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Effect of Steam Blanching and Sulphiting on the Antidiabetic Potentials of Aerial Yam (*D. bulbifera*) Amala Flour Fed Alloxan Induced Diabetic Rats

Chidimma Juliet Igbokwe[1,*], Peter Isah Akubor[2], Ifeoma Elizabeth Mbaeyi-Nwaoha[1]

[1]Department of Food Science, University of Nigeria, Nigeria
[2]Department of Food Science and Technology, Federal University Wukari, Nigeria

Abstract This research work investigated the effect of processing (sulphiting and steam blanching) of aerial yam (*Dioscorea bulbifera*) amala flour on the hypoglycemic and hypolipidemic properties of alloxan induced diabetic rats. Yellow fleshed *Dioscorea bulbifera* bulbs were processed by sulphiting for 30 minutes and steam blanching for 10 minutes. The unprocessed sample served as control. The flours were used to make stiff doughs which were dried and used to feed alloxan induced diabetic rats. The hypoglycemic and hypolipidemic properties of the flours as well as some biochemical indices of the rats were studied. The LD_{50} of the samples was determined using acute toxicity study. Diabetic rats fed sulphited, steam blanched and untreated *Dioscorea bulbifera* flours had significantly ($p>0.05$) reduced fasting blood glucose level which decreased from 385 to 75.67 mg/dl, 654.33 to 80.00 mg/dl and 616.67 to 86.00 mg/dl, respectively. Diabetic rats fed untreated, steam blanched and sulphited *Dioscorea bulbifera* flours had significantly ($p<0.05$) reduced serum cholesterol (4.73, 4.66 and 4.43 mmol/L), low density lipoprotein (2.80, 2.70 and 2.33 mmol/L) and triacylglycerol (1.43, 1.41 and 1.42 mmol/L) respectively compared to the diabetic control. Diabetic rats fed steam blanched and untreated *Dioscorea bulbifera* flour had reduced alkaline phosphatase (75.00 and 81.33 IU/L), aminotransaminase (39.00 and 44.00 IU/L), creatinine (4.37 and 115.67 mg/dl) and urea (2.23 and 62.00 mg/dl) respectively. However, diabetic rats fed sulphited *Dioscorea bulbifera* flour had increased alkaline phosphatase (88.00 IU/L), aminotransaminase (53.66 IU/L), creatinine (7.57 mg/dl) and urea (207.67 mg/dl) relative to diabetic control. Steam blanched and sulphited *Dioscorea bulbifera* showed significant hypoglycaemic effect while the sulphited sample showed an adverse effect on the liver and kidney.

Keywords *Dioscorea bulbifera*, Hypoglycemic, Hypolipidemic, Diabetes, Sulphiting and Steam Blanching

1. Introduction

Diabetes is one of the most common non communicable diseases globally and a leading cause of death in many countries [1]. *Diabetes mellitus* is a metabolic disease characterized by hyperglycaemia and eventual glycosuria arising from relative or absolute lack of insulin. Bowman and Rand [2] defined diabetes as a disease characterized by hyperglycaemia, glycouria, polyuria, polydipsia and ketosis, all of which are a consequence of relative or absolute deficiency of insulin activity arising from abnormality in the Islets of Langerhans-insulin system. The International Diabetes Federation (IDF) estimated in 2011 that the number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030, if no urgent action is taken. Approximately, more than 471 billion USD is spent on healthcare for diabetes [3], with a large portion of the cost of diabetes being associated with its complications [4].

