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Effect of Aqueous Leaf Extract of *Vernonia Amygdalina* on Blood Glucose and Lipid Profile of Alloxan-Induced Diabetic Rats.

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Abstract: *The effects of orally administered aqueous leaf extract of Vernonia amygdalina on serum glucose and lipid profile of albino rats were determined. Adult healthy rats of both sexes of about eleven weeks old were randomly assigned into six groups following acclimatization to laboratory and handling conditions. Animals in group A were not intoxicated (normal control) while group B were intoxicated and were gives access to food and water. Animals in groups C, D, E and F were intoxicated and were treated with insulin (40mg/kg), Atorvastatin(30mg/kg), low dose (200mg/kg) and high dose (400mg/kg) extracts of Vernonia amygdalina respectively. Diabetes was induced with a single dose of alloxan (100mg/kg) body weight and serum glucose was taken 72h after induction of confirm diabetes. Treatment regimes of hyperglycemia and hypalipidemia started on the 4th and 8th day of the experiment respectively. The result obtained from the phytochemical analysis showed that the aqueous extract of Vernonia amygdalina gave positive reactions for tannins, flavonoids, alkaloids, terpenoids, Saponins and polyphenols. The extracts decreased blood glucose in a dose independent fashion. The extract exhibited significant decrease in triglyceride (TG) total cholesterol (TC), low density lipoprotein (LDL) in the alloxan monohydrate intoxicated rats whereas high density lipoprotein (HDL) was increased. The bioefficacy of the herb extracts was significant when compared to the reference drugs (insulin and atorvastatin). These findings are of medical, nutritional and industrial importance in view of its observed possible applications.*

Keywords: *Vernonia amygdalina, phytochemicals, antidiabetic and hyperlipidemic activities*

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I. Introduction

Diabetes is one of the major disorders of carbohydrate, protein and lipid metabolism. It is characterized by persistent elevation of blood glucose resulting from partial or complete stoppage of insulin secretion or weak response of tissues to insulin hormone [(1.15).]

In diabetes, lipid abnormalities are common among patients, as result of interventions in biochemical processes and these precipitate to diabetes metabolic disorder. This diabetes metabolic disorder is apparent as lipoprotein lipase is activated by insulin, therefore insulin resistance and/or insulin deficiency may result in extremely elevated triglyceride levels[(2)]. Similarly, cholesterylester hydrolase within adipocytes is inhibited by insulin (this hydrolyses triglyceride to produce NEFA and glycerol)[(3)]. Therefore in insulin deficiency resistance increased levels of Non-Esterified Fatty Acids (NEFA) are releases from the adipocytes. Hence there will be lowered levels of triglyceride clearance as a result of reduced activation of lipoprotein lipase[(3)].

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. Subsequently, the evaluation of medicinal plants used traditionally in treating diabetes is of growing interest [(4)].

Vernonia amygdalina, a member of the daisy family and it is a small shrub that grows in tropical Africa. *Vernonia amygdalina* typically grows to a height of 2.5m (6.6-164ft). The leaves are elliptical and up to 20cm. (7.9inch) long. It grows well in Africa tropics and other parts of Africa, particularly Nigeria, Cameroon and Zimbabwe.[(5)] The use of *Vernonia amygdalina* has both hypoglycemic and hypolipidemic properties in experiment animals and so could be used in the management of diabetes[(6)].

The objective of this work is to investigate the therapeutic effects of the aqueous extract of *Vernonia amygdalina* on diabetes mellitus and lipid abnormalities on laboratory induced albino rats and to compare the effect of this leaf extract to synthetic drugs.

II. Materials And Methods

Materials/Apparatus

Fresh leaves of *Vernonia amygdalina*, Beakers, conical flasks, crucible, filter papers, pipettes, oven, weighing balance, soxhlet extractor, measuring cylinder, centrifuge, electric grinder syringes, and needles, water bath, sterile sample bottles, dessicator.

Reagents/Chemicals utilized

Alloxan monohydrate (St Louis, MD, USA) cholesterol reagent (Teco Diagnotics, USA) Triglyceride lipo reagent (Teco Diagnostics USA), and HDL cholesterol reagent (Agape Diagnostics, Switzerland) insulin (Novolog USA), Atorvastatin (Unipex USA).

All other reagents used in the study were of high analytical grade.

Plant sample collection

Fresh leaves of *Vernonia amygdalina* (Bitter leaves) were collected from Umungasi Market. Aba North L.G.A of Abia State.

Experimental animals

Adult healthy Albino rats (Wistar strain) of both sexes of about eleven weeks old with an average body weight of 120-200g were collected from the animal house of Biochemistry Department, University of Part Harcourt, Nigeria. The animals were allowed to accamatize for one week in the Departments of Biochemistry of the Abia State Polytechnic, Aba Abia State. They were kept in different ages with high hygiene and of standard housing conditions of temperature (22 to 28^0c) and 12hours light/12 hour dark regime. They were fed with standard rat feed and water libitum throughout the duration of the study.

