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Dioscorea dumetorum-Fed Rats Exhibited Decreased Body Weight, Blood Glucose, and Insulin in STZ-induced Diabetes

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ABSTRACT

Background: Preventive measures that could slow down the rising incidences of diabetes mellitus are essential. The use of neglected local foods, which have effects on this chronic disease beyond basic nutrition as dietary controls, is desirable.

Objective: The effect of *Dioscorea dumetorum* (Kunth) Pax *(Dioscoreaceae)* feed on satiety, weight, blood glucose, and insulin levels were investigated in streptozotocin-induced diabetic rats.

Methods: Twenty adult male rats in four groups of five were used for the experiment. Three groups – *D. dumetorum*, glibenclamide, and standard pellet-fed rats were induced with diabetes by i.p. administration of 50mg kg^{-1} streptozotocin, while the fouth group (?) served as a non-diabetic control. *D. dumetorum* was fed at 15g daily for ten days before induction, and after induction, feeding continued. Glibenclamide was orally administered 5mg kg^{-1} daily. Both the untreated and non-diabetic rats were kept on standard rat pellets. Feed intake, weight, and blood glucose concentration were monitored daily, while insulin level was measured on day two and day six after inductions.

Results: Average feed intake for non-diabetic rats was 15g for *D. dumetorum* per day, which dropped to 10.3g after induction of diabetes. Weight of normal non-diabetic rats consistently increased (142.61 ± 4.37g – 169.43 ± 8.61g) for the duration (17 days) of the experiment. The *D. dumetorum*-fed rats showed weight reduction of 5.4%, glibenclamide 4.0%, and untreated diabetic 6.15%. Non-diabetic rats blood glucose levels ranged between 70 to 100mg dL^{-1}.

Streptozotocin (STZ) (i.p.) administration increased blood glucose levels from 370% to 626% in the rats. *D. dumetorum*-fed rats showed reduced ($p<0.05$) blood glucose levels of 22.6%. Glibenclamide had 5.5% reduction ($p<0.05$). Insulin was absent in *D. dumetorum*-fed rats, whereas 0.95ng ml^{-1} of insulin was detected in glibenclamide-administered rats. These quantities were lower ($p<0.001$) than 1.40ng ml^{-1} in the non-diabetic rats.

Conclusion: This study revealed that *D. dumetorum* tuber caused decreased hunger, weight reduction, and displayed hypoglycemic property in diabetic rats, even after heat treatment. Its probable mechanism of anti-hyperglycemic activity might not be through increased insulin secretion.

Key words: *D. dumetorum*, streptozotocin-induced diabetes, weight, blood glucose, insulin.

Background:

The increased prevalence of chronic non-communicable diseases, such as type 2 diabetes, hypertension, and other cardiovascular diseases (CVD), [1] has made it essential to search for methods of control and management of these diseases in ways that are cheap, safe, and easily accessible. Diet control is becoming a major strategic approach to manage these diseases among caregivers, nutritionists, and researchers.

The basis of the abnormalities in carbohydrate, fat, and protein metabolism in type 2 diabetes is deficient action of insulin on target tissues. This results in inadequate insulin secretion and/or diminished tissue response to insulin at one or more points on the complex pathway of hormone action [2]. The global prevalence of the disease is estimated to increase from 4% in 1995 to 5.4% by the year 2025 [3]. One of the reasons adduced for this rise is increased urbanization linked with lifestyle where daily routine requires less physical activity and greater access to foods with higher energy densities [4]. This has created a challenge of balancing both energy intake and expenditure leading to more people becoming overweight. Obesity and lack of physical activity have consistently been implicated in type 2 diabetes mellitus [5].

Glycemic control is a key to management of the disease. Reports state that intensive management of hyperglycemia in diabetes can significantly reduce complications, which suggest that most complications arising from the disease are not a part of the primary diabetic syndrome but secondary to the effect of chronic hyperglycemia [6]. Multiple interventions have been developed over the years to achieve glycemic control. However, most of these interventions have not been successful due to numerous factors, such as undesirable side effects of hypoglycemic agents [3], cost, life style, and disregard of the occurrence of the disease.

