.037

The values are the Means ± SD for five rats in each group.

Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT),Mean cell volume(MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration(MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD) Platelet count (PLT), Mean platelet volume (MPV),platelet Distribution Width (PDW) and Plateletcrit (PCT)

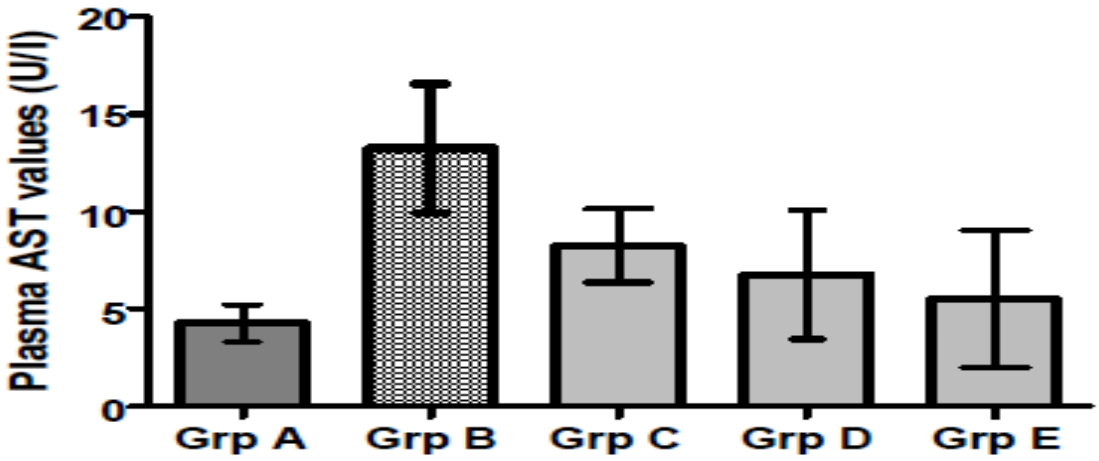

Figure 11. Plasma Aspartate aminotransferase(AST) vaLues of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 200mg/kg body weight of treated albino rats.

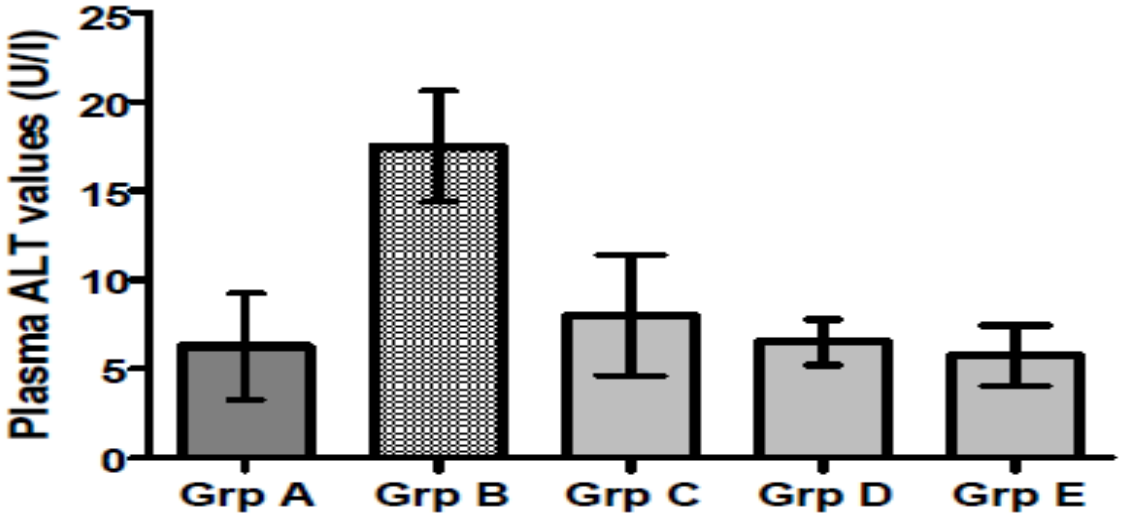

Figure 111. Plasma Alanine aminotransferase (ALT) values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 200mg/kg body weight of treated rats

