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The Effect of *Vernonia amygdalina* Extract on Blood Glucose, Liver and Kidney Biochemical Markers in Alloxan-induced Hyperglycaemic Albino Rats

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ABSTRACT

This study evaluated the effects of an aqueous extract from *Vernonia amygdalina* leaves, sourced from Kashere, Gombe State, on alloxan-induced hyperglycemic albino rats. A qualitative phytochemical screening was conducted using standard method and it confirmed the presence of alkaloids, flavonoids, saponins, tannins, and reducing sugars. Hyperglycemia was induced via intraperitoneal injection of 150 mg/g alloxan, and no high glucose spike after three days. The extract was administered orally at 200 mg/kg (Grp-A) and 400 mg/kg (Grp-B), with negative (untreated, alloxan-injected) and normal (untreated) controls. By Day 7 and 14, significant reductions in blood glucose levels were observed in treated groups compared to the negative control. The extract improved liver and kidney biochemical markers, increasing serum total protein, globulin, and bilirubin while reducing aspartate transaminase (AST) and alanine transaminase (ALT) enzyme levels. It also elevated electrolytes like K^+ and HCO_3 and decreased urea and creatinine levels ($p \leq 0.05$). In conclusion, *V. amygdalina* extract contains vital phytochemicals that lower blood glucose and enhance liver and kidney function in diabetic rats.

Keywords: Blood glucose, biochemical markers, hyperglycaemia, *Vernonia amygdalina*

INTRODUCTION

Hyperglycaemia is a medical condition characterized by an increase or elevated glucose level in the blood. It is also known as diabetes mellitus [1]. This condition is characterized by an impaired release of insulin by the pancreatic β cells, production of inactive insulin or absolute insulin deficiency, and inadequate or defective insulin receptors [2]. Diabetes has become a

global health threat worldwide as the number of cases increases every year in all age groups [3]. The total number of people with diabetes has been predicted to rise to 643 million by 2030 and 784 million by 2045. It has also been reported that 81% of people with diabetes live in low-income and middle-income countries. Undiagnosed adults living with diabetes are estimated to be 44% of which 90% live in low-income and middle-income countries [4]. In Nigeria, 3.6 million cases were recorded in 2021 with about 48,375 deaths reported [5].

The surge in diabetes has necessitated research into different approaches to the treatment of the disease and one of the approaches is the use of anti-diabetic drugs [6]. Nevertheless, these drugs have their inadequacies which result in detrimental health effects. Aside from this, most of these drugs are not readily available and affordable in developing countries and also in some developed countries [7]. Therefore, research is presently conducted on medicinal plants with hypoglycaemic activities which are less toxic, affordable, readily available, and accessible as a suitable substitute [8, 9].

Vernonia amygdalina, commonly known as bitter leaf, is a valuable medicinal plant that is widespread in West Africa. The leaves have a characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial, antidiabetic and anti-parasitic agent [10]. *V. amygdalina* contains complex bioactive phytochemicals that are useful pharmacologically [10]. The phytochemical studies of *V. amygdalina* reveals the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes [11]. Some other phytochemicals have been isolated and characterized from the leaves of the plant which include vernonioside D, vernodalol, luteolin, vernodalin, vernolepin and luteolin 7-O- β-glucoside [12].

Bitter leaf is majorly grown in the southern part of Nigeria where the leaves are mostly used to prepare unique delicacy [13]. Amaechi et al [14] conducted a study in southeast Nigeria and reported *V. amygdalina* to significantly reverse the nutritional indices and biochemical parameters which were compromised in diabetic rats. Likewise, Johnson et al [15] assessed the antidiabetic effect of *V*. amygdala collected from a farm in southwest Nigeria and revealed that the extract produced hypolipidemic effect, increased blood parameters and decreased blood sugar in male albino mice. Therefore, this study is important given the existence of various bitter leaf plant varieties/accessions, with only a few

representatives found in the northern part of Nigeria, and the lack of such research in Gombe State, Nigeria.

The aim of this study was to assess the phytochemicals present in the aqueous leaf extract of *V. amygdalina* collected from Gombe State, evaluate the hypoglycaemic effect of aqueous leaf extract of *V. amygdalina* on fasting blood glucose levels in alloxan-induced hyperglycaemic Wistar rats and the residual effect of the plant extract on kidney and liver biochemical markers in the experimental albino rats.

Figure 1: The whole plant (*Vernonia amygdalina*), plant processing and experimental animal

MATERIALS AND METHODS

Preparation of *Vernonia amygdalina* extract

Vernonia amygdalina leaves were harvested from a garden in Kashere, Akko Local Government Area Gombe state. The leaves were rinsed with distilled water, air-dried and pulverized into powder. Distilled water (2 litres) was used to extract the constituents of the powder (1 kg) with Microwave assisted extraction method [16]. The colloidal solution was passed through cotton wool to obtain a filtrate that was evaporated to obtain a semi-solid extract [17].

