



## Evaluation of Antihyperlipidaemic Activities of Hydromethanolic Extracts of *Dioscorea bulbifera*

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

**Introduction:** Hyperlipidemia is a leading cause of cardiovascular diseases (CHDs) with treatment ranging from dietary management and the use of antihyperlipidaemic drugs. The desire for anti-hyperlipidaemic drugs with less side effects has led to the screening of medical with anti-hyperlipidaemic properties.

**Aim:** The present study is aimed at evaluating the effects of hydromethanolic extracts of *Dioscorea bulbifera* on high fat diet, tyloxapol and dexamethasone induced hyperlipidaemia using Wistar rat models.

**Methodology:** Fifty five (55) adult male Wistar rats weighing 180-250 g were used for the study. Natural induction of hyperlipidaemia was done using a formulated High fat diet made from commercial rat chow and rendered cow fat while chemical induction of hyperlipidaemia was done using tyloxapol (200 mg/kg) and dexamethasone (20 mg/kg). The Wistar rats were divided into eleven (11) groups comprising four (5) control groups and seven (6) experimental groups. The extracts were used to treat the hyperlipidaemic rats at 200 mg/kg and 400 mg/kg while Simvastatin was used as a standard. Blood samples of the animals were analyzed for Total cholesterol (TC),

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Triglycerides (TG), High density lipoproteins (HDL), Low density lipoproteins (LDL), Very low density lipoprotein (VLDL) and Serum glucose were determined by standard enzymatic methods.

**Results:** The results indicate that the hyperlipidaemic rats treated with extracts of *Dioscorea bulbifera* had significantly reduced TC, TG, LDL, VLDL and serum glucose compared with the control (untreated hyperlipidaemic rats) ( $P < 0.05$ ). In the same way, the HDL was found to be significantly higher among the treated hyperlipidaemic rats compared with the untreated controls.

**Conclusion:** The present study shows that hydromethanolic extracts of *Dioscorea bulbifera* has a possible antihyperlipidaemic potentials as demonstrated by its ability to significantly improve lipid profile and lower serum glucose levels in hyperlipidaemic rat models.

**Keywords:** *Dioscorea bulbifera*; lipid profile; serum glucose; hyperlipidaemia.

## 1. INTRODUCTION

Hyperlipidemia refers to abnormally high levels of lipids (fats) in the blood stream. Triglycerides, cholesterol esters, phospholipids and or lipoproteins are the various forms of lipids in the blood [1-3]. Lipids are generally divided into cholesterol and triglycerides. While cholesterol are the forms which circulate in the blood stream carried or attached to lipoproteins, triglycerides are stored in fat cells where they function mostly as energy stores [4-6]. The excessive buildup of these lipids in the blood leads to thickening and narrowing of blood vessels leading high blood pressure, atherosclerosis, ischemic and coronary heart diseases [2,7,8]. Studies have shown that reduction a blood cholesterol significantly reduced mortality due to atherosclerosis, coronary heart diseases and other lipid associated disorders [9-11]. Dietary management of hyperlipidaemic patients in addition to the use of antihyperlipidaemic drugs has remained the main stay of the treatment and management of hyperlipidaemia [12,13]. However, the undesirable side effects associated with many synthetic anti-hyperlipidaemic drugs continues to mask their effectiveness [14,15]. These side effects include but not limited to various degree of gastrointestinal disorders, choestryamine drug interactions, hepatic injury, gall stone formation as well as arrhythmias [12,15-17]. These undesirable effects associated with anti-hyperlipidaemic drugshas continued to fuel research in natural plants and herbs with antihyperlipidaemic properties.

*Dioscorea bulbifera* commonly known as aerial yam has remained one of the available yam in the West Africa, Asia and the Caribbean where they are grown mostly for food their wild range of application in folk remedy [18-20]. Common names in Nigeria include; adu, aduinu, isu-emina, emina, isu-ahun [21,22]. It is used in many parts of the world alone or in combination

with other herbs in the treatment and management of various ailments. In Nigeria, it is used in the treatment of fever, constipation as well as for memory enhancement [21]. In Zimbabwe, Cameroun and Madagaster it is used in the management of wounds and sores [23,24].