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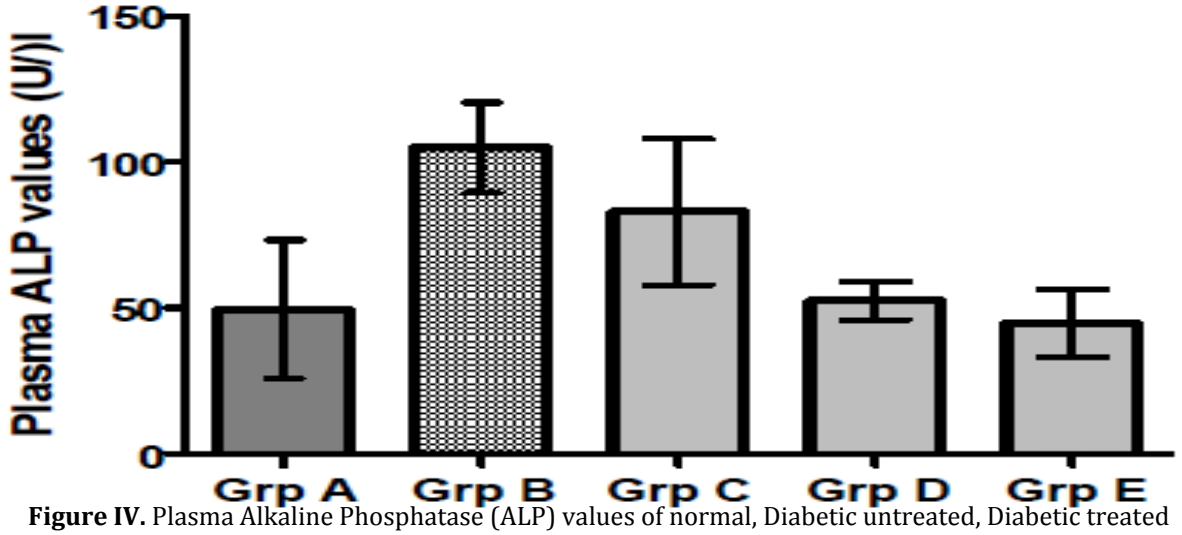

Figure IV. Plasma Alkaline Phosphatase (ALP) values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 200mg/kg body weight of treated rats.

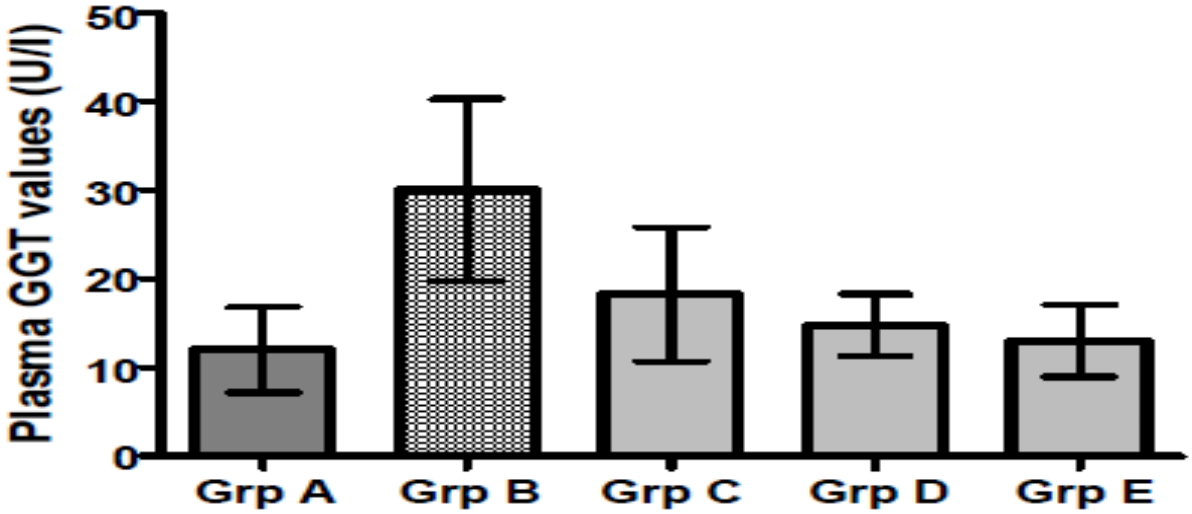

Figure V. Plasma Gamma Glutamyltransferase (GGT) values of normal, Diabetic untreated, Diabetic treated with glibenclamide, 100 and 200mg/kg body weight of treated rats

Figure II-V, show the liver maker enzymes for the determination of hepatic damage. The significant reduction in plasma values of AST, ALT, ALP and GGT is an indication that the extract has hepatoprotective effect.

DISCUSSION

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine have been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Ebong et al., 2008 showed clearly that medicinal plants formed the basis of health care throughout the world and have considerable importance. Some of these herbal preparations have been found to exert biological actions against diabetes mellitus and its complications (Ojiako and Nwanjo,2006).

The phytochemical screening of methanolic leaf extract of *V. amygdalina* indicated the presence of tannins, saponins, alkaloids, flavonoids, anthraquinones, Glycosides, Cyanogenic glycosides, polyphenols and Terpenoids etc (Table 1). Igile et al. (1995) reported that the leaves of bitter leaf (*V. amygdalina)* contain sesquiterpene lactones, tannins, sterols, saponin, glycosides, and flavonoids. It has also been reported that the plant extract is generally non-toxic, but excess consumption could be purgative. Phenols are reported to inhibit alpha (α-) amylase, sucrase, as well as the action of sodium glucose transporter (S-GLUT-1) of the intestinal brush border cells and this is responsible for their anti-diabetic action (Tiwari and Rao, 2002).