There is increasing awareness about the links between diet and diseases, such as certain fats and CVD, calcium and osteoporosis, and fiber and gastrointestinal health [4]. Therefore, the need to investigate indigenous foods that could act as functional foods has arisen from the relationship between diet, specific foods, and health. Currently, people's interest in healthy living is moving towards these specific foods. Moreover, scientific evidence lends support that these foods contain bioactive compounds that have positive effects on our health and well-being beyond simple nutritional requirement [4]. These types of foods that have positive effects on health beyond basic nutrition are termed 'functional foods'.

The concept of functional foods is predicated on considering that food is not only necessary for living but also as a source of mental and physical well-being and contributes to prevention and reduction of several risk factors of diseases [7]. They are not pills, capsules, or any other type of dietary supplements [4]. Currently, the trend in management of chronic diseases like diabetes is shifting from the use of synthetic agents to diet for glycemic control. This shift is to use foods from the local diet (What do you mean by this sentence?).

Many indigenous foods in Nigeria have been used for management of diabetes in the traditional healing system. Many of these foods have become neglected and are almost at the point of extinction. This work is an attempt to revert this trend by identifying and scientifically corroborating the potentials of these foods on health and diseases. One of these foods is *Dioscorea dumetorum (Kunth)* Pax (*Dioscoreacea). D. dumetorum* is a tuber with fleshy edible parts which can be yellow or white [8]. The tuber is commonly called cluster yam (because it occurs naturally in clusters), bitter yam or trifoliate yam, and it is called *"ona*" by the Igbos of Southeast Nigeria [9]. *D. dumetorum* is boiled and eaten as a snack in Southeast Nigeria. Its extract is used for the treatment of diabetes mellitus in traditional medicine [10]. The tubers are also reported to be rich in fiber and contain an alkaloid, *dioscorentine*, which possesses hypoglycemic activity [11] [10].

Thus, the focus of the present study is to evaluate the potential of *D. dumetorum* to act as a functional food in the control of diabetes using STZ-induced diabetic rat model. Previous studies on *D. dumetorum* have concentrated on the evaluation of different solvent extracts of the tuber for its hypoglycemic property and not as food. Moreover, the effect of heat from cooking the bioactive component of the tuber, to the best of our knowledge, has also not been reported. The objective of this study is to assess the effect of feed from the boiled tubers on satiety, weight, blood glucose level, and serum insulin concentration in STZ-induced diabetic rats.

MATERIALS AND METHODS:

Chemicals:

Hypoglycemic agent Glibenclamide (GlaxoSmithKline), D-glucose (Evans), Tween 80 (Sigma-Aldrich, Germany), Streptozotocin (Sigma-Aldrich, Germany), and Eliza immunoassay kit ELX405^{TM} (Accubind, USA).

Sample collection and authentication:

The tubers of *D. dumentorum* were collected from a farm in Itu Ezinihitte Mbaise, Imo State. The sample was identified by a taxonomist, Professor Okeke in the Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of feed:

The tubers (1000g) were properly washed, cooked, and the bark peeled and thinly sliced. The slices were air-dried at room temperature for two weeks on the laboratory bench. The dried slices were stored in an airtight container and refrigerated.

Animals:

Wistar albino rats (20) were purchased from the animal house at the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra State. The animals were kept in the animal laboratory with normal (twelve hour) daylight, housed in standard rat cages, and handled according to the guidelines for proper conduct of animal experimentation [12]. They were kept on commercial rat pellet and water *ad libitum* and acclimatized for seven days.

Experimental design:

All animals were weighed and sorted into four groups of five rats each. Sliced *D. dumetorum* feed was fed to the test (Group 1) rats for 10 days before induction of diabetes.

Group 1– *D. dumetorum*-fed diabetic rats, Group 2– Glibenclamide-administered (oral) diabetic rats (Positive control), Group 3– Standard pellet-fed diabetic rats (Negative control), Group 4- Non-diabetic rats.

Induction of experimental Type 2 Diabetes mellitus:

The rats were fasted for 15 hours but were allowed access to water. The initial blood glucose levels of the rats were recorded (One Touch 2, Ultra glucometer Ifescan, Inc. USA) before induction of diabetes. Diabetes was induced intraperitoneally with streptozotocin 50mg kg^{-1} body weight [13]. The animals were allowed food and water after the streptozotocin administration. Hypoglycemia in the rats was prevented by oral administration of 20ml 75% glucose solution one hour after induction. Blood glucose levels of the animals were determined 48 hours after induction. When the blood glucose level of the induced rats was higher than 200 mg dL^{-1}, diabetes was confirmed.

