Anti-hyperglycaemic and anti-hyperlipidemic effect of aqueous leaf extract of *Vernonia amygdalina* in Wistar rats

Ibegbu, Madu D.¹, Nnaemeka, Emmanuel J.², Ikele, Ikenna T.³ and Nwachukwu, Daniel C.²

¹Department of Medical Biochemistry, College of Medicine, University of Nigeria Enugu Campus (UNEC) Enugu, Nigeria.
²Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus (UNEC) Enugu, Nigeria.
³Department of Anatomy, College of Medicine, University of Nigeria Enugu Campus (UNEC) Enugu, Nigeria.

Received 28 March, 2018; Accepted 14 May, 2018

Diabetes mellitus is a complex metabolic disorder characterised by impaired glucose tolerance and hyperglycemia, which is 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 mellitus 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 (40, 80 and 120 mg/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 7th, 14th and 21st days; 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 anti-hyperglycemic and lipid lowering activities, with 80 mg/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.

**Key words:** *Vernonia amygdalina*, diabetes mellitus, blood glucose, anti-hyperglycaemic, anti-hyperlipidemic.

**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 (Saltiel and Kahn, 2001). This disease has been reckoned as one of leading health problems in Africa, which contributes significantly to morbidity and mortality (Garcia et al., 1974) and adversely affecting both quality and length of life. The prevalence of diabetes mellitus in
Nigeria has been reported to have increased from 2.2% in 1997 to 5.0% by 2013 (Oputa and Chinenye, 2015), diabetes mellitus is amongst the leading cause of mortality in Africa (Hall et al., 2011). Unlike in Africa, diabetes mellitus prevalence in India has reached a pandemic level, with number of diabetic patients reaching over 62 million (Kaveeshwar and Cornwall, 2014) and reflecting the global burden of the disease. Various types of this disease have been identified (National Diabetes Data Group, 1979) and some prevention and treatment measures have been recommended (Oputa and Chinenye, 2015; Pan et al., 1997).

The chronic hyperglycemia that results in this ailment is associated with many abnormalities in various organs of the body (Pignone et al., 2009). Some studies have shown that lipid profile is also altered in diabetics (Nakhjavani et al., 2004), and this dyslipidemia predisposes the diabetic patients to cardiovascular complications (Barrett-Connor, 1983; Rader, 2007). Most diabetic patients especially in Nigeria are grossly faced with inadequate medicine, and cost of managing the disease is high. In addition, the uses of available antidiabetic drugs like metformin have several side effects, which compounds the existing problems faced by health care-givers (Robertson, 1995). The rise in prevalence of diabetes mellitus 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.

Vernonia amygdalina (VA) is a perennial shrub commonly known as bitter leaf, which belongs to the family of Asteraceae (Iwalokun et al., 2006). In ethnomedicine, V. amygdalina leaves are consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for treatment of various illnesses (Igile et al., 1995). In North America, all the known 17 species of Vernonia have been shown to possess properties like blood purifier, uterus toner, and also ability to prevent atherosclerosis (Erasto et al., 2007; Nwanjo, 2005). In herbal medicinal practice, aqueous leaf extract of V. amygdalina 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 V. amygdalina as possessing anti-pathogenic and other beneficial medicinal effects; for example, leaf extract of V. amygdalina has been shown to suppress, delay or kill cancer cells, possess anti-fungal, anti-plasmodia, anti-bacterial (Kupchan et al., 1969; Ijeh et al., 1996; Akinpelu, 1999; Jisaka et al., 1993); antioxidant (Torel et al., 1986; Igile et al., 1994; Iwalokun et al., 2006; Adaramoye et al., 2008; Owolabi et al., 2008), hepato and nephron-protective effects (Ijeh and Obidio, 2004; Iwalokun et al., 2006). Bioactive peptide of aqueous leaf extract of V. amygdalina is a potent anti-cancer agent (Izevbegie, 2003; Izevbegie et al., 2004). Dietary incorporation of V. amygdalina has been reported to lower serum triacylglycerol and LDL level, normalize cholesterol concentration and concomitantly increased HDL (Ugwu et al., 2010; Nwanjo, 2005) while ethanolic leaf extract of V. amygdalina has been reported to keep the lipid profile of rats in normal range (Ekpo et al., 2007). Lowering of blood sugar has also been reported by Akah et al., (2004). The utilization of herbal extracts to treat diabetes related illness has therefore increased over the years (Rates, 2001). According to WHO (1993), 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. Considering the use of V. amygdalina leaves in ethnomedicine, this study further investigated experimentally, the capability of aqueous leaf extract of V. amygdalina in treating of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Fresh leaves of V. amygdalina were purchased from Kenyatta Market in Uwani, Enugu State, Nigeria. They were identified and authenticated by Mr. Onyeukwu Ojiokwe 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 Akah et al. (1992). 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 1200 g of the V. amygdalina leaf powder and boiled for 30 min under reflux at 80°C and then allowed to cool for 20 min. Thereafter, 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