III. Methods

Plant sample preparation

The healthy leaves of *Vernonia amygdalina* collected were properly washed under running water and air dried at room temperature for several days. The dried leaves were then pulverized using an electric blender and stored in an air tight container and kept in a cool-dry place for further analysis.

Preparation of plants aqueous extract

A portion of the powered leaves (50g) was weighed and dissolved in 400ml of hot distilled water of temperature (40^0C-60^0C) and allowed to cool for 60minutes and then filtered before being used in administration. These aqueous extracts were prepared daily to avoid turbidity and bacteria actions due to poor storage systems.

Phytochemical screening

Phytochemical tests for bioactive constituents like tests for saponins tannins, terpenoids, flavonoids, alkaloids, and polyphenols were carried out on a portion of the powered sample material using standard phytochemical procedures as described by Ukpabi *et al* [16].

Induction of diabetes mellitus

Diabetes was induced in rats by injecting freshly prepared alloxan monohydrate intraperitionally at dosage of 100mg/kg body weight. Development of diabetes was confirmed after 72hours of alloxanization by using Accuchek active glucometer (Roche diagnostic). Rats that had blood glucose levels above 250mg/dl were considered diabetic and was selected for the study.

Experimental design and treatment

The animals were grouped into six groups of five animals per group as shown below.

Group A –(Normal control): This group was not intoxicated and was given access to food and water.

Group B-(Negative control): This group was induced with 1ml each of 100mg/kg alloxan IP without treatment.

Group C-(Positive control 1): This group was induced with 1ml each of 100mg/kg alloxan IP and treated with insulin (40mg/kg).

Group D-(Positive control 2): This group was induced with 1ml each of 100mg/kg alloxan IP and treated with Ator vastatin (30mg/kg).

Group E- (Test group 1): This group was induced with 1ml each of 100mg/kg alloxan IP and treated with 200mg/kg of *V. amgydalina*.

Group F (Test Gruop2): This group was induced with 1ml of 100mg/kg allocan IP and treated with 400mg/kg of *V. amygdalina*.

Subsequently, treatment regimes of hyperglycemia and hyperlipidemia started on the 4th and 8th day of the experiment respectively.

Biochemical analysis

Collection of Blood Samples

After the administration of the sample, the animals were fasted for 24hours. Blood was collected by cardiac puncture into clean tubes, and was later centrifuged at 3000rpm for 10 minutes using a centrifuge to remove cells and recover serum for biochemical assay.

Glucose estimation

Glucose is a major carbohydrate present in the blood and serves as a primary source of energy. Glucose oxidase is an enzyme highly specific for glucose. It catalyses the oxidation of Beta D-glucose present in the plasma to D glucono-1,5,-lactone with the formation of hydrogen peroxide. The lactone is then slowly hydrolyzed to D-gluconic acid. In the presence of the enzyme peroxidase, the hydrogen peroxidase is broken down and oxygen released reacts with 4-amino-phenazone and phenol to give a pink colour. The absorbance of the colour produced in measured in a spectrophotomer.

Lipid Profile analysis

The plasma total cholesterol (TC), Triglycerides (TG) and HDL (High density lipoprotein) were measured using commercial kits, via enzyme coupled reactions and the coloured complex, measured by spectrophotometry. LDL (low density lipoprotein) was calculated using Friedwald's equation [(7)].

Statistical Analysis

Results from the analyses were expressed as Mean +SD and $p<0.05$ being considered as statistically significant.

IV. Result

Phytochemical composition of aqueous leaf extract of *Vernonia amygdalina*.

The results obtained from the phytochemical analysis showed that the aqueous extract of *Vernonia amygdalina* gave positive reactions for tannins, flavonoids, alkaloids, terpenoids, saponins and polyphenols. Saponins reactions showed the deepest colouration as seen in (Table 1).

Table 1: Qualitative Phytochemical constituents of aqueous leaf extract of *Vernonia amygdalina*

Phytoconstituent	Qualitative abundance
Tannins	++
Flavonoids	++
Alkaloids	++
Terpenoids	++
Saponins	+++
Polyphenols	++

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

Serum glucose concentrations of alloxan induced albino rats following oral administration of aqueous leaf extracts of *V. amygdalina*.

Results obtained from the diabetic study on the albino rats showed that the extract of *Vernonia amygalina* decreased blood glucose in a dose-dependent fashion. The efficiency of the herb extracts was significant when compared to the reference drug (Insulin).

Table II: Effects of aqueous leaf extract of *Vernonia amygdalina* on blood glucose concentrations of alloxan induced albino rats

Group	Initial glucose conc (mg/dl)	Glucose conc. after 4days of treatment(mg/dl)	Glucose conc. after 8days of treatment(mg/dl)	Glucose Conc. after 12days of treatment (mg/dl)
A	83.85±3.36	83.40±3.20	84.00+1.98	85.50±2.91
B	283.33±1.34	278.33±1.74	271.23±1.15	266.85±3.69
C	280.00± 1.36	210.25±2.96	160.12±1.33	100.13±1.91
E	278.00±3.62	209.00±2.00	152.00±1.27	107.23±0.01
F	282.00±0.52	201.00+0.73	148.00±0.82	94.60±0.00

Result are expressed as mean ± SD

Serum lipid levels in albino rats following the induction of alloxan monohydrate (100mg/kg).
The induction of the albino rats with alloxan monohydrate produced an elevated total cholesterol, total triglyceride and low density lipoprotein and decreased high density lipoprotein concentrations.