Phytochemical screening

Qualitative phytochemical compositions of the aqueous leaf extract of *V. amygdalina* were determined using the methods previously described by Balamurugan [18].

Administration of alloxan and plant extract

The twenty (20) Albino rats weighing between 99 g - 205 g were randomly divided into 4 experimental groups (Grp-A, Grp-B, Grp-C and Grp- D) of 5 rats each. Diabetes was induced

in rats assigned to groups A, B and C by intraperitoneal injection of 150 mg/g of alloxan monohydrate following 12 hours of fasting while rats in Grp-D (normal control) were not injected with alloxan [19]. Blood samples from the rats were collected through the tail vein and tested for hyperglycaemia with a glucometer three days after alloxan administration [20]. The rats in Grp-A and Grp-B were treated with 200 mg/kg and 400 mg/kg of aqueous leaf extract *V. amygdalina* respectively while rats in Grp-C (negative control) and Grp-D were not treated with the extract. All treatments were made once daily for 14 days through oral gavage. The diabetic animals were allowed free access to tap water, pellet diet, and were maintained at room temperature in cages.

Analysis of fasting blood glucose and biochemical markers

The experimental albino rats were fasted before collecting blood samples on Day 1, 7 and 14 after treating the rats with plant extract for the assessment of FBG (fasting blood glucose) through tail snip and analysis was done using a glucometer [21, 22]. At the end of the experiment (Day 15), blood samples were collected into lithium heparin bottles through cardiac puncture of the anesthetized experimental animals for further biochemical analysis. To assay for liver biochemicals, serum was poured into a pre-washed tube and used to determine the levels of liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), albumin, globulin and total protein. ALT was determined using colorimetric method and the principle of the reaction is that ALT catalyses the transfer of the amino group between L-alanine and a-ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with 2,4 dinitrophenylhydrazine in sodium hydroxide to give a complex with a reddish-brown colour. The absorbance of the sample was read against the reagent blank at 546 nm using a calorimeter (C401 ThermoFisher, USA).

AST was also determined using spectrophotometric method and the principle of the reaction is that the AST catalyzes an exchange reaction of an amino group between aspartate and a-ketoglutarate forming oxaloacetate and glutamate. The oxaloacetate formed reacts with 2,4-dinitrophenylhydrazine in sodium hydroxide to form oxaloacetatehydrazone which has a reddish brown colour. The absorbance of the sample was read against the reagent blank at 546 nm using a UV spectrophotometer (SP-VG722, China).

The biuret method was employed for the estimation of albumin, globulin and total protein [23]. The serum total bilirubin and conjugated bilirubin were determined using Randox kit [24]. The level of serum sodium (Na^+), potassium (K^+), chloride (Cl^-),

bicarbonate (HCO_3), urea, and creatinine were measured using an SKU:SM100 autoanalyzer for kidney function biomarkers [23].

Statistical analysis

Statistical analyses were performed by One-way Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to separate mean where significance exist. All the values of the experimental results were expressed as Mean ± SEM (Standard Error Mean). Results were analyzed using Statistical Package for Social Sciences (SPSS Inc. Chicago IL) version 23.0.

RESULTS AND DISCUSSION

Phytochemical constituent of *V. amygdalina* aqueous leaf extract

The phytochemical screening conducted for the *V. amygdalina* aqueous leaf extract in this study contains phytochemical compounds such as alkaloid, flavonoid, saponins, tannins and reducing sugars (Table 1). These phytochemicals are naturally occurring substances that have been discovered in plants parts such as roots, stems and leaves. These substances are ubiquitous and serve as protective factors in plants against heat, ultraviolent light and external pathogens [25]. Studies have also reported that *V. amygdalina* have high content of pharmacologically active phytochemicals such as saponins, tannins, alkaloids and flavonoids, triterpenoids, steroids and cardiac glycosides that are also effective as supplements in human and animal nutrition [26, 27]. A number of compounds with antidiabetic activities which belong to various classes of compounds detected have been isolated and characterized from the plant [28].