Table II shows significant decrease in blood sugar level after periods of 3,7 and 14 days of treatment. The results showed that the leaf extract exhibited a profound reduction ($P<0.005$) in blood glucose level of the diabetic albino male rats. It has been suggested that the antihyperglycemic effects attributed to 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)

A number of other plants have been reported to have antihyperglycemic and insulin stimulatory effects.
(Venkateswaran S, 2002 and Latha M, Pari L: 2003)

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Graph 1 above shows that there was a significant reduction (P <0.005) in the body weight of the untreated group (group B) compared to other groups. Inspite of the increased food consumption, group B animals lost body weight and this could be due to the defect in glucose metabolism and excessive breakdown of tissue protein which is a characteristic condition of diabetics (Swanston-Flat. *et al* 1990).

Table III shows that the methanolic leaf extract of *Vernonia amygdalina* lowered plasma TC, TG, LDL-Chol, atherogenic index levels and increase HDL-Chol concentrations in the treated rats, and this could account for its use in folk medicine for the treatment of diabetes and hypertension. The results of this study clearly indicate that the administration of methanolic leaf extract of *V. amygdalina* produces hypoglycemic and hypolipidaemic effect and may prevent cardiovascular diseases. Different studies have shown that increased in the risk factor of cardiovascular disease correlate with increase in plasma TC, TG, LDL-Chol, atherogenic index level and a decrease in HDL-Chol concentrations (Massing MW et al 2001).

Lipid abnormalities under diabetic condition may be due to the unlimited action of lipolytic hormones on the fat deposits'. Lipoprotein lipase an enzyme that help in the hydrolysis of triacylglycerol is in-activated due to insulin defficiency and this result to hypertriglyceridemea.(Al-Shmaony et al 1994).

The study shows that there is a significant increase p(<0.005) in the level of RBC, Hb, HCT of the positive control group and the treated groups compared to the untreated group (Table IV). These maybe as a result of onset of glycosylation process in the untreated diabetic rats or ensuing anemia of diabetes. Anaemia has been identified as a common complication of chronic kidney disease (CKD), affecting over half of all patients (USRDS, 2002) and the most common cause of CKD in about 2/3 of cases is diabetes mellitus (Thomas et al., 2005).

Alloxan diabetogenesis may cause perturbation in the bone marrow stem cells (Edet et al., 2011). The significant reduction (P < 0.05) in WBC levels of diabetic rats treated with methanolic extract when compared to the diabetic control group gave credence to the abilities of the above treatment groups in curtailing hematological abuses in the defense system of the diabetic rats. The treated groups showed that there is no significant effects of *V.amygdalina* treatment on red blood cells (RBC) counts and indices relating to it (Hb, HCT, MCV, MCH and MCHC) when compared with the control (group A). This is an indication that there was no destruction of RBC and no change in the rate of production of RBC (erythropoiesis).

This also shows that the extract does not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996). Also, the methanolic leaf extract of *V.amygdalina* treatment may not have adverse effects on the bone marrow, kidney and haemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism (Young and Maciejewski, 1997).

The non– significant change in the MCV and MCH values indicate absence of macrocytic anemia since increased in MCV an MCH values are known to be indicative of macrocytic anaemia. The extract also caused non- significant change in the MCHC value which suggests absence of hereditary spherocytosis since MCHC values are known to be elevated in hereditary spherocytosis. Other hematological parameters (RDW-CV, RDW-SD, PLT, MPV, PWD and PCT) showed no significant differences in all the different group. In conclusion, the *Vernonia amygdalina* methanolic leaf extract does possess hematopoietic activity and is not hematotoxic.

Vernonia amygdalina extract caused significant decrease (P<0.05) in the activity of AST, ALT, ALP and GGT values as shown in Figure II-V. These indicate that the extract has hepato-protective potentials. In medicine, the presence of elevated values of ALT and AST is indicative of liver damage (Giboney, 2005).Increased level of ALP has been attributed to the damaged structural integrity of hepatic cells because the enzyme alkaline phosphatase is located in the cytoplasm and is released into the circulation after cellular damage. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. GGT is reasonably specific to the liver and a more sensitive marker for cholestastic damage than ALP. GGT may be elevated with even minor, sub-clinical levels of liver dysfunction.

CONCLUSION

The results suggest that the methanolic leaf extract of *Vernonia amygdalina* not only possesses antihyperglycemic and hypolipidemic properties, but also have hepatoprotective effect.

Recommendations for further studies

Further studies are needed to determine the exact component in *Vernonia amygdalina* responsible for the observed effect and, such component may be use as a prophylactic agent against hypercholesterolemia.

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