To determine alkaloid content, 5 g of the sample was weighed into a 250-mL beaker and 200-mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. 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 (Harbone, 1973).

Flavonoid determination

To determine flavonoid content, 10 g of the sample was treated with 100 mL 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; thereafter, it was weighed until constant weight was obtained (Boham and Kocipai-Abyazan, 1994).

**Tannin determination**

To determine tannin content, 500 mg of the sample was weighed into a 50-mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50-mL volumetric flask and made up to 50 mL with distilled water. 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M K₃[Fe(CN)₆]·3 H₂O. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1998).

**Glycoside determination**

The method of El-Olemy et al. (1994) was used. 1 g of the V. amygdalina powder was soaked in 10 mL of 70% alcohol for 2 h and then filtered. The extract obtained was then purified using lead acetate and Na₃H₂PO₄ solution before addition of freshly prepared Baljet reagent (containing 95 mL aqueous 1% picric acid + 5 mL 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 Obadoni and Ochuko, (2001). 20 g of the V. amygdalina leaf powder was added into a conical flask and 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 mL of 20% ethanol. The mixture of the extracts was reduced to 40 mL over water bath at 90°C. The concentrate was transferred into a 250-mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously; also, the aqueous layer was recovered while the ether discarded. The purification process was repeated and 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL 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**

To determine the phenolic compound, 10 g of the extract was weighed and dissolved in 100 mL of distilled water, with 1 mL of this solution transferred to a test tube; thereafter, 0.5 mL of the Folin-Ciocalteu reagent and 1.5 mL (20% of Na₂CO₃ solution) was added and the volume made up to 8 mL with distilled water followed by vigorous shaking and finally allowed to stand for 2 h after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from vigorous diluted concentrations of gallic acid (Kahkonen et al., 1999).

**Experimental animals**

Thirty-five (35) male albino Wistar rats weighing 175-285 g was used for the study. The rats were purchased from the Animal House of Department of Pharmacology, University of Nigeria Enugu Campus (UNEC) and were housed in a clean ventilated wire mesh cages, at room temperature. Here, food and water were given ad libitum, with 12 h dark and 12 h light cycle also observed. Animals were used according to the animal welfare regulation of the institution.

**Induction of diabetes**

Diabetes was induced using the method of Imaga et al., (2013). The rats were fasted overnight at least 10 h after which, 10% alloxan solution diluted in normal saline was administered to the rats intraperitoneally; and were allowed access to food and water 30 min after alloxan administration. The rats were confirmed diabetic after 24 h 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 Akah et al. (1992).

- **Group A**: The control group; 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 5 mg/kg body weight of Glibenclamide (a standard anti-diabetic drug) orally.
- **Group B**: Non-diabetic that received 80 mg/kg of aqueous leaf extract of V. amygdalina.
- **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 40 mg/kg body weight of aqueous leaf extract of V. amygdalina.
  - **Group Cii**: received 80 mg/kg body weight of aqueous leaf extract of V. amygdalina.
  - **Group Ciii**: received 120 mg/kg body weight of the aqueous leaf extract of V. amygdalina.

Tween 80 was used as a vehicle for the delivery of the drug and the aqueous leaf extract of VA to the animals. The extract was administered orally for 21 days before sacrifice.

**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 and lipid; body weight of the animals was also measured on the days as samples were collected.

**Blood glucose estimation**

The blood glucose levels were estimated with the tail prick method using glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostic, USA). Thereafter, blood glucose was determined using glucometer.
Measurement of body weight

The weight of the rats were measured and recorded to the nearest gram (g) using the electronic weighing scale Model No.: LP505A made in China.

