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EFFECTS OF AQUEOUS EXTRACT OF *VERNONIA AMYGDALINA* DELILE LEAVES IN ALLOXAN-INDUCED DIABETIC RATS

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Abstract

This present study investigates the effect of the *Vernonia amygdalina* Delile (Bitter leaf) on some biochemical parameters such as fasting blood glucose, serum lipid profile, serum electrolytes, activities of the transaminases and alkaline phosphatase, in alloxan-induced diabetic rats. Twenty adult albino rats were used and grouped into four of five (5) rats each: control, diabetic untreated rats, diabetic rats administered with *V. amygdalina* (400 mg/kg) and normal rats fed with *V. amygdalina* (400 mg/kg) daily respectively. All rats except those in control and normal rats fed with *V. amygdalina* (400 mg/kg) group were induced intraperitoneal with a single dose of 150 mg/kg of alloxan. Aqueous extract of *V. amygdalina* leaf had hypoglycaemic effect when monitored as there was significant increase in the diabetic control groups (5.07 ± 0.15 mmol/L) when compared with diabetic treated groups. Also, the effect of the *V. amygdalina* extract on the activities of the transaminases and the alkaline phosphatase reveals a considerable increase in the diabetic control group compared to the diabetic treated group. From our study, the *V. amygdalina* leaf extract proved to have hypoglycaemic effect by significantly reducing the blood glucose level in diabetic treated rats as it has a great potential of serving as a supplementary therapy to oral hypoglycaemic drugs.

Keywords: *Vernonia amygdalina*, alloxan, diabetes

Introduction

Diabetes mellitus (DM) is a metabolic syndrome defined by hyperglycemia caused by lack of insulin or disruption of insulin signalling as a result of lack of hypoglycemic agent or insensitivity of insulin hormone. DM is related to aberrant metabolism of macromolecules (1, 2). The prevalence of this syndrome worldwide is at an increasing rate, which led to the uses of several therapeutic approaches (currently available) for the management of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, improvement of insulin action at the target sites, inhibition of dietary starch and lipid degradation, and treatment with oral hypoglycemic agents (3, 4, 5). In Africa, there has been vigorous growth of phyto-pharmaceuticals with proven efficacy in a variety of medicinal problems. Among other things, demand for the use of plant natural products with antidiabetic activity due to low cost, easy availability and lesser side effects has been on the rise. Therefore, plant constituents are constantly explored and examined for their effect as hypoglycemic agents. One of such plant is *Vernonia amygdalina.*

Vernonia amygdalina commonly called Bitter leaf is a medicinal plant of the family *Asteraceae.* It is a small perennial shrub that grows in tropical Africa. It is found in Angola, Burundi, Cameroun, Central Africa Republic, Nigeria and in many other countries. It is widely called *Ewuro* in Yoruba land. The leaves of the plant may be consumed either as a vegetable (macerated leaves in soup) after rounds of washing to remove the bitter taste, or aqueous extract as tonics for the treatment of various illnesses (6). *V. amygdalina* is rich in minerals sources such as K^{+}, Ca^{2+}, Na^{+}, P^{3-}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} (7). It also contains saponins, tannins, ascorbic acid, amino acids (glycine, cysteine and nicotinamide), iodine, hydrochloric acid, sugar and oxalate (8,9). Extract of *V. amygdalina* is used traditionally to cure a number of ailments and its actions include, anti-inflammatory, wound healing, immune modulation, anti-tumour, anti-bacteria, antiviral laxative and purgative properties (10, 11, 12, 13). *V. amygdalina* can also be used as food supplements when prepared with melon (egusi soup) a nutritious African food (14). This study seeks to investigate the effect of aqueous extract of *V. amygdalina* leaves on alloxan-induced metabolic syndrome in Wistar rats.

Methods

Chemicals and Reagents

The following reagents were used; Alloxan monohydrate, Normal Saline, chloroform, Total cholesterol, High density lipoprotein (HDL)-cholesterol, Low density lipoprotein (LDL)-cholesterol, very low density lipoprotein (VLDL)-cholesterol and triglycerides assay kits used were product of Randox Laboratories Ltd., Antrim, UK. All other chemicals were of analytical grades and prepared in all-glass apparatus using sterilized distilled water (BDH, UK).

Sources of Plant Material and Identification

Fresh leaves of bitter leaf were gathered from a suburb of Naraguta Hostel Area, Plateau State. It was identified as *V. amygdalina* by Mr. Thomas Yakubu of Pharmacognosy Department, University of Jos, with the identification number, UJ/PCG/HSP/13A03.

