Full Length Research Paper

Studies of the anti-hyperglycemic and antioxidant activities of the extract of aerial yam (Dioscorea bulbifera)

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This study investigated the anti-hyperglycemic and antioxidant activities of methanol extract of Dioscorea bulbifera with a view to studying the possibility of utilizing the yam in the management of hyperglycemia. Methanol extracts of the peels (MEPL), fresh (MEFL), and the whole tuber (MEWT) were prepared, phytochemically screened and assayed for α-amylase. Hyperglycemia was induced in rats using intraperitoneal injections of streptozotocin (40 mg/kg bwt) for five days, followed by administration of various doses of extract. Fasting blood sugar, alpha amylase, total protein, lipid profiles, creatinine, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in plasma of test rats. The extracts were found to contain alkaloids, cardiac glycosides, carbohydrate, flavonoids, protein, saponins, tannins, triterpenoids and vitamin C and E. Preliminary assessment revealed that the whole tuber (MEWT) contained the highest levels of these phytochemicals and exhibited highest α-amylase inhibitory potential (47.54%, IC50 169.83 ± 18.64 μg/ml). Also, the extract possessed potent and concentration-dependent radical scavenging activities, comparable to that of the standard (Vitamin C). The extract also inhibited alpha amylase activity and caused significant improvement in elevated lipid profile and creatinine, comparable with that brought about by the standard drug Metformin. Histological examination of pancreatic section showed improved arrangement of islets of Langerhans in exocrine tissues of hyperglycemic groups treated with the extract when compared with hyperglycemic control. These results suggest that methanol extract of whole tubers possesses potent significant anti-hyperglycemic activity and that whole yam (without peeling) is appropriate for use in the management of hyperglycemia disorders.

Key words: Dioscorea bulbifera, methanol extract, Streptozotozin, hyperglycemia, diabetes mellitus, antioxidants, lipid profile.

INTRODUCTION

The incidence of chronic diseases including diabetes mellitus is on the increase globally with attendant socioeconomic and disease burden. Diabetes occurs when the body does not produce or cannot effectively utilize insulin, resulting in high glucose plasma level (hyperglycemia) and causing tissue damage over time.
Consumption of plants to reverse hyperglycemia has been practiced by herbalists/traditionalists for a long time (Rego et al., 2014). This is because such plants have been found to contain various phytonutrients that often possess and exhibit anti-hyperglycemic effects (Kooti et al., 2016). Such effects are often due to their ability to improve the performance of pancreatic tissue by increasing insulin secretion or reducing the intestinal absorption of glucose (Kooti et al., 2016).

*Dioscorea bulbifera* (L.) of the family Dioscoreaceae, commonly known as “aerial yam” is well cultivated in West Africa and Asia countries (Chinko et al., 2016). In West African traditional medicine, *D. bulbifera* is employed in the treatment of cough, fever, piles, dysentery, ulcers, asthma, diabetes, leprosy, and cancer (Ikiriza et al., 2019). The present study was undertaken to ascertain possible or likely antioxidant and anti-hyperglycemic activities of methanol extract of *D. bulbifera* tubers with a view to studying the possibility of utilizing the plant in the treatment and management of hyperglycemic disorders.

**MATERIALS AND METHODS**

**Plant materials**

Fresh tubers of *D. bulbifera* were collected between August and November 2018 from a location in Ajebamidele Area, Ile-Ife, Nigeria (7° 29’37” N and 4° 30’8” E), identified and authenticated at Forestry Research Institute of Nigeria, Forestry Hill, Jericho, Ibadan, where a specimen copy was deposited and voucher number (FHI 112569) obtained. The methanol extract of *D. bulbifera* was prepared according to a modified method of Oyedapo and Amos (1997). Briefly, powdered plant material flesh (198 g), peels (141 g) and whole tuber (180 g) were separately soaked in 80% (v/v) methanol at room temperature for three days and stirred daily. The suspensions were filtered through double-layered cotton cloth. The residues were re-extracted with same solvent 80% (v/v) methanol until the filtrate became colourless. Filtrates were then concentrated in vacuo to dryness under reduced pressure at 40°C on Buchi (Switzerland) Vacuum Pump V-700 Rotary Evaporator to yield methanol extracts of flesh, peels and whole tuber and kept in sterile petri dishes at 4°C for further analysis.

**Phytochemical screening**

The extract was screened for the presence of alkaloids, cardiac glycosides, carbohydrates, flavonoids, saponins, tannins, terpenoids using standard procedures (Oyedapo et al., 1999; Tiwari et al., 2011 and Ahmed et al., 2020).