Due to the progressive nature of diabetes, nutrition therapy still remains a critical component of treatment among non-pharmacological measures in diabetes treatment. This is owing to the fact that it aids in effective glycaemic control, simplicity and affordability. *Dioscorea bulbifera* is an under-utilized agricultural produce that is available locally. Its utilization is presently limited to household level. *Dioscorea bulbifera*is rich in phytonutrients and has being shown to possess physiological functions to certain diseases other than its nutritional functions [5, 6]. It is commonly regarded as food for the poor and being eaten mostly when there is scarcity of food. *Dioscorea bulbifera* which are cultivated for their bulbils are consumed once cooked like potatoes in water with oil and local ingredients [7], charcoal roasted [8], and rarely prepared as dough ("*amala*"). In Asia, *Dioscorea bulbifera* is highly recommended for treating diabetes disorder and has been traditionally used to lower glycemic index, providing a more sustained form of energy and better protection against obesity and diabetes [5, 9]. *Dioscorea bulbifera* has prospects as an alternative meal for

the management of diabetes and can also promote food security among vulnerable groups or household.

In Nigeria, sulphiting and steam blanching are popular processing treatments used to inactivate enzymes and preserve colour prior to drying. Inspite of the importance of these pre-treatments, scanty reports exist in literature on the biochemical evaluation of these pretreatment methods using bioassay. It is important to understand the antidiabetic effect of foods based on processing methods invivo. The objective of this work was to steam blanch and sulphite *Dioscorea bulbifera* flour and produce stiff dough ("*amala*") and to determine the serum glucose lowering and hypolipidaemic potentialas well as some biochemical indices in alloxan induced diabetic rats fed these flours.

2. Materials and Methods

2.1. Sample Procurement

Dioscorea bulbifera bulbs were purchased from Eke market, in Obollo Eke, Udenu Local Government Area, Enugu State, Nigeria. The specie was authenticated at the Department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria. The duration of the research was from August 2014 to January 2015.

2.2. Sample Preparation

Dioscorea bulbifera bulbs were divided into three portions and each portion was washed, peeled and sliced manually into thin slices with sharp knife. The first portion was immersed in sodium metabisulphite solution (1.0 %) for 30 minutes [10]. The second portion was steam blanched in a metal sieve over boiling water at 100^0C for 10 minutes, while the third portion which was untreated served as control. The slices were oven dried at 40^oC to constant weight, milled in an attrition mill (Bentall Superb, Model 200L 09) and then sieved through a 200 μm sieve. The flours were then packed in air tight containers.

2.3. Preparation of Stiff Dough ('*Amala'*)

The stiff dough (*amala)* samples were prepared according to the method described by Malomo et al. [11]. Water (500 ml) was boiled in a pot over a gas cooker. Flour (100 g) was gradually poured into the boiling water and stirred continuously till it gelled into thick dough. Twenty millilitres (20 ml) of water was added to allow the flour cook properly under low heat. The paste was stirred until semi dough was obtained. It was cooled and dried in an oven to a constant weight, milled in an attrition mill (Bentall Superb, Model 200L 09) to obtain the flour and used for the rat study. Each group of diabetic treated rat received 100 g of the various dried sample each day.

The various '*amala'* flour samples were fed to the diabetic induced rats as shown below.

Group 1: Normal control rats + normal rat feed
Group 2: Diabetic rat + normal rat feed
Group 3: Diabetic rat + Sulphited *D. bulbifera* flour
Group 4: Diabetic rat + Steam blanched *D. bulbifera* flour
Group 5: Diabetic rat + Untreated D*. bulbifera* flour

2.4. Bioassay

2.4.1. Animals, Housing and Diet

Thirty (30) male albino rats of the wistar strain (supplied by the Department of Zoology, University of Nigeria Nsukka) were divided into five groups. All the rats were initially fed commercial rat diet for one week for acclimatization after which they were weighed. The animals were housed in cages and fed diets and tap water *ad libitum* for twenty one (21) days. The experimental diet consisted of *D. bulbifera* '*amala'* flour samples as sole source of food. The control diet for the 21 days study was commercial rat chow.

2.4.2. Induction of Diabetes in Rats

The baseline blood glucose levels were determined before the induction of diabetes. Four groups of six rats each were fasted overnight. Diabetes was induced in each fasted rat by administering alloxan monohydrate (150 mg/ kg body weight; intraperitoneal) in normal saline. Blood samples collected from tail veins were used to confirm diabetes induction after three days and rats with fasting blood glucose levels above 300 mg/dL were considered diabetic and used in the experiment. A group of ten rats that received no alloxan treatment served as control. The treatment lasted for twenty-one (21) days in which blood glucose levels of the rats were determined at day 0, 7, 14 and 21.

