Biochemical Evaluation of the Effects of Hydromethanolic Extracts of *Dioscorea bulbifera* in Wistar Rats

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**Abstract:** The use of plants for medicinal purposes in Africa and other parts of the world have continued to be seen as safe alternatives to conventional medicine. *Dioscorea bulbifera* remains one of the widely used therapeutic plants in Africa and Asia. The present study is aimed at studying the biochemical effects of hydromethanolic extract of *Dioscorea bulbifera* on biochemical parameter using Wistar rats. Forty (40) albino Wistar rats were used for the study and were divided into 4 groups of 10 rats each. The control group received distilled water while the experimental groups received hydromethanolic extracts of *Dioscorea bulbifera* at 20mg/kg, 50mg/kg and 100mg/kg. Serum glucose, proteins, lipid profile and liver enzymes were analyzed using standard laboratory methods. Results of the present study indicate that the mean values for serum glucose, albumin, TG, TC, LDL, VLDL and atherogenic index was found to be statistically higher in experimental group compared to control (p<0.05) while the mean values for total protein, ALT and AST was found to be significantly higher in experimental group compared to control (p<0.05). These results show a potent hypoglycaemic and hypolipidaemic activity of the extract.

**Key words:** *Dioscorea bulbifera*, lipid profile, serum glucose, liver enzymes

I. Introduction

The use of plants for medicinal purposes in African predates the recent global use of a variety of herbal products and home remedies[1]. Traditional medicine evolved from environmental resources, which the people of a community adapted in desperation for survival from disease and remained the sole medical system for health care before the advent of modern medicine[2]. Today herbal products are often promoted as being “natural” and completely “safe” alternatives to conventional medicines, however many are potentially toxic[3]. The source of contamination may due to an inadvertent contamination by microbial or chemical agents during any of the production stages or by other species or plant parts through misidentification with resultant unsafe consequences. While herbal remedies have a wide range of therapeutic uses and are suitable for chronic treatments; the occurrence of undesirable side effects seems to be less frequent. However, well-controlled, randomized clinical trials have revealed that side effects also exist.[1, 3, 4]

*Dioscorea bulbifera* also known as aerial or air potato is one of the highly grown yam in West Africa and Asia. They are not only grown for food but also for its use in herbal preparations in many parts of the world[5, 6]. In Nigeria, herbal preparation using *Dioscorea bulbifera* is used for memory enhancement, anti-gasting, constipation and fever[7] while in Zimbabwe it used as an infusion to apply to cuts and sores[6]. In Cameroon and Madagascar, the pounded bulbils are applied to abscesses, boils and wound infections[8]. In traditional Indian and Chinese medicine, it used in the treatment of goiter, sore throat, gastric cancer and carcinoma of the rectum[9, 10].

Reports on the extracts of *Dioscorea bulbifera* have demonstrated its anti-fungal activity[11], antibacterial potential[12], protective capability in myocardial ischemic reperfusion injury[13], wound healing ability[14], analgesic and anti-inflammatory properties[5] and antioxidant and gastroprotective effects[15, 16]. Despite its widely acclaimed therapeutic potential, information regarding its effects on biochemical parameters is meagre. The present study therefore is an attempt to evaluate the effects of hydromethanolic extract of *Dioscorea bulbifera* on biochemical parameters using Wistar rat models.

II. Materials And Methods

Research Animals

Forty (40) albino Wistar rats weighing 180-300g were used for the study. The rats were allowed three weeks of acclimatization under standard laboratory conditions (Temp. 25 - 29°C, natural light/dark cycle) during which they were fed a balanced commercial rat pellet diet *ad libitum*. 
Preparation of Plant Extract
The aerial tubers of Dioscorea bulbifera were freshly harvested from a farm in Amiri, Imo State. They were thoroughly washed, sliced into fine small pieces, and shade dried for 2 weeks. They were then reduced to coarse powdery form by grinding followed by Soxhlet hydromethanolic extraction as described by Odebisi and Sofowora[17].

Experimental Design
The rats where divided into 4 groups (Groups1, 2, 3 and 4) of 10 rats per group.
- Group 1 (control) received distilled water
- Group 2 received 20mg/kg of Dioscorea bulbifera extract
- Group 3 received 50mg/kg of Dioscorea bulbifera extract
- Group 4 received 100mg/kg of Dioscorea bulbifera extract
The oral administration of the extracts lasted for 20 days.

Collection of Blood Sample and Laboratory Analysis
The animals were sacrificed using chloroform anesthesia followed by cardiac puncture. Blood was collected into a dry sample bottle for biochemical parameters using standard laboratory kits (Randox Laboratories Ltd., UK).

Histopathological examination of the Liver
The animals were dissected by the method described by Patel et al[18]. The liver was harvested and cut into sections. The sections were fixed directly on a slide and stained with haematoxylin and eosin, examined at x400 and photographed.