**Estimation of total phenolic, total flavonoid and tannin contents**

The total phenolic contents of the extracts were determined using Folin-Ciocalteu’s Phenol reagent reaction method according to the spectrophotometric method of Singleton et al. (1999) with tannic acid as standard. The estimation of the flavonoid content was carried out according to the spectrophotometric method of Sun et al. (1999) based on the formation of aluminum-flavonoid yellow complex with quercetin as standard. The estimation of tannin concentration was carried out according to the method of Makkar and Goodchild (1996) with Folin-Ciocalteu’s Phenol Reagent.

**Determination of vitamins C and E concentration**

The determination of concentrations of vitamin C in the extracts was carried out according to a procedure that was based on the earlier methods of Omaye et al. (1979) and Japota and Dani (1982) with ascorbic acid as standard while the concentration of vitamin E was carried out according to the method of Baker and Frank (1968) and Santhosh et al. (2013) with vitamin E as standard. Concentrations were extrapolated from the standard calibration curve and expressed as milligram per gram of extracts (mg/g extract).

**DPPH-radical scavenging and ferric reducing power activity**

DPPH-radical scavenging activity of the extracts of whole tuber, flesh, and peels was carried out using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to modified method of Blois (1985) and with ascorbic acid as standard while ferric reducing power activity was carried out according to the procedure of Oyaizu (1986) as modified and reported by Aina and Oyedapo (2013). Standard ascorbic acid of varying concentrations (0.250 μg/ml) were used to compare with the activities of the extracts.

**In vitro evaluation of alpha- amylase inhibition**

Assay of Alpha-amylose inhibition was carried out according to the method of Nickavar et al. (2008). Varying volumes 0 - 1.0 ml of the methanol extracts or standard (Acarbose) were measured into test tubes. The volumes in each test tube was adjusted to 1 ml with distilled water and gently mixed with Alpha-amylose solution (1 ml, 10 μg/ml) and incubated at room temperature for 30 min. Then, 1 ml of starch solution was added to the mixture and incubated further at 37°C for 3 min. The reaction was terminated with the addition of 1 ml of colour reagent and further incubated for 15 min at 85°C. Absorbance was read at 540 nm against reagent blank. The inhibition percentage of α-amylose was calculated using the expression:

\[
\% \text{ inhibition of } \alpha-\text{amylose} = \frac{\Delta A_{\text{sample}} - \Delta A_{\text{control}}}{\Delta A_{\text{control}}} \times 100
\]

Where \( \Delta A_{\text{sample}} \) and \( \Delta A_{\text{control}} \) are change of absorbance of control and sample respectively.

**Experimental animals**

Thirty albino rats purchased from the animal house of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife were housed in plastic cages and maintained under standard conditions.

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and fed with standard rat chow and water *ad libitum*. All animals were acclimatized for three weeks before the study.

**Induction of hyperglycemia in rats with streptozotocin (STZ)**

Hyperglycemia was induced by multiple, low-dose intraperitoneal injections of streptozotocin (40 mg/kg bwt) for five days (Fuman, 2015). STZ (40 mg/kg bwt) was dissolved in 0.1 M sodium citrate buffer (0.5 ml/kg bwt pH 4.5) and injected immediately to twenty of the rats within 5 min of dissolution to avoid degradation. Hyperglycemia (blood glucose level of 180 mg/d L and above) was confirmed in animals after 72 h of streptozotocin injection using a digital glucometer (Accu-chek, Roche Diagnostic, Germany). The rats were then divided into groups 2 to 5.

**Grouping and treatment of STZ-induced hyperglycemic rats**

The experimental rats (30) were divided into six groups of five animals each and treated as follows:

- **Group 1**: Control + vehicle (sodium citrate buffer pH 4.5, 0.5 ml/kg bwt)
- **Group 2**: Hyperglycemic rats
- **Group 3**: Hyperglycemic rats + standard drug (metformin, 10 mg/kg bwt);
- **Group 4**: Hyperglycemic rats + extract (125 mg/kg bwt);
- **Group 5**: Hyperglycemic rats + extract (250 mg/kg bwt); and
- **Group 6**: Normal rats + extract (250 mg/kg bwt).

The animals were treated daily with the methanol extract of whole tuber for 21 days. The body weight and blood glucose concentration of the animals were taken on a weekly basis throughout the experimental period using a standard bathroom scale and digital glucometer respectively. On day 22, fasted animals were sacrificed under mild diethyl ether anesthesia. Blood was collected by cardiac puncture into heparinized bottles. Liver and kidney were excised, perfused in normal saline (0.85% NaCl) to remove blood, blotted on tissue paper and wrapped with aluminum foil paper. It was then kept frozen -20°C for further biochemical analyses. Also, the pancreas was surgically removed and fixed with 10% (v/v) formal-saline for histological study. Plasma was centrifuged at 3000 rpm for 10 min and the supernatant was carefully removed into plain tubes and kept frozen for biochemical analyses. Liver and kidney homogenates were prepared and used for total protein, ALT and AST analyses.