2.4.3. Biochemical Analysis for the Bioassay

2.4.3.1. Determination of Blood Sugar Level

Changes in blood glucose levels during the feeding period were measured weekly using blood glucose monitoring system (ACCU-CHEK Sensor; Roche Diagnostics GmbH, Mannheim, Germany) and ACCU-CHEK test strips were used for the assay. Blood was collected from rat tail veins.

2.4.3.2. Cholesterol Determination of Experimental Rats

On the last day of treatment, experimental rats were fasted overnight but allowed access to water, blood samples were collected by ocular puncture, transferred into plain ethylene-diamine-tetra-acetic acid (EDTA) bottles and centrifuged at 3000 rpm for 5 minutes. The clear sera was aspirated and stored frozen for serum biochemical analysis. Serum cholesterol was determined by the method of Abellet al. [12] low density lipoprotein (LDL) cholesterol and serum total high density lipoprotein **(**HDL) were determined by the method described by Zlatkis et al. [13]. Total triglyceride content was determined using the method of Gotfried and Rosenberg [14]. Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by the method described by Reitman and Frankel, [15]. The modified jaffe method for the in vitro determination of creatinine in serum [16] using the Quimica

Clinica Applicada (QCA) creatinine test kit (QCA Spain) was used. The modified method of Berthelot-Searcy for the in vitro determination of urea in serum [17] using the Quimica Clinica Applicada (QCA) urea test kit (QCA, Spain) was used.

2.4.4. Acute Toxicity Test

Three healthy female albino mice weighing between 150 and 180 g maintained under standard laboratory conditions were used in each stage for the acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 [18]. The LD_{50} determination for each of the fractions was done using the method of Lorke [19]. The evaluation was done in two phases. In phase one, three groups of three mice each, were treated with 100, 500 and 1000 mg extract/kg body weight orally, respectively. The mice were observed for clinical signs and symptoms of toxicity within 24 hours and death within 72 hours. Based on the results of the phase one study for the extract, nine fresh mice with three mice per group were treated with 1600, 2900 and 5000 mg extract/kg orally, respectively. Clinical signs and symptoms of toxic effects and mortality were observed for two days.

2.5. Experimental Design and Statistical Analysis

Experiments were based on completely randomised design. All data were subjected to Analysis of Variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) computer software version 20 and means were separated using Duncan's Multiple Range Test (DMRT). Significance was accepted at $p < 0.05$.

3. Results

3.1. Effect of *Dioscorea bulbifera Amala* Flours on Fasting Blood Glucose

Table 1 shows the effect of *Dioscorea bulbifera* '*amala'* flours on the fasting blood glucose of the diabetic induced rats. The fasting blood glucose increased significantly ($p<0.05$) in the diabetic control group compared with the normal rat group with single dose of intraperitoneal alloxan (150 mg/kg body weight) administration. Feeding of diabetic treated groups with sulphited, steam blanched and untreated *D. bulbifera amala* flour samples significantly ($p < 0.05$) reduced the fasting blood glucose level compared to the diabetic control group fed with normal rat feed.

3.2. Effect of Sulphited, Steam Blanched and Untreated *Dioscorea bulbifera* 'Amala' Flour on the Lipid Profile of Diabetic Rats

Table 2 shows that the diabetic control group had significantly ($p < 0.05$) increased cholesterol, low density cholesterol (LDL), triacylglycerol (TAG) and decreased high density cholesterol (HDL) compared to the normal control. The diabetic treated groups had significantly ($p < 0.05$) decreased cholesterol, low density cholesterol (LDL), low triacylglycerol (TAG) and significantly ($p<0.05$) increased high density lipoprotein when compared to the diabetic control.