Statistical Analysis
The mean and standard error of mean were determined using SPSS v.20. The one way ANOVA followed by an LSD post hoc analysis was used to determine the difference among the groups. The results were considered significant at p-value <0.05. Results are presented as mean±standard error of mean.

III. Results
The result of the phytochemical screening revealed the presence of carbohydrates, cholesterol, alkaloids, steroids/triterpenoids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, phenols, proteins and amino acids.

Table 1: The effects of hydromethanolic extract of Dioscorea bulbifera on serum total protein, albumin, alkaline phosphatase, alanine transaminase and aspartate transaminase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2 (20mg/kg DB)</th>
<th>Group 3 (50mg/kg DB)</th>
<th>Group 4 (100mg/kg DB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>41.92±1.30</td>
<td>37.50±0.96</td>
<td>44.25±0.75</td>
<td>33.33±0.71</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>73.17±2.42</td>
<td>93.75±3.21</td>
<td>85.17±2.77</td>
<td>82.58±1.51*</td>
</tr>
<tr>
<td>Alkaline Phosphatase (ALP) (U/L)</td>
<td>42.83±3.93</td>
<td>44±2.93</td>
<td>49.00±1.32</td>
<td>46.17±2.93</td>
</tr>
<tr>
<td>Alanine Transaminase (ALT) (U/L)</td>
<td>21.33±1.04</td>
<td>16.75±0.96</td>
<td>22.80±3.52</td>
<td>36.25±6.88</td>
</tr>
<tr>
<td>Aspartate Transaminase (AST) (U/L)</td>
<td>79.23±1.46</td>
<td>76±0.01</td>
<td>81.92±1.85</td>
<td>81.92±1.81</td>
</tr>
</tbody>
</table>

Table 2: The effects of hydromethanolic extract of Dioscorea bulbifera on serum glucose, lipid profile and atherogenic index

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2 (20mg/kg DB)</th>
<th>Group 3 (50mg/kg DB)</th>
<th>Group 4 (100mg/kg DB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.82±0.14</td>
<td>6.86±0.28*</td>
<td>6.52±0.24</td>
<td>6.28±0.32*</td>
</tr>
<tr>
<td>Total Cholesterol (TC) (mmol/L)</td>
<td>3.82±0.05</td>
<td>3.54±0.10*</td>
<td>3.34±0.08*</td>
<td>3.28±0.12*</td>
</tr>
<tr>
<td>Total triglycerides (TG) (mmol/L)</td>
<td>0.80±0.04</td>
<td>0.49±0.05*</td>
<td>0.45±0.05*</td>
<td>0.47±0.03*</td>
</tr>
<tr>
<td>High density Lipoproteins (HDL) (mmol/L)</td>
<td>2.66±0.17</td>
<td>3.01±0.15</td>
<td>2.67±0.17</td>
<td>2.86±0.12</td>
</tr>
<tr>
<td>Low density Lipoproteins (LDL) (mmol/L)</td>
<td>0.79±0.14</td>
<td>0.31±0.12*</td>
<td>0.47±0.10*</td>
<td>0.21±0.05*</td>
</tr>
<tr>
<td>Very Low density Lipoproteins (VLDL) (mmol/L)</td>
<td>0.37±0.02</td>
<td>0.22±0.02*</td>
<td>0.21±0.02*</td>
<td>0.22±0.01*</td>
</tr>
<tr>
<td>Atherogenic Index</td>
<td>0.52±0.04</td>
<td>-0.81±0.07*</td>
<td>-0.79±0.07*</td>
<td>-0.79±0.03*</td>
</tr>
</tbody>
</table>

Table 3: The effects of hydromethanolic extract of Dioscorea bulbifera on body weight of wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control, n=10)</th>
<th>Group 2 (20mg/kg, n=10)</th>
<th>Group 3 (50mg/kg, n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>Week 1: 275.8±21</td>
<td>Week 2: 283.3±49.48</td>
<td>Week 3: 280.6±72.7</td>
</tr>
<tr>
<td></td>
<td>Week 1: 275.8±21</td>
<td>Week 2: 283.3±49.48</td>
<td>Week 3: 280.6±72.7</td>
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</tbody>
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Table 1 shows the effect of hydromethanolic extract of *Dioscorea bulbifera* on serum total protein, albumin, alkaline phosphatase, alanine transaminase (ALT) and aspartate transaminase (AST). Mean values of serum total protein, albumin, ALT and AST were found to be significantly higher in experimental group compared to control (p<0.05).