Estimation of total cholesterol, HDL-cholesterol and triacylglycerol was carried out according to the method of Richmond (1973) using Randox Diagnostic kit. Estimation of total protein content of plasma, liver and kidney homogenate was carried out using Biuret reaction method as described by Tietz (1995) with bovine serum albumin as standard while the estimation of plasma albumin concentration was carried out according to the method of Doumas et al. (1971). Plasma creatinine concentration was measured according to the method of Bartels and Bohmer (1972) while plasma Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were estimated according to method of Reitman and Frankel (1957). Commercial Randox diagnostic kits were used for these estimations.

**Assay of alpha- amylase activity**

Alpha-amylase activity was measured according to the method of Bernfeld (1951) as slightly modified by Dare et al. (2014) using soluble starch as substrate. Typically, 50 µl of soluble starch (1% w/v in 20 mM phosphate buffer, pH 6.9) and 50 µl of plasma were pipetted into clean test tubes in triplicates and incubated at room temperature for 3 min. To the mixture, 100 µl of 3,5-dinitrosalicylic acid (DNSA) colour reagent was added and heated in boiling water bath for 5 min, cooled to room temperature and the volume was adjusted to 1 ml with distilled water. The absorbance was read at 540 nm against the reagent blank. Standard calibration curve was prepared using α-D-glucose as standard. The concentration released was extrapolated from standard calibration curve.

**Histopathological studies**

The histopathological studies of the pancreases were carried out at the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife.

**Statistical analysis**

All values are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA), using Graph pad prism 5. Differences were considered to be significant at *p* < 0.05.

**RESULTS**

**Yield of extracts**

The extraction of peels (141 g) of *D. bulbifera* yielded 11% (15 g) of the starting material. The extraction of 198 g flesh of *D. bulbifera* yielded 13% (25 g) of the starting material and also whole tuber (180 g) yielded 17% (30 g) of the starting material.

**Phytochemical constituents and analysis of *D. bulbifera***

Table 1 gives a summary of the phytochemical constituents of *D. bulbifera* revealing the presence of alkaloids, cardiac glycosides, carbohydrate, flavonoids, protein, saponins, tannins and terpenoids. Whole tuber contained the highest levels of phenolic, flavonoids, tannins, and vitamin C compared with peels and fleshes of *D. bulbifera* and hence was used for all other analyses (Table 2).

**Antioxidant activities of methanol extract of *D. bulbifera***

Results reveal that the extracts exhibited potent and appreciable radical scavenging activities, with the peel being more potent. The activities were concentration dependent and comparable with that of the standard (ascorbic acid) (Figure 1). The reducing power of the methanol extracts of *D. bulbifera* was concentration dependent, with the flesh being more potent, although lower than that of the standard (ascorbic acid) as depicted in Figure 2.
Table 1. Phytochemical screening of methanol extract of *D. bulbifera*.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Peels</th>
<th>Flesh</th>
<th>Whole tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Dragendorff’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b. Mayer’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c. Wagner’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
+ Present.

Table 2. Phytochemical composition of *D. bulbifera*.

<table>
<thead>
<tr>
<th>Phytonutrient</th>
<th>Peels</th>
<th>Flesh</th>
<th>Whole tuber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic (mg TAE/g)</td>
<td>5.18 ± 0.07</td>
<td>18.01 ± 0.75</td>
<td>25.04 ± 0.47</td>
</tr>
<tr>
<td>Total flavonoids (mg QE/g)</td>
<td>5.65 ± 1.26</td>
<td>29.48 ± 0.44</td>
<td>35.54 ± 0.24</td>
</tr>
<tr>
<td>Tannin (mg TAE/g)</td>
<td>0.17 ± 0.02</td>
<td>0.33 ± 0.12</td>
<td>0.37 ± 0.13</td>
</tr>
<tr>
<td>Vitamin C (mg/g)</td>
<td>0.28 ± 0.02</td>
<td>1.43 ± 0.05</td>
<td>2.05 ± 0.04</td>
</tr>
<tr>
<td>Vitamin E (mg/g)</td>
<td>9.14 ± 0.03</td>
<td>9.57 ± 0.16</td>
<td>9.21 ± 0.14</td>
</tr>
</tbody>
</table>

Each value represents the Mean ± SEM of 3 readings, TAE- Tannic Acid Equivalent; QE- Quercetin Equivalent.