Over one hundred (100) compounds have been isolated from *Dioscorea bulbifera* conferring on it a wide range of documented pharmacological properties [25] as reports have shown that extracts of *Dioscorea bulbifera* has haematopoietic potential [26], wound healing potential [27,28], analgesic and anti-inflammatory properties [18] and antioxidant and gastroprotective effects [29,30], antitumor [31,32] and antibacterial properties [33,34]. Though recent studies have shown its ability to improve lipid profile [35,36], no study have demonstrated its efficacy in the possible improvement of lipid profile of natural and chemically induced hyperlipidaemia. The aim of this study therefore is to determine the effects of hydromethanolic extracts of *Dioscorea bulbifera* on high-fat-diet, tyloxapol and dexamethasone induced hyperlipidaemia using Wistar rat models.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Extract Preparation

The aerial tubers of *Dioscorea bulbifera* were freshly harvested from a farm in Amiri, Imo State, Nigeria. They were sliced into smaller pieces and shade dried for a period of 2 weeks. They were grinded into a coarse powder and then extracted to exhaustion with a soxhalet apparatus using hydromethanol (80:20). Extraction and phytochemical screening were carried out according to methods described by Odebiyi and Sofowora [37]. The extracts were then filtered and concentrated using a rotatory evaporator at 40-50°C to dryness. The hydromethanol extracts were stored at 2-5°C until required.

## 2.2 Experimental Animals

Fifty five (55) adult male Wistar rats weighing 180-250 g were source from the Animal house of the Department of Human Physiology, University of Port Harcourt and used for the study. The rats were allowed three weeks of acclimatization before the start of the study under standard laboratory conditions: Temperature at 25 - 29°C, 55 - 65% relative humidity under natural light/dark natural cycle. During the period of acclimatization, the animals were fed a balanced commercial rat chow (Top Feed LTD., Sapele, Nigeria) ad libitum.

## 2.3 Induction of Hyperlipidaemia

Natural induction of hyperlipidaemia was done using a formulated High fat diet [38] using 80% commercial rat chow (Top Feed LTD., Sapele, Nigeria) and 20% rendered cow fat. Cow fat was sourced from Slaughter market, Port Harcourt, Nigeria. The fat was dried, rendered and thoroughly mixed with the rat chow. The animals were allowed to feed on the formulated diet *ad libitum* for eight (8) weeks. Animals with a weight increase up to 30% were considered obese/hyperlipidaemic and used for the study.

Chemical induction of hyperlipidaemia was done using tyloxapol and dexamethasone. A single intra-peritoneal administration of Tyloxapol at 200 mg/kg [39-41] (Carbosynth, Ltd., UK) was used while a continuous five (5) days oral administration of dexamethasone [14,42] (Carbosynth, Ltd., UK) was used at 20 mg/kg. A standard ant-hyperlipidaemic drug, Simvastatin (TEVA, UK) at 10 mg/kg was used as the positive control. All experiments were examined and approved by the appropriate ethics committee.

## 2.4 Experimental Design

The male Wistar rats were divided into eleven (11) groups comprising four (5) control groups and seven (6) experimental groups as follows:

- Group 1 – Control [Distilled water]
- Group 2 – Negative control I [High fat diet only]
- Group 3 – Negative control II [Tyloxapol only]
- Group 4 – Negative control III [Dexamethasone only]
- Group 5 – Positive control [Simvastatin]

