Anti-Hyperglycaemic and Anti-Hyperlipidemic Activities of Vernonia Amygdalina in Wistar Rats

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Abstract
Diabetes is a syndrome of impaired substrate metabolism caused by either lack of or resistance to insulin in tissues; this disease causes significant morbidity and mortality largely due to its end-organ complications. Traditional treatment of diabetes has involved the use of several plant extracts; in this study the efficacy of aqueous leaf extract of Vernonia amygdalina (VA) was sought as a treatment module for alloxan induced diabetic rats. The aqueous leaf extract of VA was administered in three (3) different doses (40mg/kg, 80mg/kg, and 120mg/kg) to non-diabetic and alloxan-induced diabetic rats. The body weights of the experimental animals were taken along with blood samples collection at baseline, on day 7, 14 and 21, thereafter the blood glucose and serum lipid levels were determined. The aqueous leaf extract of VA showed statistical significant (p< 0.05) reduction of blood glucose, serum triglyceride and cholesterol, as well as body weight in both non-diabetic and alloxan-induced diabetic rats. This study showed that aqueous leaf extract of VA administered at different doses contains hypoglycemic, anti-hyperglycemic and lipid lowering activities, with 80mg/kg body weight dosage appearing to be the minimum effective dose; suggesting that aqueous leaf extract of VA is likely to contain actives that could be important in the control of blood glucose and serum lipid levels in diabetics.

Keywords: Vernonia amygdalina, Diabetes mellitus, Blood glucose, Lipid profile

Introduction
Diabetes mellitus (DM) is a disease caused by either lack of insulin secretion or decreased sensitivity of tissues to insulin in which glucose metabolism is impaired [1]. This disease has been reckoned as one of leading health problems in Africa, which contributes significantly to morbidity and mortality and adversely affecting both quality and length of life [2]. The prevalence of diabetes mellitus in Nigeria has been reported to have increased from 2.2% in 1997 to 5.0% by 2013, diabetes mellitus is amongst the leading cause of mortality in Africa, [3,4]. Unlike in Africa, diabetes mellitus prevalence in India has reached a pandemic level, with number of diabetic patients reaching over 62 million this reflects the global burden of the disease [5]. Various types of this disease have been identified; some prevention and treatment measures have been recommended [6,7]. The chronic hyperglycemia that results in this ailment is associated with many abnormalities in various organs of the body [8]. Some studies have shown that lipid profile is also altered in diabetics, and this dyslipidemia predisposes the diabetic patients to cardiovascular complications [9-11]. Most diabetic patients especially in Nigeria are grossly faced with inadequate medicine, and cost of managing the disease is high, in addition the use of available antidiabetic drugs like metformin have several side effects, which compounds the existing problems faced by health care-givers [12,13]. The rise in prevalence of diabetes has necessitated the need for development of adequate and sophisticated methods for its management and treatment to forestall the danger and health complications involved with the disease.

VA is a perennial shrub commonly known as bitter leaf, which belongs to the family of Asteraceae [14]. In ethnomedicine, VA leaves are consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for treatment of various illnesses [15]. In North America, all the known 17 species of Vernonia have been shown to possess properties like blood purifier, uterus toner, also ability to prevent atherosclerosis [16,17]. In herbal medicinal practice, aqueous leaf extract of VA is recommended for patients to treat anemia, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems. A number of experimental findings have presented VA as possessing anti-pathogenic and other beneficial medicinal effects; for example, leaf extract of VA has been shown to suppress, delay or kill cancer cells, possess anti-fungal, anti-plasmodia, anti-bacterial; antioxidant, hepato and nephron-protective effects [14,18-26]. Bioactive peptide of aqueous leaf extract of VA is a potent anti-cancer agent [27,28]. Dietary incorporation of VA has been reported to lower serum triacylglycerol and LDL level, normalize cholesterol concentration and concomitantly increased HDL while ethanolic leaf extract of VA has reported to keep the...
lipid profile of rats in normal range [17,27,30]. Lowering of blood sugar has also been reported [31]. The utilization of herbal extracts to treat diabetes related illness has therefore increased over the years, according to WHO, due to poverty and lack of access to modern medicine, moderate percentage of world population found in the developing countries depend mostly on plants for primary health care [32]. Considering the use of VA leaves in ethnomedicine, this study further investigates experimentally, the capability of aqueous leaf extract of VA in treating of alloxan- induced diabetic rats.

Methods
Fresh leaves of VA were purchased from Kenyatta market in Uwani, Enugu state, Nigeria. They were identified and authenticated by Mr. Onyekwuu Chijioke of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium for future reference with number: (UNH7a).