Table 1: Phytochemical reports of aqueous *Vernonia amygdalina* leaves extract

Phytochemical compounds	Result
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Reducing sugars	+

Note: + and - indicates the presence and absence of the compounds respectively

Effect of *V. amygdalina* aqueous leaf extract on blood glucose in diabetic albino mice

The fasting blood glucose (FBG) levels of diabetic rats treated with *V. amygdalina* aqueous leaf extract is presented in Table 2. Before treatment, Grp-B (5.10±0.27 mmol/L) and Grp-C (5.60±0.35 mmol/L) had the highest blood glucose level amongst all treatment groups. Moreover, there was no significant spike in blood glucose of all groups of rats injected with alloxan. For the Day 1 after treatment regime, the FBG of group treated with 200mg/kg (11.50±1.04 mmol/L) and 400mg/kg (11.20±0.95 mmol/L) of *V. amygdalina* aqueous leaf extract was significantly higher than other groups. However, for the 7- and 14-days post-treatment, the FFBG of Grp-A (10.40±1.03 mmol/L, 9.80±0.05 mmol/L) and Grp-B (9.60±1.05 mmol/L, 9.10±0.12 mmol/L) significantly decreased than the negative control (16.20±0.07 mmol/L, 15.00±0.04 mmol/L). These findings reveal the hypoglycaemic potency of *Vernonia amygdalina* aqueous leaf extract at 200mg/kg and 400mg/kg at Day 7 and 14. The results are in agreement with those obtained in studies that also assessed the hypoglycemic effect of *V. amygdalina* when administered to diabetic rats [29, 30]. It may also be possible that the leaf extract might possess the potency of inducing the synthesis of insulin or act as oral hypoglycaemic drug by mediating some of the actions discussed above. The antidiabetic role of *V. amygdalina* can be explained by the presence of several biochemicals resident in the plant and the level of chemical interactions both at the cellular and molecular levels [30].

Table 2: Blood glucose level of diabetic albino rats treated with *V. amygdalina* aqueous leaf extract

Groups	Pre-treatment (mmol/L)	Day 1 (mmol/L)	Day 7 (mmol/L)	Day 14 (mmol/L)
Grp-A	4.80±0.52^{b}	11.50±1.04^{a}	10.40±1.03^{b}	9.80±0.05^{b}
Grp-B	5.10±0.27^{a}	11.20±0.95^{a}	9.60±1.05^{b}	9.10±0.12^{b}
Grp-C	5.60±0.35^{a}	9.10±1.98^{b}	16.20±0.07^{a}	15.00±0.04^{a}
Grp-D	4.40±0.17^{b}	4.90±0.08^{c}	4.00±0.08^{c}	4.40±0.03^{c}

Note: Values are represented as Mean ± SEM, n= 5, values with different superscript shows significant difference, Grp-A= 200mg/kg of extract, Grp-B= 400mg/kg of extract, Grp-C= negative control, Grp-D= Normal control.

Effect of *V. amygdalina* aqueous leaf extract on liver biochemical markers in diabetic albino mice

The mean values of biochemical parameters for liver function of experimental diabetic animals treated with *V. amygdalina* aqueous leaf extract is demonstrated in Table 3. Total protein in the serum of rats that were not administered alloxan and plant extract (78.30±0.5 mmol/L was significantly higher than other groups except the rats administered 400mg/kg (78.00±2.0 mmol/L) of the plant extract. Albumin does not differ significantly for all groups but globulin was significantly higher in normal control (36.00±1.0 mmol/L) than all groups and the least concentration of globulin occurred in animals treated with 400mg/kg (13.00±1.5 mmol/L) of the extract. Total bilirubin and conjugated bilirubin were significantly higher in Grp-B (87.00±1.0 mmol/L, 32.00±1.0 mmol/L) than negative control (56.00±3.0mmol/L, 20.00±0.5mmol/L). Liver enzymes such as AST and ALT were significantly reduced in serum of experimental animals in Grp-A (79.00±0.5 U/L, 57.00± 0.6 U/L) and Grp-B (81.00±1.5 U/L, 58.00±0.5 U/L) than negative control (114.00±2.0 U/L, 87.00±1.5 U/L). In other words, the administration of *Vernonia amygdalina* aqueous leaf extract at specific concentration to diabetic albino rats caused a higher level of serum total protein (400 mg/kg), globulin (200 mg/kg), total bilirubin and conjugated bilirubin (400 mg/kg) and reduced concentration of AST and ALT enzymes at both concentrations. Therefore, this study suggest that the plant extract could cause an increase in the production of liver proteins and bilirubin levels at certain concentrations. In contrast, methanol leaf extract of *V. amygdalina* was reported to have no effect on albumin, total bilirubin and conjugated bilirubin but total protein to increase in animals treated with 80 mg/kg and 320 mg/kg dose of the plant extract [30]. A reduction in serum liver enzymes was also reported by Akah [30] and Atang [31] who both studied the ameliorative effect of *V. amygdalina* in diabetic rats. Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids [32] and this accounts for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increase levels of transaminases which are marker enzymes that are important in heart and liver damage [33].