TABLE III: Effect of alloxan monohydrate induction on lipid profile of albino rats.

Group	Total cholesterol (mg/dl)	Total triglyceride (mg/dl)	Low density Lipoprotein (mg/dl)	High density Lipoprotein (mg/dl)
A	92.00±2.1	87.00±3.1	44.00± 0.7	51.00±2.3
B	132.00±6.5	149.00±10.1	117.00±0.3	25.00±1.5

Serum lipid levels in diabetic rats following oral administration of the aqueous extracts of *V. amygdalina*
Results showed that total triglyceride, total cholesterol, and low density lipoprotein in the alloxan monohydrate intoxicated rats were significantly decreased when compared to normal control whereas high density lipoprotein was increased.

Table IV: Effect of atorvastatin and aqueous leaf extracts treatments of *Vernonia amygdalina* on diabetic albino rats.

Group	Total cholesterol (mg/dl)		Total triglyceride (mg/dl)		Low density lipoprotein(mg/dl)		High density lipoprotein (mg/dl)	
	13th day	18thday	13th day	18th day	13th day	18thday	13th day	18th day
D	110±6.3	99±2.4	122±8.1	107±4.7	79±3.7	53±1.1	26±2.1	34±1.1
E	103±7.4	95±1.8	114±7.1	90±2.7	73±2.6	42±1.3	32±2.31	45±1.8
F	109±6.8	96±2.6	119±7.2	100±1.9	74±3.1	42±1.4	29±1.15	40±1.3

V. Discussion

Medicinal plants are known to contain wide species of chemicals. Some of these chemicals come into being by natural selection through biosynthetic metabolic pathways. These phytochemical compounds have been found to possess medicinal properties and health promoting effects [8.17]

The phytochemical screening of aqueous leaf extract of *V. amygdalina* indicated the presence of tannins, Saponins, flavonoids, alkaloids, polyphenol and terpenoids (table 1). These compounds have been found to have free radical scavenging and anti-microbial activities [10,16]. The prevalence of flavonoid rich components in the human diet has recently been associated with significant reductions of the risks factors in significant chronic human pathologies, such as diabetes, cancer, neuroegenerative and cardiovascular diseases [20].

Studies suggests that alloxan selectively destroys pancreatic B cells, therefore making it a suitable drug for induction of experimental diabetes, Alloxan structural resemblance to glucose enables it to enter B cells, and plasma membrane via glucose transporters. In this study, rats treated with single dose of alloxan monohydrate developed significant pancreatic damage which was observed from a substantial increase in blood glucose levels. The present data indicated that the aqueous extracts of *Vernonia amygdalina* significantly reduced the elevated blood glucose levels with respect to those of the diabetic control rats. The efficiency of the herb extracts was significant when compared to the reference drug (insulin). This antihyperglycemic action may be attributed to the potentiation of pancreatic secretion of insulin from the existing or regenerated cells of insulin and its action or to the extra pancreatic mechanisms like enhanced transport of blood glucose to peripheral tissue, increased peripheral utilization of glucose via different enzymatic pathways or inhibition of intestinal absorption of glucose[11,12].

Another characteristic feature of severe diabetes is an elevated concentrations of serum lipid parameters[8]. The abnormally high concentration of serum lipids in diabetes is mainly due to the increase in the production of free fatty acids from the peripheral fat depots [9,21].

In this study, the rise in blood sugar was accompanied by marked increase in cholesterol, triglycerides, low density lipoprotein and a reduction in high density lipoprotein (table III)

Table IV shows that the aqueous extracts of *Vernonia amygdalina* and the reference drug (Atorvastatin) lowered plasma total cholesterol total triglyceride, low density lipoprotein levels and increased high density lipoprotein concentrations in the treated rats, and this could account for the use of *Vernonia amygdalina* in folk medicine for the treatment of diabetes and its complications such as hyperlipidemia. This observation is consistent with earlier report that diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids[13,18] and this accounts for the atheroscolerosis, arteriosclerosis and severe coronary heart disease. The results suggests that the leaf extracts of *Vernonia amygdalina* is a potent hypoglycemic agent which is capable of normalizing hyperlipdiemia abnormalities associated with diabetes mellitus thus could be prescribed as adjunct to dietary therapy and/or main therapy for diabetes mellitus. These results confirm earlier report [14,19]

on the use of leaf extracts of *Vernonia amygdalina* in traditional medicine for the treatment of diabetes mellitus and its complications.

VI. Conclusion

Vernonia amygdalina may be considered as an important component of preventive therapy in management of diabetes and its associated complications. Our studies were able to prove the hypoglycemic and hypolipidemic effects in alloxan induced diabetic rats.

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