Preparation of V. amygdalina (Bitter Leaf) Extract

Fresh leaves of *V. amygdalina* were cut off their stalk and stripped their mid rib. The stripped leaves were thoroughly rinsed in clean water and left over night to be properly drained of the washed water. Thus prepared leaves were then pulverized to powder using an electric blender. Weighed samples were macerated in known volume of distilled water and left for 24 hrs (15). It was then filtered with a what man no1 filter paper. The filtrate was oven dried at 50 $^{\circ}$C to obtain the dry paste of the extract which was used to prepare a stock solution of the extract. The weight of the dry paste extract was 11.7g. The dried extract was then diluted with distilled water (10g dissolved in 100 mL), poured into a clean well labelled container and stored until when needed.

Experimental animals

Twenty adult albino rats were used in this study. They were obtained from the cage yard of Dr (Mrs.) Johnson of the Department of Biochemistry, University of Jos, Nigeria. Their weight was between 120g and 170g. The animals were kept in large clean spacious cages and were given food and water *ad libitum*. The animal room was well ventilated with a 12 h light/dark cycle, throughout the period of the

experiment. They were fed *ad libitum* on rat pellets (Top Feeds, Nigeria) and water. The handling of animals was conformed to the standards of National Institute of Health (NIH publication 85-23, 1985) for experimental animal maintenance.

Induction of diabetes

A single dose of 150 mg/kg of alloxan monohydrate was dissolved in normal saline (0.9%) and induced into the already fasted rats by single intraperitoneal injection. Forty-eight hours after the induction, the animals were fasted and the blood glucose level was checked using Accu chek Glucometer. Only the animals with fasting blood glucose level ≥ 200 mg/dl was used in this study (16).

Animal distribution

Twenty Adult Wistar rats were randomized into four groups as follows: Healthy control (received distilled water), diabetic untreated group, diabetic + *V. amygdalina* (400 mg/kg body weight), normal + *V. amygdalina* (400 mg/kg body weight).

Collection of Blood samples and Glucose Evaluation

Blood sample from each animal was collected through their jugular vein and preserved until further processing. The blood sample was collected into a dry tube and allowed to clot for 30 min before centrifuging at 3,500 rpm for 10 min to collect the serum (16). Collection and evaluation was done in all the groups before induction with Alloxan, after 48 hrs of induction with alloxan and after administration of *V. amygdalina* extract via oral gavage for fourteen (14) days of treatment. The rats were anaesthetized with chloroform to maintain calmness during blood collection and 3.5 mL of blood were collected from each rat for further biochemical examinations.

Biochemical Parameters

Serum lipid concentrations, in additional to aspartate and alanine aminotransferases and alkaline phosphatase as well as urea and creatinine concentrations, sodium (Na^+), potassium (K^+), chloride (Cl^-) were measured by means of an Automated Chemistry Analyzer.

Low Density Lipoprotein-cholesterol was estimated according to equation as shown below:

LDL-Cholesterol (mg/dl) = TC- HDL- (TG/5)

Whereas, TG/5 is equivalent to the concentration of Very Low Density Lipoprotein-cholesterol. TC: total cholesterol; TG means triacylglycerol (17).

Data analysis

All data are expressed at mean ± standard error of mean (SEM). The data were analyzed by one-way analysis of variance via a statistical software package (SPSS, Version 20.0, IBM Corporation, NY, USA) using Duncan multiple range *post-hoc* test (DMRT). Values were considered to be significantly different at $p < 0.05$.

Results

Table 1 shows extremely significant increase ($p < 0.05$) in the fasting blood glucose level of diabetes-induced groups when compared with the healthy control. High serum levels of cholesterol were obtained in diabetic control compared with the diabetic treated and healthy controls. Diabetic untreated rats show a significant increase ($p < 0.05$) in triglycerides and LDL concentrations when compared to the healthy control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract Table 2. Diabetic untreated group also shows a significant decrease ($p < 0.05$) in HDL (good cholesterol) concentrations when compared to healthy control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract.

Table 3 shows the effect of the *V. amygdalina* extract on the activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in normal and diabetic rats. There was considerably increase in the activity of ALP, AST and ALT in the diabetic control group compared to healthy control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract.