**Alpha-amylase inhibition activity**

Figure 3 gives a summary of α-amylase inhibitory activity of methanol extract of *D. bulbifera*. Results revealed that the extracts of *D. bulbifera* displayed appreciable, concentration dependent α-amylase inhibitory effect. Whole tuber extracts exhibited the highest inhibitory effect.

**Effect of extract on blood glucose level (mg/dL) in rats**

There was marked reduction in blood glucose concentration across all hyperglycemic animals treated with standard metformin (10 mg/kg bwt) and methanol extract of *D. bulbifera* (125 and 250 mg/kg bwt) (Table 3).

**Effects of methanol extract of *D. bulbifera* on plasma lipid profile, albumin, creatinine and total protein in streptozotocin induced hyperglycemic rats**

Plasma total cholesterol, triacylglycerol, LDL, VLDL cholesterol levels and creatinine were increased significantly in streptozotocin induced hyperglycemic rats compared to normal rats. In hyperglycemic rats treated with the *D. bulbifera* extract/metformin, there was significant reduction in the elevated levels of plasma triacylglycerol, total cholesterol, LDL, VLDL cholesterol and creatinine (Tables 4 and 5). Decreased HDL (Table 4), albumin and total protein (Table 5) in plasma during streptozotocin induced hyperglycemia were found to be significantly increased by methanolic extract and metformin treatment.

**Effects of methanol extract of *D. bulbifera* on plasma and liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities**

There were significant (p<0.05) increases in the activities of AST and ALT in both plasma and liver in the STZ-induced hyperglycemic rats (Group 2) when compared with the control (Group 1) as represented in Table 6. These increases significantly (p<0.05) reduced in the groups of animals treated with the extracts (Groups 4 and 5) and compared favourably with that of metformin (Group 3). There were no significant difference between the group of animals treated with the extract only (Group 6) and the control (Group 1).
Figure 1. DPPH radical scavenging activities of the ascorbic acid, peels, flesh and whole tuber of methanol extracts of *D. bulbifera*. IC₅₀ values are 8.69±0.13, 12.33±0.10, 16.58±0.05 and 7.35±0.36 for ascorbic acid, whole tuber, flesh and peel extracts respectively. Each value represents Mean ± SEM of n = 3 readings.

Figure 2. Ferric reducing antioxidant power of methanol extract of *D. bulbifera*. Each value represents Mean ± SEM of n = 3 readings.
Alpha-amylase inhibitory effects of *dioscorea bulbifera* extract

Table 7 shows significant decrease in the activities of plasma alpha-amylase in hyperglycemic rats treated with 125 mg/kg bwt of extract, 250 mg/kg bwt and standard drug Metformin (10 mg/kg bwt) when compared untreated hyperglycemic rat (Group 2).

Histological structure of pancreas in normal control, hyperglycemic control, and extract treated albino rats

From the histological examination (Plate 1) it was observed that the cells in the normal control (Group 1) and treated normal control (Group 6) animal pancreas islets showed a normal arrangement of the islets of Langerhans of various sizes densely packed. However, degenerated islet of Langerhans and loss of beta cells were detected in hyperglycemic control (Group 2). This abnormality was corrected in treated groups with extract of *D. bulbifera* (125 and 250 mg/kg) of Group 4 and 5 respectively. The standard drug (metformin) group also showed signs of recovery.

DISCUSSION

The bio-prospecting of ethno-medicinal plant as anti-hyperglycemic agents is imperative especially because most diabetic patients in developing countries find it increasingly difficult to manage hyperglycemic conditions due to high cost of synthetic drugs coupled with various side effects. Plant extracts on the other hand are safe and easily accessible. Therapeutic effects of medicinal plant extracts are due to their phytochemical constituents with varieties of biological activities (Gollo et al., 2020).

In this study, phytochemical screening of methanol extract of *D. bulbifera* revealed the presence of alkaloids, cardiac glycosides, carbohydrate, flavonoids, protein, saponin, tannins and terpenoids. This is in agreement with the observations of Okon and Ofeni (2013) who noted that tuber extract of this plant contained alkaloids, flavonoids, tannins, saponins, terpenoids, phenols and cardiac glycosides. Natural antioxidants such as flavonoids, phenolic acids, tocopherols, vitamin C and tannins found in plants (Lourenço et al., 2019) are reported to possess both potent anti-hyperglycemic and free radical scavenging activity (Ghosh et al., 2013). Ascorbic acid (vitamin C), a water soluble antioxidant scavenges harmful free radicals, reactive oxygen species (ROS) and regenerates tocopherol to its functional state (Eboh, 2014) while vitamin E is a major powerful membrane bound antioxidant employed by humans. Both were found in appreciable amounts in *D. bulbifera* extracts.