Preparation of the extract
Preparation of the extract was done using the method of [33]. The leaves were washed and dried under shade for 7 days. It was pulverized into powder using electric blender. Five and half (5.5) liters of distilled water was added to 1200g of the VA leaf powder and boiled for 30 minutes under reflux at 80 °C and then allowed to cool for 20 minutes. Then, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated using water bath at a temperature of 50 °C; then evaporated to dryness to give a dark green solid paste with a yield of 11.3%.

Phytochemical Analysis

Alkaloid Determination
5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of its original volume. Concentrated NH₄OH was added drop-wise to the extract and the precipitate was collected and washed with dilute NH₄OH and then filtered. The alkaloid residue was dried and weighed [34].

Flavonoid determination
10g of the sample was treated with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through a whatman filter paper no 42 the filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed until constant weight was obtained [35].

Tannin determination
500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to 50ml with distilled water. 5ml of the filtrate was pipetted into a test tube and mixed with 2ml of 0.1mFeCl₃ in 0.1NHCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 mins [36].

Glycoside determination
The method used was that of [37]. 1g of the VA powder was soaked in 10ml of 70% alcohol for 2 hours and then filtered. The extract obtained was then purified using lead acetate and Na₃HPO₄ solution before the addition of freshly prepared Baljet reagent (containing 95ml aqueous 1% picric acid + 5ml of 10% aqueous NaOH). The difference between the intensity of colors of the experimental and blank (distilled water and Baljet reagent sample) gives the absorbance and is proportional to the concentration of the glycoside.

Saponin determination
The method used was that of [38]. 20g of the VA leaf powder was added into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The mixture of the extracts was reduced to 40ml over water bath at 90 °C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously, the aqueous layer was recovered while the ether discarded. The purification process was repeated; 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous NaOH. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage weight.

Determination of the phenolic compound
10g of the extract was weighed and dissolved in 100ml of distilled water, 1ml of this solution was transferred to a test tube, then 0.5ml of the folin-ciocalteu reagent and 1.5ml (20% of Na₂CO₃ solution) was added and the volume was made up to 8ml with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from vigorous diluted concentrations of Gallic acid [39].

Experimental animals
Thirty five (35) male albino wistar rats weighing 175-285g were used. The rats were purchased from the animal house of Department of Pharmacology, UNEC. They were housed in a clean ventilated wire mesh cages at room temperature, food and water were given ad libitum, 12h hour dark and light cycle were also observed. Animals were used according to the animal welfare regulation of the institution.

Induction of diabetes
Diabetes was induced using the method of [40]. The rats were fasting overnight at least 10 hours after which, 10% alloxan solution diluted in normal saline was administered to the rats intraperitoneally; and were allowed access to food and water 30 minutes after alloxan administration. The rats were confirmed diabetic after 24 hours with glucometer.

Animal grouping and administration of the extract
The rats were divided into 3 major groups (A, B and C) with groups A and C having sub-groups. The administration of the extract was done according the method of [33].

Group A: The control group; it was divided into 3 sub-groups (AI, AII and AIII) of 5 rats per group. Group AI: Non-diabetic that received water only. Group AII: Untreated diabetic that was given water only. Group AIII: Diabetic that was treated with 5mg/kg body weight of Glibenclamide (a standard anti-diabetic drug) orally, Group B: Non-diabetic that received 80mg/kg of aqueous VA leaf extract.

Group C: The diabetic group treated with different doses of aqueous
leaf extract of VA. It was divided into 3 sub-groups: CI, CII, and CIII of 5 rats per group. Group CI received 40mg/kg body weight of aqueous VA leaf extract; CII received 80mg/kg body weight aqueous VA leaf extract while CIII received 120mg/kg body weight of the aqueous VA leaf extract. Tween 80 was used as a vehicle for the delivery of the drug and the aqueous VA extract to the animals.

Collection of blood sample
Blood samples were collected from the media canthus of the eye by retro orbital puncture for serum preparation. Samples were taken at baseline, on day 7, 14, and 21 and the following parameters determined: blood glucose, lipid profile and body weight of the animals was also measured on the days as samples were collected

Blood glucose estimation
The blood glucose level were estimated with the tail prick method using glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostic, USA). Then blood glucose was determined using glucometer

Measurement of body weight
The weight of the rats were measured and recorded to the nearest (g) using the electronic weighing scale model no: LP505A made in China.