Table 4 shows the effect of the *V. amygdalina* extract on the activities of Billirubin (Total), Billirubin (Conjugated) in healthy control and diabetic rats. There was significant increase in the diabetic control groups compared to healthy control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract.

Table 5 shows the effect of the *V. amygdalina* extract on the activities of sodium, potassium, chloride, bicarbonate, urea and creatinine in normal and diabetic rats. Significant increase ($P < 0.05$) in sodium, potassium, chloride, bicarbonate, urea and creatinine was observed in the diabetic control when compared to healthy control, diabetic rats

administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract.

Discussion

The result confirms that *V. amygdalina* has anti-diabetic properties and these was seen in the experiment conducted on the various groups of rats. Alloxan-induced metabolic syndrome is characterized by hyperglycemia, polydipsia, polyphagia and polyuria. In the present study, considerably elevated fasting blood glucose levels was efficiently induced. Administration of aqueous extract of *V. amygdalina* leaves significantly decreased blood glucose levels to almost their basal blood sugar levels Table 1. This hypoglycaemic effect may be due to depression of key gluconeogenic or the increase in the levels of glucose transports and stimulation of uptake in peripheral tissues (18). Another possible effect of this plant extract may be that it preserves the cells of islets of Langerhans of β -cells functions, which result in a significant increase in insulin activity (16, 19, 20).

Abnormal lipid metabolism, resulting in amassing of LDL-C and total cholesterol in addition to reduced HDL-cholesterol, is often related to diabetes mellitus (21, 22). Elevated levels of LDL, and total cholesterol are thought of as main menace for cardiovascular disease (CVD). On the contrary, elevated HDL-cholesterol that functions in the transport of cholesterol from the periphery to the liver reduces the chance of CVD (23). Consistent with this, a considerable raise on serum lipid profiles with a decrease on serum HDL-cholesterol in diabetic untreated compared with normal control. Oral intervention of *V. amygdalina* leaf aqueous extract averts dyslipidemia, and amplified serum HDL-cholesterol level. This is in accordance with newly published studies (21, 24). This could be due to the effect of Cuisine which is a component of *V. amygdalina*, which reduces low density lipoprotein cholesterol by 50% (25), while also boosting good high density lipoprotein cholesterol.

Elevated serum transaminase, alanine aminotransferase (ALT), aspartate aminotransaminse (AST), alkaline phosphatase (ALP), urea and creatinine levels are thought of as biomarkers of hepato-renal damage, related to liver disease and hyperglycaemia (26, 27). There was significant increase in the three liver enzymes of diabetic control when compared to normal control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract. This indicates that the extract has the effect of preventing hepatocellular injury and obstructive liver disease in liver damaged conditions in a diabetic state.

The effect of the *V. amygdalina* extract on the kidney was monitored on the electrolyte parameters such as sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO^{3-}), urea and creatinine as there was significant increase in the diabetic control compared to normal control, diabetic rats administered with *V. amygdalina* and normal rats fed with *V. amygdalina* extract. It has been postulated that increased levels of serum urea and creatinine are linked to kidney disease (26, 28, 29). In this study, it has been shown that *V. amygdalina* leaf extract reduces blood glucose level in diabetic rats, by proving its hypoglycaemic effect. Therefore, *V. amygdalina* leaf extracts has a great potential to be used as supplementary treatment to oral hypoglycaemic drugs in the management of diabetes mellitus in humans as well as preventing hepatocellular injury and obstructive liver disease in diabetic conditions due to the suppression of hepatic enzymes in diabetic state.

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Table 1. Effects of aqueous extract *V. amygdalina* leaves on the fasting blood glucose level of alloxan-induced diabetic rats before, during and after induction of alloxan.

Groups	Initial fasting blood glucose level (mg/dl)	Fasting blood glucose at 72 h after induction (mg/dl)	Final fasting blood glucose at 15 days after induction (mg/dl)
Healthy Control	71.28 ± 0.14[a]	75.25 ± 0.10[a]	77.18 ± 1.14[a]
Diabetic control	82.21 ± 1.01[a]	262.80 ± 1.01[c]	355.42 ± 3.14[d]
Normal + Extract	71.14 ± 0.21[a]	73.20 ± 0.45[a]	70.20 ± 1.10[a]
Diabetic + Extract	78.24 ± 1.42[a]	224.23 ± 1.32[b]	91.26 ± 0.76[c]

Data are presented as the mean ± SEM of 5 rats per group. [a-c]Values with different letters along a row for a given parameter are significantly different from each other (Duncan multiple range post hoc test, $p < 0.05$).