Group 6 – High fat diet + 200 mg/kg of *Dioscorea bulbifera*

Group 7 - High fat diet + 400 mg/kg of *Dioscorea bulbifera*

Group 8 – Tyloxapol + 200 mg/kg of *Dioscorea bulbifera*

Group 9 – Tyloxapol + 400 mg/kg of *Dioscorea bulbifera*

Group 10 – Dexanmathasone + 200 mg/kg of *Dioscorea bulbifera*

Group 11 – Dexanmathasone + 400 mg/kg of *Dioscorea bulbifera*

The oral treatment of hyperlipidaemic rats with of hydromethanolic extracts of *Dioscorea bulbifera* and standard drug lasted for fourteen (14) days. All the animals fasted overnight before being sacrificed by cardiac puncture under chloroform anaesthesia. Blood samples were collected into dry sample bottles and allowed to clot for about 20mins. They were centrifuged at 5000 rpm and the supernatant serum was collected and stored at 4°C prior to biochemical analysis. Total cholesterol (TC), Triglycerides (TG), High density lipoproteins (HDL) and Serum glucose were determined by enzymatic methods, using standard laboratory test kits (Randox, UK). Low density lipoproteins (LDL) was calculated as TC-HDL/TG/2.2 [43], Very low density lipoprotein (VLDL) was calculated as TG/2.2 [44] while atherogenic index was calculated as  $\log_{10}TG/HDL$ .

## 2.5 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 were used to determine the difference in means among the groups. The results were considered significant at  $P < 0.05$ .

## 3. RESULTS AND DISCUSSION

The result of the preliminary phytochemical screening of hydromethanolic extract of *Dioscorea bulbifera* showed the presence of carbohydrates, cholesterol, alkaloids, steroids/triterpenoids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, phenols, proteins and amino acids as reported elsewhere [18,23,45-47].

The lipid profile, serum glucose and atherogenic index of high fat diet, tyloxapol and

dexamethasone induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera* are shown in Tables 1, 2 and 3 respectively.

The mean values for Total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins, serum glucose and atherogenic index were found to be significantly lower in all experimental groups (groups 6-11) compared to untreated hyperlipidaemic rats (groups 2-4) ( $P<0.05$ ). However the mean values obtained for high density lipoproteins was found to be higher in all experimental groups (groups 6-11) compared to untreated hyperlipidaemic rats (groups 2-4) ( $P<0.05$ ). For the high fat diet induced hyperlipidaemic rats (Table 1), treatment with the extract caused significant reduction in the mean values for triglycerides, low density lipoproteins, very low density lipoproteins in the experimental groups (groups 6,7) when compared with the antihyperlipidaemic standard drug, simvastatin ( $P<0.05$ ) and a significantly increased high density lipoprotein among the rats treated with 400 mg/kg of *Dioscorea bulbifera*

(group 7) when compared with the hyperlipidaemic standard drug, simvastatin ( $P<0.05$ ). Similarly, it was observed that mean values for triglycerides and low density lipoproteins among tyloxapol induced hyperlipidaemic rats (Table 2) were found to be significant lower compared with the antihyperlipidaemic standard drug, simvastatin ( $P<0.05$ ) with a significantly increased high density lipoprotein among the rats treated with hydromethanolic extract of *Dioscorea bulbifera* (groups 8,9) ( $P<0.05$ ). For the dexamethasone induced hyperlipidaemic rats (Table 3), it was observed that the mean values for total cholesterol, triglycerides and low density lipoproteins and very low density lipoproteins were significantly lower in rats treated with hydromethanolic extract of *Dioscorea bulbifera* (groups 10,11) compared with the hyperlipidaemic standard drug, simvastatin ( $P<0.05$ ) with a significantly increased high density lipoprotein among the rats treated with hydromethanolic extract of *Dioscorea bulbifera* compared with the hyperlipidaemic standard drug, simvastatin ( $P<0.05$ ).

**Table 1. Lipid profile, serum glucose and atherogenic index of high fat diet induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera***

Parameters	Group 1 Control	Group 2 High fat diet only	Group 5 Simvastatin	Group 6 200 mg/kg <i>D. bulbifera</i>	Group 7 400 mg/kg <i>D. bulbifera</i>
TC (mmol/l)	5.50±0.10	7.20±0.34	5.84±0.14	5.50 <sup>a</sup> ±0.07	5.20 <sup>a</sup> ±0.09
TG (mmol/l)	2.44±0.12	3.12±0.26	2.50±0.08	2.14 <sup>ab</sup> ±0.12	1.43 <sup>ab</sup> ±0.26
HDL (mmol/l)	1.46±0.09	1.06±0.09	1.98±0.07	2.05 <sup>a</sup> ±0.90	2.30 <sup>ab</sup> ±0.07
LDL (mmol/l)	2.70±0.22	5.58±0.62	3.01±0.24	1.76 <sup>ab</sup> ±0.33	1.20 <sup>ab</sup> ±0.05
VLDL (mmol/l)	1.10±0.68	2.41±0.17	1.14±0.04	0.97 <sup>ab</sup> ±0.19	0.75 <sup>ab</sup> ±0.17
Serum glucose (mmol/l)	6.40±0.10	8.80±0.11	6.54±0.17	5.90 <sup>a</sup> ±0.11	5.41 <sup>a</sup> ±0.11
Atherogenic Index	0.22±0.02	0.47±0.04	0.24±0.03	0.18 <sup>ab</sup> ±0.3	-0.48 <sup>ab</sup> ±0.04