Animal feeding:

The diabetic rats in the test group received 15g of *D. dumetorum* feed each per day. The reference drug group (positive control) was orally administered 5mg kg^{-1} body weight of glibenclamide (a standard hypoglycemic agent) dissolved in 10% between 80% (?) and were fed standard rat pellets. The untreated (negative control) did not receive any treatment and were fed standard rat pellets. The non-diabetic rats were fed standard rat pellets and served as the base line control rats.

Determination of feed intake:

This study was carried out for 10 days. The test group rats *(D. dumetorum)* were housed individually and 5g of the feed was given to each rat. This quantity (g) was increased daily to 7, 9, 11, 13, 15, 17, 19 and 21 depending on the leftovers of each animal. The leftovers were weighed every day and noted. The quantity of feed consumed daily by each rat was determined by deducting the weight of the leftovers from the initial weight of feed given daily. The average *D. dumetorum* feed consumed was then determined. After induction of diabetes and during the experiment, the average consumed feed was also determined.

Death rate, weight assessment and blood glucose concentration determination: The number of animals that died in each group per day during the experiment (17 days) was

recorded. The weights (g) of the animals were determined before and after induction of experimental diabetes and daily for the duration of the experiment. Blood glucose levels of the animals were monitored daily from tail blood using the ultra-glucometer.

Insulin assay:

Blood was collected by the retro-orbital plexus method from the rats two days after induction of diabetes and at the end of the experiment. Serum was collected and insulin concentration (ng) was determined by immunoassay using the Elisa ELX405^{TM} kit.

Statistical analysis:

Data collected after three measurements were analyzed with SPSS (version 17). Analysis was with one-way (ANOVA) for weight, blood glucose level and insulin concentration, and $p<0.05$ was considered significant.

RESULTS

Feed consumption:

The normal (non-diabetic) rats consumed an average 15g of *D. dumetorum* feed per day. However, after induction of diabetes, the *D. dumetorum* feed consumption dropped to 10.3g daily.

Death rate:

The summary of the animals' death per day during the experiment is presented in Figure1. The *D. dumetorum*-fed rats had 60% survival rate by day six, glibenclamide had 20% while standard pellet-fed 0%.

Table 1: DEATH RECORD OF DIABETIC RATS

Day	1	2	3	4	5	6	7
D. dumetorum	ND	ND	1D	2D	ND	3D	NS
Glibenclamide	ND	ND	2D	1D	1D	1D	NS
Standard rat pellet	ND	2D	3D	NS	NS	NS	NS
Non-diabetic	ND	ND	ND	ND	ND	ND	ND

ND – No death**; D –** Death**; NS –** No survivor

Weight Changes:

The result of the weight assessment of the rats during the experiment is shown in figure 1. The weight of the normal non-diabetic rats consistently increased (142.61 ± 4.37g – 169.43 ± 8.61g)