Table 3: Liver biochemical markers of diabetic albino rats treated with *V. amygdalina* aqueous leaf extract

Group	Total protein (mmol/L)	Albumin (mmol/L)	Total bilirubin (mmol/L)	Conjugated bilirubin (mmol/L)	AST (U/L)	ALT (U/L)	Globulin (mmol/L)
Grp-A	73.40±1.5^{b}	45.90±1.0^{a}	48.00±1.0^{b}	17.00±1.5^{c}	79.00±0.5^{b}	57.00±0.6^{b}	28.00±1.5^{b}
Grp-B	78.00±2.0^{a}	42.70±1.0^{a}	87.00±1.0^{a}	32.00±1.0^{a}	81.00±1.5^{b}	58.00±0.5^{b}	13.00±1.5^{d}
Grp-C	51.10±0.5^{c}	42.60±1.0^{a}	56.00±3.0^{b}	20.00±0.5^{b}	114.00±2.0^{a}	87.00±1.5^{a}	23.00±0.5^{c}
Grp-D	78.30±0.5^{a}	44.50±0.5^{a}	51.00±0.5^{b}	19.00±1.3^{b}	25.00±1.0^{c}	40.00±2.5^{c}	36.00±1.0^{a}

Note: Values are represented as Mean ± SEM, n= 5, values with different superscript shows significant difference, Grp-A= 200mg/kg of extract, Grp-B= 400mg/kg of extract, Grp-C= negative control, Grp-D= Normal control, AST= Aspartate transaminase, ALT= Alanine transaminase.

Effect of *V. amygdalina* aqueous leaf extract on kidney biochemical markers in diabetic albino mice

The kidney biomarkers analysed for this study are the electrolytes, urea and creatinine (Table 4). There was no significant difference in the mean value of Na^{+} and Cl^{-} electrolytes in serum of experimental animals. However, K^{+} and HCO_3 electrolytes in Grp-A (3.90±0.05 mmol/L, 29.00±2.5 mmol/L) and Grp-B (4.40±0.25 mmol/L, 31.00±1.0 mmol/L) were significantly higher than those of Grp-C (2.00±0.03 mmol/L, 28.00±1.0 mmol/L). Urea and creatinine in the serum of rats treated with *V. amygdalina* aqueous leaf extract reduced significantly than untreated control.

The treatment of diabetic rats with *Vernonia amygdalina* aqueous leaf extract does not have effect on Na^{+} and Cl^{-} electrolytes but an increase in serum level of K^{+} and HCO_3 was observed. In addition, the urea and creatine levels of extract treated rats were significantly

reduced than the untreated group. In diabetes, there is a decrease in the level of electrolytes as a result of osmotic diuresis with subsequent loss of water and electrolytes induced by glycosuria [34].

Another characteristic of severe diabetic is an elevated blood urea whose concentration may be five times higher than the normal value [35]. Therefore, the plant extract may have influence on electrolyte balance, acid base regulation and amelioration of renal function due to diabetes. In support, certain electrolytes (Cl, K^+ and HCO_3), were also increased while urea and creatinine reduced significantly in albino rats treated with methanol leaf extract of *V. amygdalina* [30].

Table 4: Kidney biochemical markers of diabetic albino rats treated with *V. amygdalina* aqueous leaf extract

Group	Sodium (Na) (mmol/L)	Potassium (K) (mmol/L)	Chloride (Cl) (mmol/L)	Bicarbonate (HCO3) (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Grp-A	146.00±1.0^a	3.90±0.05^b	108.00±2.0^a	29.00±2.5^a	4.90±0.7^b	41.60±0.5^c
Grp-B	148.00±1.0^a	4.40±0.25^a	109.00±2.5^a	31.00±1.0^a	5.30±0.15^b	45.80±0.5^b
Grp-C	144.00±3.0^a	2.00±0.03^c	107.00±0.5^a	28.00±1.0^b	7.30±0.5^a	64.40±0.6^a
Grp-D	142.00±2.0^a	3.90±0.25^b	104.00±0.5^a	29.00±0.5^b	4.10±0.3^c	40.00±1.0^c

Note: Values are represented as Mean ± SEM, n= 5, values with different superscript show significant difference, Grp-A= 200 mg/kg of extract, Grp-B= 400 mg/kg of extract, Grp-C= negative control, Grp-D= Normal control.

CONCLUSION

This study observed that *V. amygdalina* leaves collected and from the specific area in Gombe State and extracted with aqueous solvent also possess vital phytochemicals. It reduced the concentration of blood glucose and ameliorate the biochemical anomalies associated with liver and kidney function in diabetic rats. Further studies should characterize the different varieties/accessions of *V. amygdalina* in Nigeria and conduct a comparative study on phytochemical screening, and hypoglycaemic potency.

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