Estimation of serum triglyceride

It was done using the method of McGowan et al. (1983). 5 µL of the sample was pipetted into test tubes using micro pipette. 1000 µL of triglyceride reagent was added. The mixture was incubated for 10 min at 25°C, and the absorbance of the samples and standard against blank was recorded using spectrophotometer.

Triglyceride concentration = Sample/Standard x Standard Concentration (mmol/L)

Estimation of serum cholesterol

Serum cholesterol level was determined by the method of Allain, (1974). 5 µL of serum was pipetted into a test tube. Thereafter, 500 µL of cholesterol reagent was then added, 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 x Concentration of Standard (mmol/L)

Estimation of high density lipoprotein (HDL)

HDL was determined using the method of Assmann, (1984). 500 µL of serum plus 500 µL diluted precipitant were pipetted into tubes and centrifuged for 10 min at 4000 rpm. The clear supernatant (100 µL) plus 1000 µL HDL reagent was mixed and incubated for 10 min at 25°C. The absorbance of the sample and standard supernatant was measured against the reagent blank using spectrophotometer.

Concentration of HDL Cholesterol Supernatant = Sample/Standard x Concentration 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 Dunnett’s test was used to compare the difference between groups. The p values ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Quantitative screening and analysis of aqueous leaf extract of V. amygdalina revealed that it contains a high percentage of saponin and alkaloid, mild percentage of tannin and few percentages of phenol, flavonoid and glycoside (Table 1).

The anti-hyperglycemic effect of aqueous leaf extract of V. amygdalina 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 80 mg/kg of leaf extract of V. amygdalina) compared with Group Ai (non-diabetic without treatment) (Table 2). A decrease in blood glucose level was also observed in Group Cii (Diabetic treated with 80 mg/kg body weight of aqueous leaf extract of V. amygdalina) when compared with Groups Ai and Aii on days (7, 14 and 21) (Table 2). Tannin has been reported to inhibit alpha-amylase, sucrase, as well as the action of SGLUT-1 of the intestinal brush border (Tiwari and Rao, 2002) and some other enzymes (Zheng et al., 2009; Oprea et al., 2008).

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 V. amygdalina. The phytochemical constituents responsible for this effect is yet unknown (Akah et al., 2004; Akah and Okafor, 1992); however, Erasto et al., (2009) suggested that antidiabetic property of extract of V. amygdalina could be associated with its ability to enhance glucose utilization and uptake by muscles and liver cells 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 (Muniappan et al., 2004). Anti-hyperglycemic activity of the aqueous leaf extract of

<table>
<thead>
<tr>
<th>S/N</th>
<th>Constituent</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>4.717</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>5.660</td>
</tr>
<tr>
<td>3</td>
<td>Glycoside</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoid</td>
<td>0.084</td>
</tr>
<tr>
<td>5</td>
<td>Phenol</td>
<td>0.188</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>1.703</td>
</tr>
</tbody>
</table>

Table 1. Percentage composition of detectable phytochemical constituent of aqueous leaf extract of VA.
V. amygdalina in this study was more effective at 80 mg/kg body weight of the extract (Table 2). Mechanism of action of leaf extract of V. amygdalina is not clearly understood (Akah and Okafor, 1992); thus, a devoted study to understand its mechanism of action is required.

There was a significant decrease in body weight in non-diabetic treated with aqueous leaf extract of V. amygdalina (Group B) compared to non-diabetic without treatment (Group Ai) on Day 7 and 21 as shown in Table 3. The decrease in body weight of non-diabetic treated with V. amygdalina compared to non-diabetic without treatment could be as a result of decreased feed intake by the animals. Phytochemical tannin could be responsible for weight loss because it affects nutrient utilization.

Significant decrease in body weight was observed among diabetic treated with V. amygdalina groups (Ci, Cii and Ciii) compared to diabetic treated with the reference drug as shown in Table 3 on the 7th, 14th and 21st days. In diabetes mellitus, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst centre on the brain and polydipsia which leads to increase in water intake. The catabolic effects then prevailed, resulting in weight loss (UK Prospective Diabetes Study Group (UKPDS), 1998).