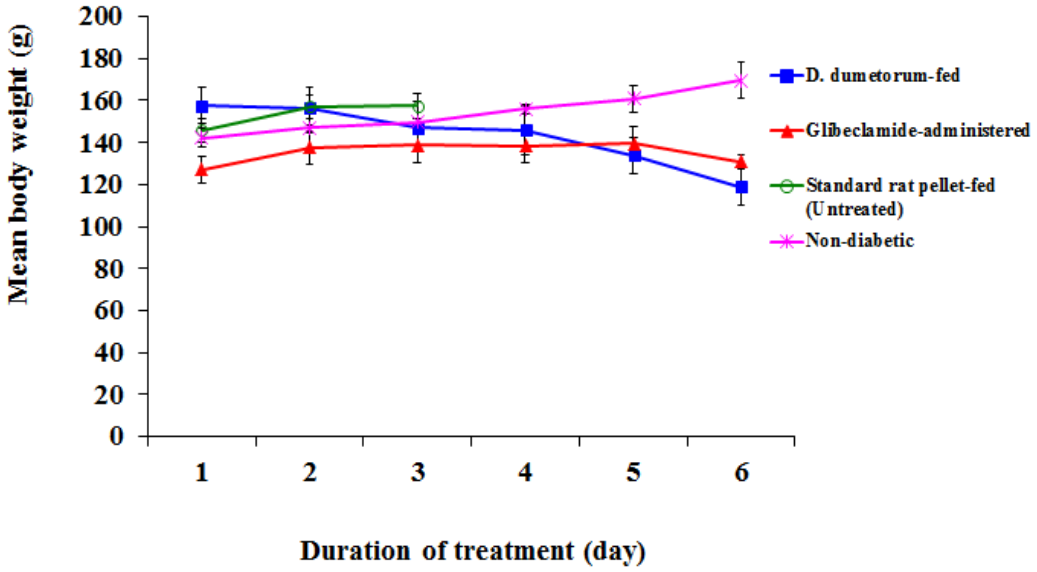

Fig. 1: Mean body weight of *D. dumetorum*-fed diabetic rats and control groups

throughout the duration of the experiment. The *D. dumetorum*-fed rats showed weight reduction of 5.4%, glibenclamide exhibited a lower percentage in weight reduction (4.0%) and the reduction was consistent throughout the experiment. The diabetic untreated (negative control)

rats exhibited 6.15% reduction in weight, which was higher than both the *D. dumetorum*-fed and glibeclamide-administered rats.

Blood glucose levels:

Results presented in figure 2 show that normal rat blood glucose levels (70 – 100mg dL^{-1}) were observed in the control group of rats that were kept on the standard rat pellets throughout the duration of the experiment. Intraperitoneal administration of streptozotocin (50mg kg^{-1} body weight) to the animals produced appreciable increases (370% to 626%) in their blood glucose levels. *D. dumetorum* tuber feed reduced the blood glucose levels of the diabetic rats by 22.6% by the sixth day of feeding (i.e. from 492.22 ± 20.00 to 381.00 ± 17.00). This reduction in blood glucose by *D. dumetorum* feed was significant ($p<0.05$) when compared with the untreated diabetic group. All the untreated diabetic rats died before the end of the experiment. Glibenclamide produced 5.5% reduction, which was lower ($p<0.05$) than the decrease caused by *D. dumetorum*.

Insulin concentration:

All the diabetic rats had higher levels of insulin than the non-diabetic group on day two after streptozotocin administration (Figure 3). By day six, insulin was completely absent in the diabetic rats fed with *D. dumetorum* tuber feed, whereas 0.95ng ml^{-1} of insulin was detected in

the group that received glibenclamide. These quantities were ($p<0.001$) lower than 1.40ng ml^{-1} observed in the non-diabetic rats.

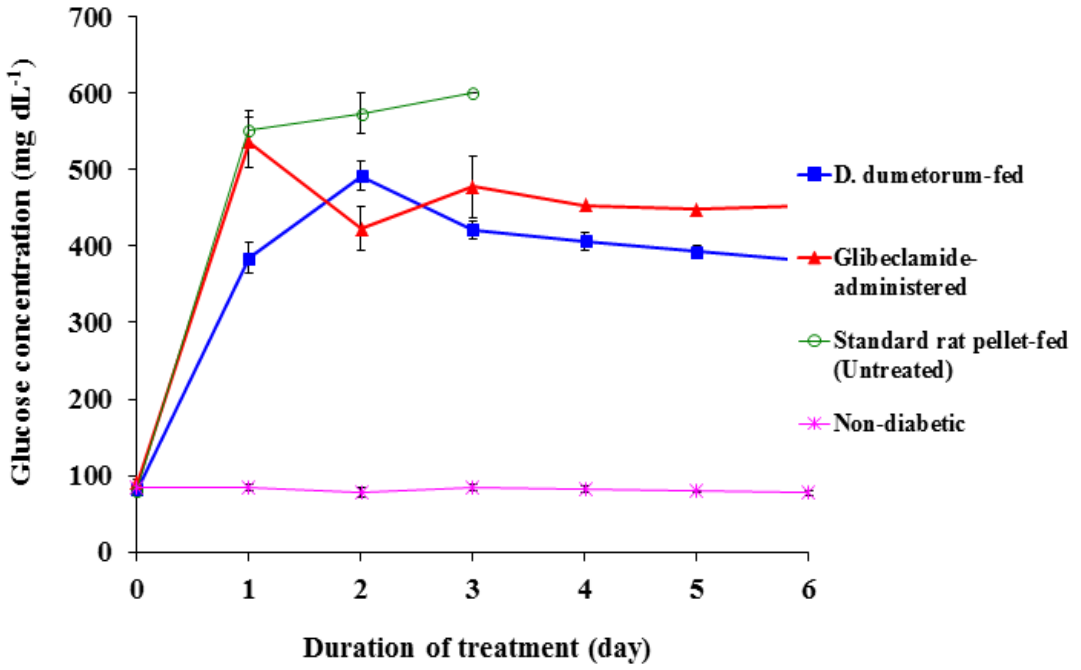

Fig. 2: Glucose concentration (mg dL^{-1}) in *D. dumetorum*-fed, glibenclamide-administered, standard rat pellet-fed and Non- diabetic rats