Table 1. Effect of *Dioscorea bulbifera* '*amala'* flours on fasting blood glucose (mg/dl) of alloxan-induced diabetic rats

		Fasting glucose (days)			
Rat group	**Baseline**	**0**	**7**	**14**	**21**
1	43.67^{c} ±7.09	80.00^{c}±3.62	106.00^{b}±15.39	76.33^{b} ±2.08	74.33^{b}±2.89
2	72.67^{b} ±7.57	430.33^{b}±21.48	382.33^{a}±20.43	330.69^{a}±36.69	308.33^{a}±12.74
3	76.00^{b} ±6.08	385.00^{b}±57.97	307.00^{a}±19.32	79.67^{b}±0.58	75.67^{b}±4.93
4	100.67^{a}±4.16	654.33^{a}±80.83	414.00^{a}±70.62	108.00^{b}±28.16	80.00^{b}±17.35
5	100.00^{a}±11.28	616.67^{a}±46.06	324.67^{a}±81.01	130.33^{b}±54.50	86.00^{b}±15.13

Values are expressed as Means ± Standard Deviation of 3 rats per group. Means within a column with the same superscript were not significantly ($p>0.05$) different. Group 1- Normal control, Group 2- Diabetic control, Group 3- Untreated *D. bulbifera,* Group 4- Steam blanched *D. bulbifera*, Group 5- Sulphited *D. bulbifera*.

Table 2. Effect of sulphited, steam blanched and untreated *Dioscorea bulbifera* 'amala' flour on the lipid profile of diabetic rats

Rat Group		**Lipid profile**		
	Cholesterol (mmol/L)	**HDL (mmol/L)**	**LDL (mmol/L**	**TAG (mmol/L)**
1	4.63^{b}±0.38	1.33^{a}±0.57	2.63^{b}±0.38	1.38^{b}±0.04
2	4.96^{a}±0.25	1.10^{b}±0.10	3.40^{a}±0.44	1.73^{a}±0.19
3	4.73^{b}±0.47	1.33^{a}±0.05	2.80^{b}±0.36	1.43^{b}±0.25
4	4.66^{b}±0.25	1.30^{a}±0.10	2.70^{b}±0.10	1.41^{b}±0.11
5	4.43^{b}±0.25	1.40^{a}±0.10	2.33^{b}±0.15	1.42^{b}±0.03

Values are expressed as Means ± Standard Deviation of 3 rats per group. Means within a column with the same superscript were not significantly ($p>0.05$) different. Group 1- Normal control, Group 2- Diabetic control, Group 3- Untreated *D. bulbifera*; Group 4- Steam blanched *D. bulbifera,* Group 5- Sulphited*D. Bulbifera*, HDL- high density lipoprotein; LDL- low density cholesterol, TAG- triacylglycerol

Table 3. Effect of sulphited, steam blanched and untreated *Dioscorea bulbifera'amala'* flour on liver and renal functions of diabetic rats

Rat group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dl)	Urea (mg/dl)
1	65.33^{b}±4.16	52.33^{c}±3.21	35.67^{a}±8.1	1.00^{c}±0.17	27.67^{c}±6.66
2	85.67^{a}±4.93	68.66^{a}±9.01	43.33^{a}±4.16	6.20^{a}±1.20	167.67^{a}±28.15
3	75.00^{ab}±4.79	56.00^{bc}±1.73	39.00^{a}±4.36	4.37^{b}±0.51	115.67^{b}±8.50
4	81.33^{ab}±4.15	59.67^{bc}±2.08	44.00^{a}±1.00	2.23^{c}±0.40	62.00^{c}±11.14
5	88.00^{a}±1.73	63.00^{ab}±3.46	53.66^{a}±5.63	7.57^{a}±1.55	207.67^{a}±45.21

Values are expressed as Means ± Standard Deviation of 3 rats per group. Means within a column with the same superscript were not significantly ($p>0.05$) different. Group 1- Normal control, Group 2- Diabetic control, Group 3- Untreated *D. bulbifera*, Group 4- Steam blanched *D. bulbifera*, Group 5- Sulphited *D. bulbifera* ALP-Alkaline phosphatase, ALT- alanine aminotransaminase, AST- aspartate aminotransaminase