Significant decrease in serum triglyceride was observed in Group Ci (diabetic treated with 40 mg/kg of aqueous leaf extract of V. amygdalina) and Cii (diabetic treated with 80 mg/kg body weight aqueous leaf extract of V. amygdalina) compared with diabetic treated with glibenclamide (Group Ai) and diabetic treated with the reference drug (Table 4). Adequate treatment of diabetes dyslipidaemia through diet is critical in reducing risk and complications, and the role of medicinal plants in the treatment of diabetes is an emerging important therapeutic approach. Aqueous leaf extract of V. amygdalina has been reported to possess anti-hypertriglyceridemic and hypolipidemic effects in alloxan induced diabetic model (Aka et al., 2004). Improved glycemic control following V. amygdalina therapy has been shown to decrease VDL and total triglyceride levels (Huupponen et al., 1984).

There was no significant decrease in serum cholesterol level of non-diabetic treated with aqueous leaf extract of V. amygdalina (Group B) compared to non-diabetic without treatment (control group) (Group Ai) (Table 5). Significant decrease in serum cholesterol level was observed in diabetic rats treated with (40 mg/kg) of aqueous leaf extract of V. amygdalina (Group Ci) on Day 21 compared to the diabetic treated with the reference drug (5 mg/kg body weight of glibenclamide) and diabetic without treatment (Group Ai) (Table 5). The level of serum cholesterol has been reported to be reduced on

### Table 2. Mean±SEM of changes in blood glucose level (mg/dL) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>121</td>
<td>600</td>
<td>339</td>
<td>115</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Day (7)</td>
<td>99</td>
<td>101</td>
<td>104</td>
<td>87</td>
<td>539</td>
<td>83</td>
<td>446</td>
</tr>
<tr>
<td>Day (14)</td>
<td>87</td>
<td>319</td>
<td>193</td>
<td>92</td>
<td>600</td>
<td>142</td>
<td>575</td>
</tr>
<tr>
<td>Day (21)</td>
<td>146</td>
<td>254</td>
<td>131</td>
<td>99</td>
<td>445</td>
<td>101</td>
<td>500</td>
</tr>
<tr>
<td>Mean</td>
<td>113.25</td>
<td>318.5</td>
<td>191.75</td>
<td>98.25</td>
<td>546</td>
<td>231.5</td>
<td>530.25</td>
</tr>
<tr>
<td>Std. error</td>
<td>12.99</td>
<td>104.37</td>
<td>52.50</td>
<td>6.10</td>
<td>36.61</td>
<td>123.45</td>
<td>35.21</td>
</tr>
</tbody>
</table>

Ai, Normal control (Non-diabetic without treatment); Aii, Diabetic without treatment; Aiii, Diabetic treated with drug (Glibenclamide); B, Non-diabetic treated with 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.

### Table 3. Mean±SEM of changes in body weight (g) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>265.53</td>
<td>190.09</td>
<td>242.83</td>
<td>261.69</td>
<td>173.27</td>
<td>203.82</td>
<td>202.53</td>
</tr>
<tr>
<td>Day (7)</td>
<td>297</td>
<td>188.5</td>
<td>259</td>
<td>287</td>
<td>151</td>
<td>210</td>
<td>192.5</td>
</tr>
<tr>
<td>Day (14)</td>
<td>316.31</td>
<td>199.36</td>
<td>262.56</td>
<td>316.62</td>
<td>163.63</td>
<td>201.45</td>
<td>192.85</td>
</tr>
<tr>
<td>Day (21)</td>
<td>335.03</td>
<td>200.22</td>
<td>264.27</td>
<td>277.66</td>
<td>153.98</td>
<td>200.55</td>
<td>199.06</td>
</tr>
<tr>
<td>Mean</td>
<td>303.4675</td>
<td>194.5425</td>
<td>257.165</td>
<td>285.7425</td>
<td>160.47</td>
<td>203.955</td>
<td>196.735</td>
</tr>
<tr>
<td>Std. error</td>
<td>14.83859</td>
<td>3.05203</td>
<td>4.90278</td>
<td>11.54286</td>
<td>5.04676</td>
<td>2.13386</td>
<td>2.44976</td>
</tr>
</tbody>
</table>