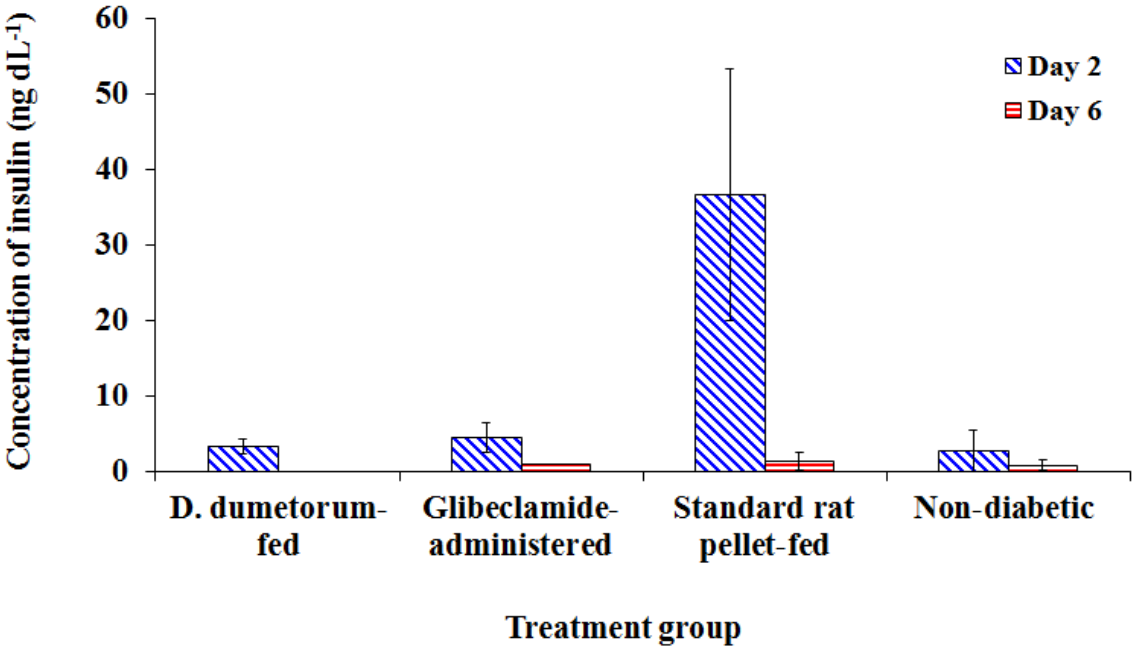

Fig. 3: Insulin concentration of *D. dumetorum*-fed diabetic rats and control groups

DISCUSSION:

From results of the present study, *D. dumetorum* exhibited important characteristics that point to its ability to act as an appropriate intervention tool. The tuber had positive effects on the amount of feed consumed, body weight, blood glucose concentration, and insulin concentration of diabetic rats. The findings support that the tubers might be useful as a functional food in the animals used for this investigation.

The reduction in *D. dumetorum* feed eaten by the diabetic rats observed in this experiment might be due to decreased hunger. *D. dumetorum* tuber has been reported to be composed of significant amount of fiber (3.47%), which is comparably higher than that of polished rice, water yam, and sweet potatoes [14]. Studies have it that the consumption of dietary fibers have a stomach filling effect, which might have resulted in reduced feed intake by the rats [15]. Consequently, dietary fiber helps in weight loss and decreases the risk of diabetes and cardiovascular diseases [16]. Furthermore, the reduction in feed intake might be an indication that *D. dumetorum* feed suppresses appetite, thereby resulting in weight loss and the lowering of blood glucose levels. This is similar to the reduction in feed intake and body weight reported by Olubobokun *et al*., [17] following the treatment of diabetic rats with *D. alata* extract.