Whole tuber extracts of the plant were found to contain higher quantities of the phytonutrients than the flesh and peels. Similar observation was made by Okwu and Ndu (2006). In the present study, *D. bulbifera* extract was found to possess significant ferric reducing power which was concentration dependent, and this correlates with the
Table 3. Effect of extract on blood glucose level (mg/dL) in rats.

<table>
<thead>
<tr>
<th>Treatment duration</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>101.80±9.05</td>
<td>104.30±6.33</td>
<td>100.00±5.03</td>
<td>94.33±6.23</td>
<td>92.00±4.58</td>
<td>109.80±11.09</td>
</tr>
<tr>
<td>72 hr. after induction (Day 0)</td>
<td>100.15±7.04</td>
<td>436.10±4.05</td>
<td>406.20±11.02</td>
<td>409.45±17.01</td>
<td>382.40±21.16</td>
<td>109.83±9.41</td>
</tr>
<tr>
<td>Day 7</td>
<td>101.80±9.05</td>
<td>438.70±4.91</td>
<td>401.20±18.06</td>
<td>403.30±19.07</td>
<td>354.00±17.05</td>
<td>101.80±9.94</td>
</tr>
<tr>
<td>Day 14</td>
<td>105.80±6.56</td>
<td>403.00±19.55</td>
<td>387.30±14.33</td>
<td>399.67±17.01</td>
<td>335.30±15.01</td>
<td>99.60±7.16</td>
</tr>
<tr>
<td>Day 21</td>
<td>106.00±7.31</td>
<td>502.00±15.75</td>
<td>360.30±20.18</td>
<td>392.00±11.24</td>
<td>321.30±15.01</td>
<td>98.20±2.62</td>
</tr>
<tr>
<td>% Change blood glucose level</td>
<td>5.52</td>
<td>13.12</td>
<td>11.30</td>
<td>4.26</td>
<td>15.98</td>
<td>10.59</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5 rats. Hyperglycemic rats + Metformin (10 mg/kg bwt); Group 4: Hyperglycemic rats + extract (125 mg/kg bwt) Group 5: Hyperglycemic rats+ extract (250 mg/kg bwt); Group 6: Normal rats+ extract (250 mg/kg bwt).

Table 4. Effect of methanol extract of *D. bulbifera* on lipid profile.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>127.60 ± 4.89</td>
<td>45.30 ± 4.32</td>
<td>61.73 ± 2.15</td>
<td>56.81 ± 3.50</td>
<td>9.06 ± 0.86</td>
</tr>
<tr>
<td>2</td>
<td>181.31 ± 1.19</td>
<td>91.56 ± 12.43</td>
<td>64.95 ± 3.12</td>
<td>54.57 ± 0.05</td>
<td>15.25 ± 1.21</td>
</tr>
<tr>
<td>3</td>
<td>138.87 ± 3.90</td>
<td>76.25 ± 6.02</td>
<td>58.67 ± 1.87</td>
<td>60.27 ± 0.05</td>
<td>50.52 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>159.07 ± 3.27</td>
<td>61.88 ± 23.98</td>
<td>46.80 ± 0.18</td>
<td>54.57 ± 0.05</td>
<td>16.71 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>142.44 ± 7.98</td>
<td>51.04 ± 6.56</td>
<td>56.35 ± 6.17</td>
<td>46.93 ± 0.05</td>
<td>10.21 ± 1.31</td>
</tr>
<tr>
<td>6</td>
<td>130.51 ± 2.50*</td>
<td>48.65 ± 0.49</td>
<td>51.64 ± 1.87</td>
<td>69.14 ± 3.12</td>
<td>9.73 ± 0.13*</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of 5 readings; *P< 0.05 was considered statistically significant when compared with control (group 1); **P< 0.05 was considered statistically significant when compared with hyperglycemic group (group 2); (*) represent no significant difference when compared with the control Group 1: control rat + vehicle, Group 2: Hyperglycemic rats (HG), Group 3: HG + Metformin (10 mg/kg bwt); Group 4: HG + extract (125 mg/kg bwt) Group 5: HG + extract (250 mg/kg bwt); Group 6: Normal rats+ extract (250 mg/kg bwt). TC: Total Cholesterol; TG- Triacylglycerol; HDL-c- Hih-density Lipoprotein Cholesterol; LDL-c- Low-density Lipoprotein Cholesterol; VLDL-c- Very low-density Lipoprotein Cholesterol.