Ai, Normal control (Non-diabetic without treatment); Aii, Diabetic without treatment; Aiii, Diabetic treated with drug (Glibenclamide); B, Non-diabetic treated with 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.
Table 4. Mean±SEM of changes in serum triglyceride level (Mmol/L) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>3.55</td>
<td>4.02</td>
<td>3.64</td>
<td>4.09</td>
<td>4.81</td>
<td>4.35</td>
<td>3.26</td>
</tr>
<tr>
<td>Day (7)</td>
<td>4.19</td>
<td>3.93</td>
<td>4.38</td>
<td>4.17</td>
<td>4.34</td>
<td>4.33</td>
<td>3.17</td>
</tr>
<tr>
<td>Day (14)</td>
<td>3.89</td>
<td>4.09</td>
<td>4.09</td>
<td>4.03</td>
<td>4.15</td>
<td>3.99</td>
<td>4.11</td>
</tr>
<tr>
<td>Day (21)</td>
<td>3.6</td>
<td>4.14</td>
<td>4.28</td>
<td>4.31</td>
<td>2.04</td>
<td>2.6</td>
<td>4.41</td>
</tr>
<tr>
<td>Mean</td>
<td>3.8075</td>
<td>4.045</td>
<td>4.0975</td>
<td>4.15</td>
<td>3.835</td>
<td>3.8175</td>
<td>3.7375</td>
</tr>
<tr>
<td>Std. error</td>
<td>0.14735</td>
<td>0.04436</td>
<td>0.1644</td>
<td>0.05962</td>
<td>0.61319</td>
<td>0.41486</td>
<td>0.30751</td>
</tr>
</tbody>
</table>

Ai, Normal control (Non-diabetic without treatment); Aii, Diabetic without treatment; Aiii, Diabetic treated with drug (Glibenclamide); B, Non-diabetic treated with 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.

Table 5. Mean±SEM of changes in serum cholesterol level (Mmol/L) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>9.88</td>
<td>9.49</td>
<td>7.73</td>
<td>9.18</td>
<td>7.28</td>
<td>9.36</td>
<td>8.52</td>
</tr>
<tr>
<td>Day (7)</td>
<td>9.85</td>
<td>9.54</td>
<td>9.71</td>
<td>8.25</td>
<td>8.3</td>
<td>9.11</td>
<td>6.5</td>
</tr>
<tr>
<td>Day (14)</td>
<td>5.81</td>
<td>6.16</td>
<td>6.18</td>
<td>6</td>
<td>6.04</td>
<td>6.2</td>
<td>6.27</td>
</tr>
<tr>
<td>Day (21)</td>
<td>9.31</td>
<td>10</td>
<td>10.11</td>
<td>9.1</td>
<td>5.31</td>
<td>10.09</td>
<td>10.11</td>
</tr>
<tr>
<td>Mean</td>
<td>8.71</td>
<td>8.80</td>
<td>8.43</td>
<td>8.13</td>
<td>6.73</td>
<td>8.69</td>
<td>7.85</td>
</tr>
<tr>
<td>Std. error</td>
<td>1.01</td>
<td>0.89</td>
<td>0.91</td>
<td>0.86</td>
<td>0.66</td>
<td>0.85</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Ai, Normal control (Non-diabetic without treatment); Aii, Diabetic without treatment; Aiii, Diabetic treated with drug (Glibenclamide); B, Non-diabetic treated with 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.

Table 6. Mean±SEM of changes in serum HDL level (Mmol/L) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>4.16</td>
<td>4.92</td>
<td>3.1</td>
<td>2.93</td>
<td>4.17</td>
<td>0.84</td>
<td>1.87</td>
</tr>
<tr>
<td>Day (7)</td>
<td>4</td>
<td>4.05</td>
<td>4.2</td>
<td>4.21</td>
<td>3.74</td>
<td>4.36</td>
<td>3.73</td>
</tr>
<tr>
<td>Day (14)</td>
<td>4.6</td>
<td>4.69</td>
<td>3.53</td>
<td>3.41</td>
<td>0.99</td>
<td>0.52</td>
<td>1.95</td>
</tr>
<tr>
<td>Day (21)</td>
<td>5.14</td>
<td>2.7</td>
<td>4.52</td>
<td>4.71</td>
<td>5.12</td>
<td>5.1</td>
<td>1.95</td>
</tr>
<tr>
<td>Mean</td>
<td>4.48</td>
<td>4.09</td>
<td>3.84</td>
<td>3.82</td>
<td>3.51</td>
<td>2.71</td>
<td>2.38</td>
</tr>
<tr>
<td>Std. error</td>
<td>0.25</td>
<td>0.51</td>
<td>0.32</td>
<td>0.39</td>
<td>0.88</td>
<td>1.17</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Mean±SEM of changes in serum high density lipoprotein (HDL) level (Mmol/L) 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 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.

diabetic rats treated with aqueous leaf extract of *V. amygdalina* (Gonzalez and Feyver, 1992, Nwanjo, 2007). Excess LDL-cholesterol could be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions (Adaramoye et al., 2008). Reduction in elevated level of cholesterol could improve renal and hepatic functions. An earlier report by Iwalokun et al., (2006) has shown hepatoprotective potentials of leaf extract of *V. amygdalina* in mice.