3.3. Effect of Sulphited, Steam Blanched and Untreated *Dioscorea bulbifera* '*Amala'* Flour on Liver and Renal Functions of Diabetic Rats

Table 3 shows the effect of sulphited steam blanched and untreated *Dioscorea bulbifera* 'amala' flour on liver and renal functions of diabetic rats. There was no significant ($p>0.05$) effect on ALP values in the diabetic treated groups (75 IU/L, 81.33 IU/L, 88 IU/L) when compared to the diabetic control (85.67 IU/L). Serum aspartate aminotransaminase (AST) levels decreased significantly ($p > 0.05$) from 56 IU/L to 63 IU/L in diabetic treated rats compared to the diabetic control (68.66 IU/L).There was no significant ($p>0.05$) effect on the levels of serum alanine aminotransaminase (ALT)in diabetic treated groups when compared to the diabetic control group. However, group 5 rats fed with sulphited *amala* flour had higher ALT value than the diabetic control group. The creatinine levels of group 3 (untreated) and group 4 (steam blanched) were significantly ($p<0.05$) reduced compared to those of group 2 (diabetic control). However, the creatinine level of group 5 (7.57 mg/dl) was not significantly ($p>0.05$) different from that of the diabetic control group (6.20 mg/dl). Urea levels in group 3 (untreated) and group 4 (steam blanched) were significantly ($p<0.05$) reduced compared to diabetic control (167.67 mg/dl) but was significantly ($p < 0.05$) increased (207.67 mg/dl) in the group 5 (sulphited).

Acute toxicity refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours [20]. In this research, acute toxicity study showed that none of the animals exhibited any obvious adverse behavioural changes such as increased respiration, diarrhea, coma or convulsion and toxic reaction after 24 hours and death after 72 hours in all the phases. The extract was safe up to a dose of 5000 mg/Kg (table not shown). Ahmed *et al.* [5] had earlier reported 2000 mg/Kg limit dose of *Dioscorea bulbifera* extracts without any signs of toxicity or mortality. This study demonstrated high safety margin since the animals tolerated up to 5000 mg/Kg (table not shown) dose of the extract which indicated that *Dioscorea bulbifera* (yellow fleshed) extracts are safe for human and animal consumption.

4. Discussion

There was significant ($p < 0.05$) increase in the fasting blood glucose level of diabetic rats (groups 2, 3, 4, and 5) on treatment with alloxan which indictaed the rats were induced with diabetes. The sulphited, steam blanched and untreated *Dioscorea bulbifera amala* flour samples fed to alloxan induced rats for 21 days produced significant ($p < 0.05$) hypoglycemic effect as shown in Table 1. The fasting blood glucose of the diabetic treated groups decreased significantly from 654 to 75.67 mg/dl at the end of the study. The possible mechanism of action of *Dioscorea bulbifera* flour samples for their hypoglycemic effect could be by promoting regeneration of β-cells or the protection of these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action [5]. The hypoglycaemic potential of *Dioscorea bulbifera* could probably be due to the presence of phytonutrients such as flavonoids and tannins [21] which are known to possess antidiabetic activity. These phytonutrients may be responsible for the observed significant hypoglycemic effect of *Dioscorea bulbifera amala* flour samples either singly or in synergy.

Derangement of glucose, fat and protein metabolism in diabetes results in the development of dyslipidemia [22). There was significant ($p < 0.05$) decrease in serum concentration of total cholesterol, triacylglycerol and increased level of high density lipoprotein in diabetic treated rats which were close to the levels of normal control rats. These results agreed with the findings of Ahmed et al. [5] who reported that *D. bulbifera* extracts have potential therapeutic value in combating dyslipidemia which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level. The lipid lowering potential of *D. bulbifera* could be by inhibition of lipid absorption due to the presence of saponins and tannins [23] or modulated by flavonoid content. The mechanism of action of *D. bulbifera* in reducing plasma cholesterol concentration could be due to the ability of one or more of the phytochemicals in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption [24] or the regression of the diabetic state due to the administration of the flour samples which may have increased the utilization of glucose, thereby depressing the mobilization of fat. The results of this study show that *D.*