The ferric reducing power activity measures the ability of antioxidants to reduce ferric III to ferrous II.
Table 5. Effects of Methanol Extract of *D. bulbifera* on albumin, creatinine and total protein.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albumin (mg/dL)</td>
<td>Creatinine (mg/dL)</td>
<td>Total protein (mg/dL)</td>
</tr>
<tr>
<td>1</td>
<td>4.42 ± 0.23</td>
<td>0.52 ± 0.23</td>
<td>5.69 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>2.10 ± 0.38</td>
<td>2.28 ± 0.03</td>
<td>3.73 ± 0.37</td>
</tr>
<tr>
<td>3</td>
<td>(52.49%)a</td>
<td>(77.19%)a</td>
<td>(34.45%)a</td>
</tr>
<tr>
<td>4</td>
<td>2.95 ± 0.55</td>
<td>0.73 ± 0.02</td>
<td>4.97 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>(28.1%)b</td>
<td>(67.98%)b</td>
<td>(24.95%)b</td>
</tr>
<tr>
<td>6</td>
<td>2.44 ± 0.58</td>
<td>0.92 ± 0.05</td>
<td>4.71 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(13.93%)b</td>
<td>(59.65%)b</td>
<td>(20.81%)b</td>
</tr>
<tr>
<td>2</td>
<td>2.89 ± 0.14</td>
<td>0.87 ± 0.04</td>
<td>4.94 ± 0.22</td>
</tr>
<tr>
<td>3</td>
<td>(27.34%)b</td>
<td>(61.84%)b</td>
<td>(24.49%)b</td>
</tr>
<tr>
<td>4</td>
<td>3.90 ± 0.14</td>
<td>0.61 ± 0.17</td>
<td>5.34 ± 0.26</td>
</tr>
<tr>
<td>5</td>
<td>(11.77%)b</td>
<td>(14.75%)b</td>
<td>(6.15%)b</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of 5 readings; a p< 0.05 was considered statistically significant when compared with control (Group 1); b p< 0.05 was considered statistically significant when compared with hyperglycemic group (Group 2); Group 1: control rat + vehicle, Group 2: Hyperglycemic rats (HG), Group 3: HG + Metformin (10 mg/kg bwt); Group 4: HG + extract (125 mg/kg bwt) Group 5: HG + extract (250 mg/kg bwt); Group 6: Normal rats+ extract (250 mg/kg bwt)

Table 6. Effect of Methanol Extract of *D. bulbifera* on the Plasma and Liver Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) Activities in STZ-Induced Hyperglycemic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma (ALT) (U/L)</th>
<th>Liver (ALT) (U/g Liver)</th>
<th>Plasma (AST) (U/L)</th>
<th>Liver (AST) (U/g Liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.60 ± 2.40</td>
<td>141.49 ± 4.92</td>
<td>49.89 ± 4.22</td>
<td>102.63 ± 5.07</td>
</tr>
<tr>
<td>2</td>
<td>44.79 ± 1.14a (51.77%)</td>
<td>199.52 ± 3.07a (29.08%)</td>
<td>55.97 ± 2.01a (10.86%)</td>
<td>35.96 ± 12.88b (64.96%)</td>
</tr>
<tr>
<td>3</td>
<td>16.31 ± 3.32b (63.59%)</td>
<td>149.82 ± 6.22b (24.91%)</td>
<td>43.33 ± 8.66b (22.58%)</td>
<td>60.88 ± 5.62b (40.93%)</td>
</tr>
<tr>
<td>4</td>
<td>30.14 ± 6.45b (32.71%)</td>
<td>172.29 ± 29.22b (13.40%)</td>
<td>54.21 ± 7.26b (3.15%)</td>
<td>46.67 ± 5.24b (22.95%)</td>
</tr>
<tr>
<td>5</td>
<td>22.91 ± 3.70b (48.85%)</td>
<td>150.27 ± 1.46b (24.68%)</td>
<td>48.42 ± 3.08b (13.49%)</td>
<td>59.68 ± 5.01b (39.74%)</td>
</tr>
<tr>
<td>6</td>
<td>21.94 ± 5.77 (1.55%)</td>
<td>146.06 ± 6.97 (3.13%)</td>
<td>20.42 ± 8.04 (59.07%)</td>
<td>61.05 ± 6.02 (40.51%)</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of 5 readings. a p< 0.05 was considered statistically significant when compared with control (Group 1); b p< 0.05 was considered statistically significant when compared with hyperglycemic group (Group 2); Group 1: control rat + vehicle, Group 2: Hyperglycemic rats (HG), Group 3: HG + Metformin (10 mg/kg bwt); Group 4: HG + extract (125 mg/kg bwt) Group 5: HG + extract (250 mg/kg bwt); Group 6: Normal rats+ extract (250 mg/kg bwt)

complex. Hence, any compound with redox potential lower than that of redox pair Fe (III)/Fe (II) can theoretically reduce Fe (III) to Fe (II). The reducing ability of a compound generally depends on the presence of reductants which exhibit antioxidant potential by breaking the free radical chain, donating a hydrogen atom (Dhalaria et al., 2020).