Table 2. Effect of *V. amygdalina* extract on triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) in alloxan induced diabetic rats

Groups	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Healthy Control	10.57 ± 0.35[a]	21.33 ± 0.21[a]	58.63 ± 1.06[a]	20.91 ± 0.06[a]
Diabetic Control	54.40 ± 1.17[b]	71.60 ± 1.65[b]	21.53 ± 0.12[b]	85.17 ± 1.08[b]
Normal + Extract	10.40 ± 0.20[a]	21.12 ± 0.31[a]	58.43 ± 1.15[a]	20.97 ± 0.02[a]
Diabetic + Extract	25.47 ± 1.06[c]	42.90 ± 1.10[c]	38.50 ± 1.10[c]	41.46 ± 0.21[c]

Data are presented as the mean ± SEM of 5 rats per group. [a-c]Values with different superscript letters along a column for a given parameter are significantly different from each other. TC, Total cholesterol; TG, Triglyceride; LDL-cholesterol, Low density lipoprotein-cholesterol; HDL-cholesterol; High density lipoprotein-cholesterol.

Table 3. Effect of *V. amygdalina* leaf extract on alanine aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in alloxan induced diabetic rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Healthy Control	53.33 ± 3.89[a]	52.67 ± 1.02[a]	37.35 ± 1.35[a]
Diabetic Control	139.67 ± 10.93[b]	143.22 ± 9.64[b]	78.33 ± 3.03[b]
Normal + Extract	52.22 ± 3.57[a]	51.18 ± 2.64[a]	37.33 ± 1.62[a]
Diabetic + Extract	89.33 ± 5.16[c]	78.00 ± 5.11[c]	55.33 ± 2.45[c]

Data are presented as the mean ± SEM of 5 rats per group. [a-c]Values with different superscript letters along a column for a given parameter are significantly different from each other. ALT, Alanine transaminases; AST, Aspartate transaminases; ALP, Alkaline phosphatases.

Table 4. Effect of *V. amygdalina* leaf extract on total bilirubin, conjugated bilirubin, albumin and total protein in alloxan induced diabetic rats

Groups	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)	Albumin (mmol/L)	Total Protein (g/l)
Healthy Control	6.37 ± 0.12[a]	5.18 ± 0.30[a]	56.33 ± 2.35[a]	39.67 ± 1.51[a]
Diabetic Control	19.20 ± 1.21[b]	15.10 ± 1.45[b]	15.56 ± 1.34[b]	17.67 ± 1.02[b]
Normal + Extract	6.20 ± 0.15[a]	5.12 ± 0.31[a]	53.67 ± 2.03[a]	38.00 ± 1.72[a]
Diabetic + Extract	10.27 ± 0.95[c]	9.63 ± 1.12[c]	34.00 ± 1.46[c]	28.33 ± 1.06[c]

Data are presented as the mean ± SEM of 5 rats per group. [a-c]Values with different superscript letters along a column for a given parameter are significantly different from each other.

Table 5. Effect of *V. amygdalina* leaf extract on the activities of sodium, potassium, chloride, bicarbonate, urea and creatinine in alloxan induced diabetic rats

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO^{3-} (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Healthy Control	35.0 ± 1.12[a]	36.91±1.26[a]	34.33±1.08[a]	27.12 ± 1.65[a]	5.90 ± 0.52[a]	53.33 ± 1.09[a]
Diabetic Control	87.3 ± 1.16[b]	86.03±3.25[b]	103.67±7.64[b]	79.33 ± 2.16[b]	13.87 ± 2.06[b]	120.67 ± 9.87[b]
Normal + Extract	30.0 ± 2.34[a]	36.03±1.15[a]	34.54±1.29[a]	25.12 ± 1.45[a]	5.87 ± 0.65[a]	54.67 ± 1.21[a]
Diabetic + Extract	63.0 ± 1.20[c]	66.04±1.35[c]	65.33±1.53[c]	59.67 ± 1.59[c]	9.43 ± 1.11[c]	61.67 ± 1.87[c]

Data are presented as the mean ± SEM of 5 rats per group. [a-c]Values with different superscript letters along a column for a given parameter are significantly different from each other. Na^+, sodium, K^+, potassium, Cl^-, chloride, HCO^{3-}, bicarbonate, Urea, Creatinine.