bulbifera has lipid lowering effect on serum cholesterol, triacyl glycerol and low-density lipoprotein cholesterol of alloxan induced diabetic rats (Table 2). *D. bulbifera* treatment also increased the serum level of high-density lipoprotein cholesterol termed "good cholesterol" because it appears to help reduce the buildup of cholesterol from artery walls and transport it to the liver for excretion, regress atherosclerosis, thereby preventing cardiovascular diseases. The results suggest that *D. bulbifera* has potentials for management of dyslipidemia.

The liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes [25]. In *diabetes mellitus*, the liver is associated with abnormalities such as elevations in serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT). Injection with alloxan induced hepatocellular damage, as indicated by significant increase in AST, ALT and ALP in diabetic control group as compared to normal control group (Table 3). This result agreed with findings of Arkkila et al. [26] who reported that elevated activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are a common sign of liver diseases and observed frequently among people with diabetes than in the general population. Diabetic rats treated with steam blanched and untreated *amala* flours had decreased AST, ALT and ALP enzyme activities close to normal control rats. The improvements in the levels of the enzymes (AST, ALT, ALP) studied are consequence of an improvement in the carbohydrate, fat and protein metabolism [27].

However, diabetic rats treated with sulphited *amala* flour had elevated ALP and ALT levels compared with diabetic control. This might be due to the sodium metabisulphite used in sulphiting the flour and also the level of sodium metabisulphite (1.0%) used. The results of this study agreed with the reports of El Kadiet al. [28] that wistar rats administered with sodium metabisulphite (1% and 4%) had increased serum aminotransferases. Sulphites are unique additives used in the food industry to perform such functions as controlling enzymatic browning either by direct inhibition or by reacting with intermediates of enzyme reactions, thus preventing the formation of brown polymeric pigments [29]. Although sulphites are very efficient, they are subject to restrictions of use due to their toxic effects. The Food and Drug Administration (US) and Department for Environmental, Food and Rural Affairs (UK) both reported that sulfites are safe but should be avoided by asthmatics and those with liver or kidney dysfunction [30].

Kidneys maintain optimum chemical composition of body fluids by acidification of urine and removal of metabolite wastes such as creatinine [31]. *Diabetes mellitus* is associated with increase in plasma creatinine levels which might be a sign of impaired renal function. The significant reduction in serum creatinine and urea levels of diabetic rats treated with untreated and steam blanched D. *bulbifera* '*amala'* flours suggest protective effect of *dioscorea bulbifera* against kidney disorders associated with diabetic conditions. The elevated levels of urea and creatinine recorded in the sulphite treated group could be due to sodium metabisulphite used and also the level (1 %).The elevated level of urea and creatinine recorded by diabetic control and sulphited group might be due to diminished ability to filter urea and creatinine from blood and excrete them in urine. El Kadi et al. [27] also reported an increase in serum urea and creatinine in rats treated with 1 and 4 % sodium metabisulphite which implies abnormal renal function.

5. Conclusions

In conclusion, the results of this study showed that *Dioscorea bulbifera* '*amala'* flours had significant hypoglycemic and hypolipidemic effects on alloxan diabetic rats. The raw and steam blanched *Dioscorea bulbifera* flour samples had protective effect on the liver and kidney of the diabetic induced rats. However, the sulphited sample adversely affected liver and renal functions of the diabetic induced rats. The implication of this study is that aerial yam (D. bulbifera) could be used in the management of diabetes mellitus but the sulphited samples should be used minimally in order the avoid its adverse effect on liver and kidney organ. Aerial yam is regarded as food for the poor, research and isolation of the active components responsible for hypoglycemic and lipidaemic effect will encourage increased cultivation of the food crop thereby improving the farmer's income and the country.

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