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin

**Table 2. Lipid profile, serum glucose and atherogenic index of tyloxapol induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera***

Parameters	Group 1 Control	Group 3 Tyloxapol Only	Group 5 Simvastatin	Group 8 200 mg/kg <i>D. bulbifera</i>	Group 9 400 mg/kg <i>D. bulbifera</i>
TC (mmol/l)	5.50±0.10	8.89±0.37	5.84±0.14	6.52 <sup>a</sup> ±0.53	5.38 <sup>a</sup> ±0.08
TG (mmol/l)	2.44±0.12	4.54±0.07	2.50±0.08	2.56 <sup>ab</sup> ±0.08	2.14 <sup>ab</sup> ±0.07
HDL (mmol/l)	1.46±0.09	1.34±0.07	1.98±0.07	3.14 <sup>ab</sup> ±0.04	3.54 <sup>ab</sup> ±0.08
LDL (mmol/l)	2.70±0.22	4.59±0.16	3.01±0.24	1.98 <sup>ab</sup> ±0.14	1.23 <sup>ab</sup> ±0.16
VLDL (mmol/l)	1.10±0.68	2.06±0.04	1.14±0.04	1.16 <sup>a</sup> ±0.03	0.99 <sup>a</sup> ±0.03
Serum glucose (mmol/l)	6.40±0.10	8.39±0.16	6.54±0.17	5.19 <sup>a</sup> ±0.11	4.20 <sup>a</sup> ±0.12
Atherogenic Index	0.22±0.02	0.53±0.03	0.24±0.03	-0.09 <sup>ab</sup> ±0.01	-0.22 <sup>ab</sup> ±0.01

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin

**Table 3. Lipid profile, serum glucose and atherogenic index of dexanmathasone induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera***

Parameters	Group 1 Control	Group 4 Dexanmath- asone only	Group 5 Simvastatin	Group 10 200 mg/kg <i>D. bulbifera</i>	Group 11 400 mg/kg <i>D. bulbifera</i>
TC (mmol/l)	5.50±0.10	10.7±0.53	5.84±0.14	6.82 <sup>ab</sup> ±0.41	5.54 <sup>ab</sup> ±0.41
TG (mmol/l)	2.44±0.12	4.41±0.15	2.50±0.08	1.90 <sup>ab</sup> ±0.07	1.31 <sup>ab</sup> ±0.06
HDL (mmol/l)	1.46±0.09	1.81±0.07	1.98±0.07	2.05 <sup>ab</sup> ±0.08	3.10 <sup>ab</sup> ±0.07
LDL (mmol/l)	2.70±0.22	6.00±0.30	3.01±0.24	1.45 <sup>ab</sup> ±0.12	0.98 <sup>ab</sup> ±0.03
VLDL (mmol/l)	1.10±0.68	2.00±0.03	1.14±0.04	0.96 <sup>ab</sup> ±0.05	0.81 <sup>ab</sup> ±0.04
Serum glucose (mmol/l)	6.40±0.10	12.50±0.87	6.54±0.17	7.50 <sup>a</sup> ±0.87	6.70 <sup>a</sup> ±0.87
Atherogenic Index	0.22±0.02	0.52±0.06	0.24±0.03	-0.19 <sup>ab</sup> ±0.02	-0.44 <sup>ab</sup> ±0.03