Table 2 shows the effects of the extract on serum glucose, TC, TG, HDL, LDL, VLDL and atherogenic index. Mean values of serum glucose, TC, TG, LDL and VLDL were found to be statistically lower in experimental group compared to control (p<0.05) while mean values for HDL did not show any trend.
Table 3 shows the mean body weights of Wistar rats following the 28-days administration of hydromethanolic extract of Dioscorea bulbifera. While significantly higher mean body weights were obtained for control rats compared to the experimental group in the fourth week (p<0.05), significantly lower mean body weights were obtained for experimental rats in groups 3 and 4 in the third and fourth weeks of the administration of the extract compared to control. There was a general decrease in body weight of the rats in the experimental group compared to control (p<0.05).

Figure 1 shows the micrographs of liver histology of the animals in the various groups with 1A, 1B, 1C and D representing liver histology for groups 1, 2, 3 and 4 respectively. Normal liver histology is shown in Fig.1A while Figs. 1B, 1C and D show various forms of liver damages.

**IV. Discussion**

Biochemical markers continue to provide information regarding the state of physiological well-being following, illness, malnutrition and the effects of exogenous compounds taken into the body[19, 20]. The present study evaluated the effects of hydromethanolic extract of Dioscorea bulbifera on lipid profile, serum glucose, proteins and liver enzymes of wistar rats.

Mean values serum glucose was found to be statistically lower in experimental groups (6.68±0.28, 6.52±0.24 and 6.28±0.32) compared to control group (7.82±0.14) in a dose dependent manner. This is attributable to the effect of Dioscorea bulbifera which has been shown to be a potent potentglucosidase and α-amylase inhibitor[21] which lowers the rate of glucose absorption through delayed carbohydrate digestion. It may also be due to the an extra pancreatic mechanism of inhibition of hepatic glucose production[22, 23]. It is also possible that the extract stimulated insulin secretion from the β-cells of the islets of Langerhans [23, 24]. Dioscorea bulbifera has also be shown to have a potent hypoglycaemic activity[21, 24-26].

The mean values for TC, TG, LDL and VLD were all found to be significantly lower in the experimental group compared to control (p<0.05) as shown in Table 2. The effect of Dioscorea bulbifera to caused reduced serum cholesterol levels could be due their anti-oxidant activity[15, 16] as they prevent the oxidative modification of LDL. Also triterpenoids as found to be a phytochemical component of the extract has been demonstrated to reduce the levels of TG, TG and phospholipids.[27]. In the same way, saponins have been associated with a decreased intestinal absorption of cholesterol[28] by binding cholesterol to the lumen, reducing its absorption, increasing its fecal excretion and consequently lowering blood cholesterol[29]. Saponins have also been reported to exhibit anti-lipase activity, reduced adipocyte differentiation and lipogenesis[30, 31].

The observed hypolipidaemic activity of Dioscorea bulbifera extract consequently improved the atherogenic index in the experimental group compared to control. The mean value of atherogenic index was found to be statistically lower in the experimental group(0.81±0.07, 0.79±0.07, 0.79±0.03) compared to control (0.81±0.07) (p<0.05).

The liver is involved in the metabolic transformation of drugs and other chemicals and hence it is normally predisposed to damages. [32]. Serum levels of liver enzymes, ALT, AST and ALP have been shown to be reliable markers of hepatotoxicity[33].While the mean values for total protein, ALT and AST was found to be significantly increased in the experimental groups compared to control (p<0.05), the mean values for serum albumin was significantly decreased in the experimental group compared to control (p<0.05). This observed increase in the activities of ALT and AST in the experimental animals may have resulted from leakages from damaged hepatocytes[34]. The observed hepatocellular injury as shown in the micrograph (Fig.1) of liver histology of the experimental animals suggests a chemically-induced liver damage. The various forms of hepatocellular injury observed include the presence of inflammatory cells around the hepatocytes, perivascular inflammation and congested vessels.

The significant decrease in serum albumin levels (37.50±0.96, 38.25±0.75 and 33.33±0.71) observed in the experimental groups compared to the control group (41.92±1.36) could be due to liver damage which prevented the liver from producing albumin[35]. The mean value for total protein was also found to be statistically higher in experimental group compared to control (83.75±3.21, 83.17±2.77 and 82.58±0.71). This could be attributed to the inflammatory changes as observed in the micrograph of liver histology (Fig 1B, 1C and 1D) [36].

While the body weights of control animals expectedly increased, the body weight of the experimental groups were observed to decrease following the administration of hydromethanolic extract Dioscorea bulbifera. This could be attributed to ability of the extract to reduce serum TG, TC and LDL. Also saponins have been found to cause appetite suppression mediated via regulation of hypothalamic neuropeptide Y and serum leptin levels[37, 38].

**V. Conclusion**
The result of present study indicate that the oral administration of hydromethanolic extract of Dioscorea bulbifera reduced serum glucose, lipid profile and consequently the weight of the animals. However, the sub-chronic administration of this extract caused visible liver toxicity. While these findings suggest a potent hypoglycaemic and hypolipidaemic activity, we recommend further research on the acute, sub-acute and chronic administration of this extract on liver function.

References

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