Significant increase in serum HDL on the 7th day of the experiment was observed in Group B compared to Group Ai (Table 6). Similar observation was made in serum high density lipoprotein (HDL) on 7th and 21st days in diabetic rats treated with 80 mg/kg of aqueous leaf extract of *V. amygdalina* (Group Cii) compared to diabetic treated with the reference drug and diabetic without treatment (Table 6). This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun et al., 2006). In this study, the use of 80 mg/kg body weight dosage numerically increased the HDL-Cholesterol on 7th and 21st days 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
Table 7. Mean±SEM of changes in serum LDL level (Mmol/L) of the experimental animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>Ai</th>
<th>Aii</th>
<th>Aiii</th>
<th>B</th>
<th>Ci</th>
<th>Cii</th>
<th>Ciii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day (0)</td>
<td>3.91</td>
<td>4.82</td>
<td>2.29</td>
<td>4.38</td>
<td>0.92</td>
<td>6.51</td>
<td>4.63</td>
</tr>
<tr>
<td>Day (7)</td>
<td>3.74</td>
<td>3.68</td>
<td>3.48</td>
<td>2.51</td>
<td>2.59</td>
<td>2.78</td>
<td>1.3</td>
</tr>
<tr>
<td>Day (14)</td>
<td>2.23</td>
<td>0.68</td>
<td>0.79</td>
<td>0.76</td>
<td>3.16</td>
<td>4.76</td>
<td>0.53</td>
</tr>
<tr>
<td>Day (21)</td>
<td>0.15</td>
<td>0.06</td>
<td>0.2</td>
<td>0.04</td>
<td>0.12</td>
<td>0.13</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean</td>
<td>2.51</td>
<td>2.31</td>
<td>1.69</td>
<td>1.92</td>
<td>1.70</td>
<td>3.55</td>
<td>1.67</td>
</tr>
<tr>
<td>Std. error</td>
<td>0.87</td>
<td>1.15</td>
<td>0.74</td>
<td>0.97</td>
<td>0.71</td>
<td>1.37</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Ai, Normal control (Non-diabetic without treatment); Aii, Diabetic without treatment; Aiii, Diabetic treated with drug (Glibenclamide); B, Non-diabetic treated with 80 mg/kg of VA; Ci, Diabetic treated with 40 mg/kg of VA; Cii, Diabetic treated with 80 mg/kg of VA; Ciii, Diabetic treated with 120 mg/kg of VA.

and an increased risk of CVD has been established through epidemiological and clinical studies (Assmann and Gotto, 2004). The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways (Nofer et al., 2002). 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 (Yokozawa et al., 2006). Furthermore, 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 80 mg/kg body weight of aqueous leaf extract of *V. amygdalina*) when compared to Group Ai (non-diabetic without treatment) on Day 7 of the experiment (Table 7).

There was also significant decreases in serum (LDL) level of diabetic treated with *V. amygdalina* (Group Ci to Ciii) compared to diabetic treated with the reference drug (Group Aii) and diabetic without treatment (Group Aii) on 7th day (Table 7). Plasma LDL-cholesterol level may be used in monitoring the treatment of patients with elevated blood cholesterol levels (Adaramoye et al., 2008). This result is in line with the finding of Imafidon and Okunrobo, (2012) where they observed that *V. amygdalina* extract reduced total blood cholesterol 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) (Krolewski et al., 1994).

### Conclusion

Aqueous leaf extract of *V. amygdalina* 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 *V. amygdalina* 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 suggest that aqueous leaf extract of *V. amygdalina* could also be useful in this regard complementing its blood glucose lowering capacity. The results, therefore, justify the ethnomedicinal use of *V. amygdalina* leaves in treatment of diabetes mellitus, though further work is required to optimize the extract for extrapolation to humans, and understand its mechanism of action in enhancing positive effect in diabetes mellitus treatment.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### REFERENCES


Boham BA, Kocjiap-Abyazan R (1994). Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticum and *V.