Furthermore, it might also be possible that stimulation of the secretion, or action of the appetite–suppressing hormones (hypothalamic leptin, and intestinal mucosal cholecystokinin), as well as inhibition of the release of the appetite enhancing peptide–gherlin, may be part of the mode of action of the *Dioscorea* diet. This possibility is further strengthened by the point that some standard oral hypoglycemic agents, such as metformin, are well known for their appetite–suppressant actions, and this property is responsible for their blood sugar lowering effects [18].

The death rate showed that more *D. dumetorum*-fed animals lived longer (60%) for the period of the experiment than the glibenclamide (20%) and the standard pellet (0%) and is indicative of a positive effect of the tuber on the physiological condition of the animals.

The weight reduction observed in *D. dumetorum*-fed diabetic animals finds support in epidemiological studies conducted on diabetic management and control interventions. Franz [5] reported that intensive lifestyle intervention studies involving weight reduction of 5% decreased the overall risk of diabetes by 58% in human subjects. As stated earlier, obesity is a leading risk factor in development of diabetes. Currently, 1.1 billion people are overweight worldwide, a reason that has contributed to the increased prevalence of type 2 diabetes mellitus [5]. In addition, Eshghinia and Mohammadzadeh [19] found that weight loss programs in obese individuals produced lower incidences of type 2 diabetes mellitus and cardiovascular diseases. Moreover, the lowest percentage reduction in body weight recorded in the glibenclamide–treated diabetic rats is a confirmation that glibenclamide could encourage body weight gain. This is the most frequently reported side effect associated with the use of the second-generation sulfonylureas in the management of Type 2 diabetes mellitus [20].

The death of all untreated diabetic rats before the end of the experiment is as a result of a hyperglycemic coma, a major feature of type 2 diabetes mellitus. The blood glucose lowering effect of *D. dumetorum* is validated by earlier reports on the hypoglycemic potentials of the tubers and bulbs of the *Dioscorea* family. Extracts of *D. alata*, *D. bulbifera, D.hispida* and *D. domentorum* reportedly reduced the blood glucose levels of the alloxan-induced diabetic rats [21-24]. However, the study revealed that *D. dumetorum* diet was significantly ($p<0.05$) more

efficacious in lowering animal's blood glucose level than oral administration of the reference drug, glibenclamide (5mg/kg), which caused a 5.4% decrease.

Several modes of action of the hypoglycemic and anti-hyperglycemic medicinal plants, herbs, and foods have been elucidated. Many of these plants inhibit endogenous glucose release [25] or impede the absorption of glucose from the gastrointestinal tract [26]. Pharmacologically active ingredients present in some extracts may display insulin-like activity [27], inhibit insulinase activity, or enhance the secretion of insulin from the pancreatic β-cells of Islets of Langerhan [28, 29].

However, the elevated levels of insulin observed in all diabetic rats are more than the non-diabetic rats and could be ascribed to the pancreatic hypertrophy, usually observed after the administration of streptozotocin and alloxan and prior to the development of hyperglycemia [30].

Preliminary investigation into the mechanism of blood glucose lowering activity indicated a complete absence of insulin in the *D. dumetorum*-fed rats' serum; in contrast, glibenclamide (a known stimulator of insulin secretion by pancreatic β-cells) suggests that increased insulin secretion may not be the mode of action of *D. dumetorum* tuber feed. However, other mechanisms such as inhibition of glucose release from diet or inhibition of gastrointestinal glucose absorption would be investigated, since the efficiency of *D. bulbifera* to inhibit α-amylase and α-glucosidase (key enzymes involved in the post-prandial release of glucose into the systemic circulation) activities have been documented [22].

CONCLUSION:

In conclusion, this study confirmed the hypoglycemic property of *D. dumetorum*, even after heat treatment. This suggests that the tuber, which forms part of the population's diet in the past, could be used for the management of diabetes mellitus. However, further work on the tuber would be carried out in future studies to establish the mechanism of the action of its hypoglycemic activity.

Competing Interests: The authors have no competing interest or conflict of interest.

Authors' contributions: All authors contributed to sponsorship of the work. *OHA initiated designed and wrote the manuscript, IEM analyzed data and edited the manuscript; ADL partook in the research and carried laboratory analysis, ECN and OCS analyzed some aspects of the data in the manuscript, ACF and MSC carried out laboratory analysis.

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