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin

This observed antihyperlipidaemic activity of *Dioscorea bulbifera* could be attributed to the effect of diosgenin which has been noted as the main active saponin in the family Dioscoreaceae [48] with significant active quantities found in *Dioscorea bulbifera* [49-51]. Diosgenin suppresses cholesterol absorption and increases cholesterol secretion. It has also been reported to interfere with both exogenous and endogenous cholesterol accompanied by depressed rates of hepatic and intestinal cholesterol synthesis [52]. Also, saponins are known to exhibit cholesterol-lowering ability by binding dietary cholesterol and bile acids in the intestinal lumen. Binding of cholesterol decreases their absorption and subsequently their fecal excretion while binding of bile acids leads to an increase synthesis of bile acids from cholesterol causing cholesterol already absorbed via the gastrointestinal tract to be inhibited and secreted into feces [7,2-56]. Saponins have also been reported to exhibit anti-lipase activity, reduced adipocyte differentiation and lipogenesis leading to the low degradation of cholesterol, triglycerides and lipoprotein [7,57,58]. More so, the anti-oxidant properties of *Dioscorea bulbifera* [29,30] due to their flavonoid content has been shown to prevent the oxidative modification of LDL by scavenging free radicals [59,60], they are known to reduce LDL levels while increasing the levels of HDL [61,62]. Similarly, triterpenoids, has been demonstrated to reduce the levels of TG, TC and phospholipids [63]. The result of the present study concurs with previous studies where *Dioscorea bulbifera* was found to reduce serum cholesterol and lipoproteins [35,36,64].

The ability of extracts of *Dioscorea bulbifera* to cause a significant reduction in serum glucose could also be attributed to the presence of

diosgenin, a steroidal saponin, a potent glucosidase and  $\alpha$ -amylase inhibitor [65]. While  $\alpha$ -glucosidase inhibitors prevents the action of carbohydrate digestion enzymes  $\alpha$ -glucosidase which typically break down complex carbohydrates such as glycogen and starch to their monomers, the  $\alpha$ -amylase inhibitor prevents the action  $\alpha$ -amylase on long chain carbohydrates like amylose and amylopectin to yield simpler sugars (Rubilar et al., 2011). Hence, through delayed carbohydrate absorption, the rate of glucose absorption is reduced leading to the observed decrease in serum glucose. Also, flavonoids as contained in the extract has also been also been shown to be a potent glucosidase and  $\alpha$ -amylase inhibitors [66-69]. Alpha-glucosidase inhibitors represent the one of the most common oral agents used in ameliorating the effect of hyperglycaemia due to their lack of hypoglycemic threat and also their ability to control blood glucose without causing body weight gain and hyper insulinemia. They also do not cause any nutritional calorie loss as they only slow down carbohydrate absorption without altering the total amount of carbohydrate absorbed [66,70]. It is also possible that the extract may have through some extra pancreatic mechanism, inhibited hepatic glucose production [71,72]. Another possible mechanism of action of the extract could be the stimulation insulin secretion from the  $\beta$ -cells of the islets of Langerhans [72,73]. The result of the present study concurs with previous studies where *Dioscorea bulbifera* was found to have an antihyperglycaemic activity [47,51,65,73].

The observed antihyperlipidaemic activity of *Dioscorea bulbifera* extract consequently improved the mean values of atherogenic index in all the experimental groups compared to the

control groups. The values for atherogenic index has remained an important tool for analyzing the values for lipid profile as the association of TG and HDL reflects the important balance between risk and protective lipoprotein forces [74] and has remained a significant predictor for cardiovascular diseases [75-78]. It is useful in the evaluation of response to treatment. The result from the present study suggests that extracts of *Dioscorea bulbifera* significantly reduced the risk of cardiovascular diseases (CVDs) for the hyperlipidaemic rats.

#### 4. CONCLUSION

In conclusion, the present study has shown that hydromethanolic extracts of *Dioscorea bulbifera* has a possible antihyperlipidaemic potentials as demonstrated by its ability to significantly improve lipid profile and lower serum glucose levels in the high fat , tyloxapol and dexamethasone induced hyperlipidaemic rat models.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All experiments were examined and approved by the appropriate ethics committee.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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