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) is a well-known rapid and sensitive antioxidant assay method for evaluation of free radical scavenging activity of specific compound or plant extracts (Fajobi et al., 2017). In the present study, the result obtained from the DPPH radical scavenging activity of the extract revealed that *D. bulbifera* can be employed in preventing accumulation of free radicals in biological systems. The free radical scavenging power of the extract of *D. bulbifera* increased in a concentration dependent manner, though significantly low compared to standard ascorbic acid. The result of this study corroborates the observation of Afolayan et al. (2020) who reported a dose-dependent scavenging
activities of some medicinal plants used for malaria therapy in south-western Nigeria. Hyperglycemia results in the generation of free radicals which may exhaust antioxidant defenses, thereby leading to the disruption of cellular functions and causing oxidative damage to membrane (Yaribeygi et al., 2019). The structures and functions of an organ can be disturbed, during uncontrolled hyperglycemia. Streptozotocin has been shown to induce free radical production and cause tissue injury (Halliwell and Gutteridge, 2015). Streptozotocin has cytotoxic effects against various vital tissues of the pancreas, liver and kidney. The pancreas is especially susceptible to the action of streptozotocin-induced free-radical damage (Fuman, 2015). The induction of hyperglycemia with streptozotocin is associated with a characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins (Liu et al., 2019). The geometric increase in blood glucose concentration observed in all streptozotocin treated rats after multiple low dose injections of 40 mg/kg bwt confirmed the induction of hyperglycemia in the rats. Similar observation was made by Fuman (2015). In this study, D. bulbifera extract (125 and 250 mg/kg bwt) was found to reduce the glucose level in streptozotocin induced hyperglycemic animals. A therapeutic approach to maintain normal blood glucose levels is suppression of the production and/or absorption of glucose by inhibiting either the α-amylase or α-glucosidase enzymes (Kunyanga et al., 2011). Alpha amyrase breaks down starch into oligosaccharides and disaccharides, which are further hydrolysed by α-glucosidase to produced glucose and other monosaccharides, which are then absorbed in the small intestine (Ali et al., 2020). In this study, the tuber extract of the plant brought about 3-fold decrease in the plasma activity of α-amylase in the hyperglycemic rats. This observation, coupled with the reduction in glucose levels strongly suggests that the plant possesses antihyperglycemic effect.

Lipids play important roles in pathogenesis of Diabetes mellitus. The abnormalities in lipid metabolism could lead to increase in the levels of plasma lipid and lipoprotein that in turn play an important role in the occurrence of premature atherosclerosis, which affects hyperglycemic condition (Dare et al., 2014). The treatment of hyperglycemic rats, in addition to glycemic control, should have favorable effect on lipid profile. The high level of total cholesterol (TC) and low-density lipoprotein (LDL) are major coronary risk factors (Temme et al., 2002). Reports have shown that triacylglycerols (TG) itself is independently related to coronary heart disease (El-Harzmi and Warsy, 2001). In this study, there was increase in the concentration of total cholesterol (TC), triacylglycerol (TG), LDL, VLDL in the untreated hyperglycemic group while the treated group showed significant increase in concentration of HDL, protein values and reduced VLDL, TG, TC. It could be suggested that the reduction in the plasma TG and TC concentration shows a good clearance of the compounds from the blood thereby preventing occurrence of coronary heart blood thereby preventing occurrence of coronary heart disease.

Chronic hyperglycemia in uncontrolled diabetes causes a serious micro-vascular complication leading to glycosylation of renal basement membranes and increased creatinine concentration (Mathilli et al., 2011). Creatinine is a waste product produced by muscles from the breakdown of creatine. It is removed from the body by the kidneys, which filters all of it from the blood and releases it into the urine (Samra and Abcar, 2012). Therefore, accumulation of creatinine in the blood could be an indication of malfunctioning of the kidney (McDonald et al., 2012). While the untreated hyperglycemic control had significantly higher concentration of plasma creatinine, hyperglycemic rats treated with D. bulbifera extract (125 and 250 mg/kg bwt) and metformin exhibited significantly lowered creatinine level. This reduction could be a result of improved renal function due to a reduced blood glucose concentration and subsequent glycosylation of renal basement. The effect of D. bulbifera extract (125 and 250 mg/kg bwt) was concentration dependent, with better activity with higher dose of plant extract over the 21 day period that the test was carried out.