Estimation of serum triglyceride
It was done using the method of Assmann [41]. 5µl of the sample was pipette into a test tubes using micro-pipette. 1000µl of triglyceride reagent was added. The mixture was incubated for 10 minutes at 25°C, and the absorbance of the samples and standard against blank was recorded using spectrophotometer. Triglyceride concentration= sample/Standard × standard conc. (mmol/l).

Estimation of serum cholesterol
Serum cholesterol level was determined by the method of [42]. 5µl of Serum was pipetted into a test tube. Then, 500µl of cholesterol reagent was added into it. The mixture was incubated for 10 min at 25°C. The measurement of the absorbance of the sample and standard against reagent blank was done using spectrophotometer. Concentration of cholesterol=Sample/Standard × conc. of standard (mmol/l)

Estimation of high density lipoprotein (HDL)
HDL was determined using the method of [43]. 500µl of serum plus 500µl diluted precipitant were pipetted into test tubes and centrifuged for 10 minutes at 4000 rpm. The clear supernatant 100µl plus 1000µl HDL reagent was mixed, incubated for10 minutes at 25°C. The absorbance of the sample and standard supernatant were measured against the reagent blank using spectrophotometer. Conc. of HDL cholesterol supernatant =Sample/Standard × conc. of standard (mmol/l).

Estimation of low density lipoprotein (LDL)
LDL cholesterol = Total cholesterol – Triglyceride/5 – HDL cholesterol (mmol/l)

Data analysis
Data were analyzed using SPSS version 20. Results were expressed as mean ± SEM. One way analysis of variance (ANOVA) with post-hoc dunnette’s test was used to compare the difference between groups. The p values ≤ 0.05 was considered statistically significant.

Results
Phytochemical analysis
Quantitative screening of VA leaf extract
The quantitative screening and analysis of aqueous leaf extract of VA revealed that it contains a high percentage of saponin and alkaloid, mild percentage of tannin and low percentages of phenol, flavonoid and glycoside (Table 1).

Table 1: Percentage composition of detectable phytochemical constituent of aqueous leaf extract of VA

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloid</td>
<td>4.717</td>
</tr>
<tr>
<td>2. Saponnin</td>
<td>5.660</td>
</tr>
<tr>
<td>3. Glycoside</td>
<td>0.04</td>
</tr>
<tr>
<td>4. Flavonoid</td>
<td>0.084</td>
</tr>
<tr>
<td>5. Phenol</td>
<td>0.188</td>
</tr>
<tr>
<td>6. Tannin</td>
<td>1.703</td>
</tr>
</tbody>
</table>

Figure 1: Mean±SEM of changes in blood glucose level (mg/dl) of diabetic and non-diabetic male albino rats (Ai – Normal control (Non-diabetic without treatment); Aii – Diabetic without treatment; Aiii – Diabetic treated with Drug (Glibenclamide); B – Non-diabetic treated with 80mg/kg of VA; Ci – Diabetic treated with 40mg/kg of VA; Cii – Diabetic treated with 80mg/kg of VA; Ciii – Diabetic treated with 120mg/kg of VA)

Figure 2: Mean±SEM of change in body weight (g) of diabetic and non-diabetic male albino rats (Ai – Normal control (Non-diabetic without treatment); Aii – Diabetic without treatment; Aiii – Diabetic treated with Drug (Glibenclamide); B – Non-diabetic treated with 80mg/kg of VA; Ci – Diabetic treated with 40mg/kg of VA; Cii – Diabetic treated with 80mg/kg of VA; Ciii – Diabetic treated with 120mg/kg of VA)
Discussion

The anti-hyperglycemic effect of aqueous leaf extract of VA was evidenced in most of the treated groups. There was a significant decrease in blood glucose level on day (7 and 21) of group B (non-diabetic treated with 80mg/kg of leaf extract of VA) compared with group Ai (non-diabetic without treatment) (fig.1). A decrease in blood glucose level was also observed in group Cii (Diabetic treated with 80mg/kg of VA) when compared with groups Aii & Aiii on days (7, 14 and 21) (fig.1). Tannin has been reported to inhibit alpha-amylase, sucrose, as well as the action of SGLUT-1 of the intestinal brush border [44]. The general reduction in blood glucose level could be as a result of the combined effect of the anti-hyperglycemic and hypoglycemic effect of some of the phytochemicals constituents of aqueous leaf extract of VA suggested that antidiabetic property of extract of VA associated with its ability to enhance glucose utilization and uptake by muscles and liver cell cultures. Effective blood glucose control is the key to preventing or reversing diabetic complication and improving quality of life in patients with diabetes mellitus. Thus sustained reduction in hyperglycemia will reduce risk of developing more vascular complications [31,45-46]. Anti-hyperglycemic activity of the aqueous leaf extract of VA in this study was more effective at 80mg/kg body weight of the extract (fig. 1.)