Aminotransferases are enzymes that catalyze transamination reaction and are found mainly in the liver, as well as red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys (Liu et al., 2012). Alanine aminotransferase (ALT) and Aspartate

<table>
<thead>
<tr>
<th>Group</th>
<th>α-Amylase (U/ml/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.79 ± 2.48</td>
</tr>
<tr>
<td>2</td>
<td>161.40 ± 9.12 ( ^a )</td>
</tr>
<tr>
<td>3</td>
<td>53.54 ± 4.43 ( ^a )</td>
</tr>
<tr>
<td>4</td>
<td>70.51 ± 4.83 ( ^a )</td>
</tr>
<tr>
<td>5</td>
<td>60.35 ± 3.47 ( ^a )</td>
</tr>
<tr>
<td>6</td>
<td>31.59 ± 2.68 ( ^a )</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of 5 readings. \( ^a \) p< 0.05 was considered statistically significant when compared with control (Group 1); \( ^b \) p< 0.05 was considered statistically significant when compared with hyperglycemic group (Group 2); Group 1: control rat + vehicle, Group 2: Hyperglycemic rats (HG), Group 3: HG + Metformin (10 mg/kg bwt); Group 4: HG + extract (125 mg/kg bwt) Group 5: HG + extract (250 mg/kg bwt); Group 6: Normal rats + extract (250 mg/kg bwt)
Plate 1. Histological Structure of Pancreas (H & E. Magnification x400). A: Pancreas cross-section of normal appearance of pancreatic acini (green arrow) and islet of Langerhans (IL) in control Group 1. B: Cross-section of pancreas showing degenerated islet of Langerhans in hyperglycemic group (Group 2); C: Pancreas cross-section showing the recovery of marked degenerated islet of Langerhans (MIR) in treated hyperglycemic group with 10 mg/kg bwt Metformin (Group 3); D: Pancreas cross-section showing the recovery of degenerated islet of Langerhans in treated hyperglycemic group with extract (125 mg/kg bwt) (Group 4); E: Pancreas cross-section showing the recovery of degenerated islet of Langerhans in treated hyperglycemic group with extract (250 mg/kg bwt) (Group 5); F: Pancreas section of normal rats + extract (250 mg/kg bwt) (Group 6).

Aminotransferase (AST) activities are a valuable aid primarily in the diagnosis of liver diseases. When body tissue or an organ such as the liver is damaged or diseased, additional ALT and AST are released into the bloodstream, causing its activities to rise. Therefore, the activities of ALT and AST in the blood are directly related to the extent of the tissue damage (Liu et al., 2012). The result of the study showed a significant increase in the activities of plasma ALT in untreated hyperglycemic rats (Group 2) when compared with the normal control (Group 1) and the treated groups (Groups 4 and 5) but a decrease in treated group with metformin (Group 3). There was significant differences (p < 0.05) in the plasma and liver AST of the untreated hyperglycemic rats (Group 2) when compared with the treated groups (Groups 3, 4 and 5).
The multiple, low-dose streptozotocin induces partial damage of pancreatic islets causing a reduction of beta cells that eventually results in hyperglycemia (Fuman, 2015). From the changes in the histoarchitecture of the islets of Langerhans after the *D. bulbifera* treatment, it appears that there was a regeneration of beta cells as observed in the histological photomicrograph (Plate 1). The histological examination of pancreatic section showed normal arrangement of the islets of Langerhans of various sizes scattered throughout the exocrine tissue of normal control and treated hyperglycemic groups. However, degenerated islet of Langerhans, atrophy and vacuolation and invasion of connective tissues in parenchyma of pancreatic islets were detected in hyperglycemic control. These abnormalities were corrected in groups treated with methanol extract of *D. bulbifera* (125 and 250 mg/kg) of Groups 4 and 5 respectively. The effect of flavonoids on pancreatic β-cells leading to their proliferation and secretion of more insulin has been proposed by Ghorbani et al. (2019) as the mechanism by which they reduce hyperglycemia caused by streptozotocin in treated rats.

The study concludes that whole tuber extract of *D. bulbifera* contains high phytochemical contents that exhibited potent and appreciable antioxidant and anti-hyperglycemic activity in Streptozotocin-treated rats thus preventing the occurrence of hyperglycemic disorders. It will be rewarding to isolate the active principle(s) responsible for these activities in subsequent studies.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