There was a significant decrease in body weight in non-diabetic treated with VA leaf extract (group B) compared to non-diabetic without treatment (group Ai) on day 7 and 21 (fig. 2). The decrease in body weight of non-diabetic treated with aqueous leaf extract of VA compared to non-diabetic without treatment could be as a result of decrease feed intake by the animals.

There was a significant decrease in body weight among diabetic treated with VA groups (C i, ii and iii) compared to diabetic without treatment (group Ai) on day 7 and 21 (fig. 2). The decrease in body weight of non-diabetic treated with aqueous leaf extract of VA compared to non-diabetic without treatment could be as a result of decrease feed intake by the animals.

Significant decrease in body weight was observed among diabetic treated with VA groups (C i, ii and iii) compared to diabetic treated with the reference drug (fig.2.) on the 7th, 14th and 21st. In diabetes mellitus, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the
Significant decrease in serum triglyceride was observed in group Cii (diabetic treated with 40mg/kg of VA) compared to non-diabetic without treatment control (group Ai) (fig.4). Significant decrease in serum cholesterol level was observed in diabetic rats treated with (40mg/kg) of aqueous leaf extract of VA (group Ci) on day 21st compared to the diabetic treated with the reference drug (5mg/kg of glibenclamide) and diabetic without treatment (group Aii) (fig.4). The level of serum cholesterol has been reported to be reduced on diabetic rats treated with aqueous leaf extract of VA [49,17]. Excess LDL-cholesterol could be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions [24]. Reduction in elevated level of cholesterol that could improve renal and hepatic functions has been reported to be consistent with earlier report on hepatoprotective potentials of leaf extract of VA in mice [15].

There was a significant increase in serum HDL on day 7 of the experiment in group B compared to group (Ai) (fig.5). Significant increase in serum high density lipoprotein (HDL) was also observed on day 7 and 21 in diabetic rats treated with 80mg/kg of aqueous leaf extract of VA (group Cii) compared to diabetic treated with the reference drug and diabetic without treatment(fig.5). This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of VA in mice [14]. In this study, the use of 80mg/kg dosage numerically increased the HDL-Cholesterol on day 7 and 21 of the experimental animals compared to diabetic treated with the reference drug and diabetic without treatment. One of the important risk factors for cardiovascular disease (CVD) includes a low level HDL-Cholesterol. The association between a low level of HDL-cholesterol and an increased risk of CVD has been established through epidemiological and clinical studies [50]. The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways [51]. HDL exerts part of its anti-atherogenic effect by counteracting LDL oxidation and recent studies also showed that HDL promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL [52]. In this study, the aqueous leaf extract of VA may probably have played the anti-atherogenic role through the elevation of HDL cholesterol.

Significant reduction of serum LDL was observed in group B (non-diabetic treated with 80mg/kg of aqueous leaf extract of VA) when compared to group Ai (non-diabetic without treatment) on day 7 of the experiment (fig.6).

There was also significant decreases in serum LDL level of diabetic treated with VA (group Ci to C iii ) compared to diabetic treated with the reference drug (group A iii) and diabetic without treatment(group A ii) on day 7 (fig.6). Plasma LDL-cholesterol level may be used in monitoring the treatment of patients with elevated blood cholesterol levels [24]. This result is in line with the finding of Imafidon and Okunrobo (2012) where they observed that VA extract reduced total blood cholesterol levels revealing a hypocholesterolemic tendency. It is now widely believed that an important signal for insulin secretion may be the link between glucose and lipid metabolism; and long-term exposure of islet cells to high levels of fatty acids result in β-cell dysfunction (lipotoxicity) [53,54].

**Conclusion**

The findings from this study showed that aqueous leaf extract of VA possesses hypoglycemic, anti-hyperglycemic and lipid lowering activities, with 80 mg/kg body weight dosage showing the most potent dose at which aqueous leaf extract of VA demonstrated highest activity. Since dyslipidaemia occurs in most diabetic patients, the utilization of lipid-lowering agents is now advocated for diabetic treatment and the findings from this study suggests that aqueous leaf extract of VA could also be useful in this regard complementing its blood glucose lowering capacity. The results of this study therefore justify the ethnomedicinal use of VA leaves in treatment of diabetes, though further work is required to optimize the extract for extrapolation to humans.

**Conflict of interest**

There was no conflict of interest with this work.

**References**


47. UKPDS (UK Prospective Diabetes Study Group),. Intensive blood glucose with sulphonyl areas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). Lancet, 353: 837-853.


