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Original Research Article

Potency of Fonio Millet to Attenuate Diabetes and Diabetes Related Cardiovascular Diseases

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ABSTRACT

Diabetes has been described as one of the progenitors of cardiovascular diseases. Many researchers have focused their attention on the inherent medicinal potency of herbs in combating diseases with grains highly neglected for their medicinal values. This study is aimed at evaluating the potency of fonio millet (*Digitaria exilis*) to attenuate diabetes and diabetes related cardiovascular diseases. Twenty-five (25) Wistar rats, selected into five groups of 5 animals per group were used for the study.Streptozotocin (60 mg/kg intraperitoneal injection) was used to induce diabetes. The rats were treated daily with 500 mg/kg aqueous extract of fonio millet, 10% and 20% fonio millet supplemented diets, respectively for a period of 14days. Fasting blood glucose was monitored at intervals of 7days. Malondialdehyde (MDA), some antioxidant enzymes and nitric oxide were assayed in heart homogenate after the 14th day. Streptozotocin (STZ) caused significant ($p<0.05$) increases in fasting blood glucose from day 1-14 in relation to the basal values. Groups treated with 10% and 20% fonio millet supplemented diet demonstrated significant ($p<0.05$) fasting blood glucose reduction from day 7-14th. Oral administration of 500 mg/kg aqueous extract did not show any significant change in fasting blood glucose level. The activities of the assayed antioxidant enzymes, MDA and nitric oxide levels were unchanged in all the groups when compared with the control. These findings suggest that 500 mg of aqueous extract of fonio millet did not demonstrate anti-diabetic effect. Whereas 10% and 20% fonio millet supplemented diet demonstrated hypoglycemic and anti-diabetic potential.

Keywords: Attenuate, Diabetes, Fonio millet, Nitric oxide, Streptozotocin.

Introduction

Identified as one of the progenitors of cardiovascular disorders, the burden of diabetes is still much of concern to scientist in the twenty-first century. Although much advances had been made in the development of drugs to mitigate the debilitating health effects of the disease and its complications, permanent solution to combating the disease that is devoid of other associated side effects is yet to be achieved.[1,2] It is a general knowledge that hyperglycemia initiates tissue damage in diabetes mellitus.[3]Hyperglycemia-induced damage to tissues/cells (endothelial cells, mesangial cells, neurons and Schwann cells) give rise to various microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (cardiovascular diseases**:** atherosclerosis, ischemic heart disease and stroke) complications of diabetes.[3,4] Endothelial dysfunction which is in most cases a result of imbalance in nitric oxide (NO) bioavailability is generally known to be one of the main causes of common cardiovascular diseases.[5] Considering the putative knowledge that the mechanisms of hyperglycemia-induced tissue damage stems from a single process of overproduction of superoxide by the mitochondrial electron transport chain,[3] it suffice to say that maintenance of blood glucose homeostasis or balance between the production of superoxide and the body's natural antioxidants defense system would mitigate against diabetes and its associated complications.

Over the years, researchers in Africa have exploited herbs with glucose lowering capacity for diabetes management with grains highly neglected for their medicinal values. Fonio millet (*Digitaria exilis),* locally called acha is a West African native cereal grain that is cultivated across the regions. Nigeria, Guinea, Mali and Burkina Faso are among the West African Countries where fonio is highly cultivated.[6] According to Koréissi *et al*. (2013),[7]fonio millet is considered to contribute greatly to healthy nutritional profile as it is known to contain high content of fibre and low fat content. Fonio millet is also believed to have glycemic index that is lower than other cereals. It is also known to contain branched chain carbohydrates which release slowly to maintain blood sugar level in the body and could therefore be a suitable food for diabetics.[7]Egbebi and Muhammad (2016)[8]have reported the presence of certain phytochemicals (Alkaloids, Saponin, Tannins and Steroid) and minerals (Potassium, Calcium, Sodium) with therapeutic potential in fonio millet. Abundant evidence in literature about the nutritional and health promoting effects of fonio millet as well as it's presume suitable food for diabetics may be the reason for its wide recommendations by the locals for diabetes management when in the real sense, there is little or no substantive scientific evidence of its potency against the disease. Therefore, this study is aimed at determining the potency of fonio millet to attenuate diabetes and its related cardiovascular complications.

Materials and Methods

Chemicals and reagents

Streptozotocin, sulfanilamide, *N*-(1-naphthyl) ethylenediamine(NED), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Ellman's reagent) and Pyrogallol (All purchased from Sigma Aldrich). Trichloroacetic acid (TCA), Thiobabituric acid (TBA), Hydrochloric acid (HCl)

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(Purchased from standard chemical outlets). All other chemicals and reagents used were of standard grades.

Animals

Adult male Wistar rats weighing between 170-210 g were procured from the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. The animals were kept in standard cages and allowed to acclimatize to the new environment for two weeks. They were allowed free access to drinking water and food (vital feeds, Bukuru, Jos, Nigeria) *ad libitum.* All animals were cared for in accordance to the internationally accepted practices for use and care of laboratory animals as published in US guidelines.[9] This research was approved by the ethics and animal welfare committee of the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma (No. BCHEAWC-02/07/2018).

Medicinal plant

Fonio millet (*Digitaria exilis)* was purchased from Tudun Wada Market, Zaria, Kaduna State in July, 2018 and was identified by Mr. Edayi Frederick of Botany Unit, Federal University Dutsinma with a specimen voucher (No 10176).

Preparation and extraction of plant material

This was carried out according to the method described by Osibemhe *et al.*[10] Washed and sun-dried fonio millet grains were made into fine powdered form using a mechanical pulverizer. Measured quantity (3000 g) of the powder was macerated in distilled water (7500 mL). The solution was left for 72 hours in a refrigerator (to avoid microbial contamination) with periodic starring. The solution was subsequently filtered after 72 hours using cheese cloth. The resultant filtrate was further filtered using Whatman No. 42 (125 mm) filter paper. The collected filtrate was freeze dried using a freeze dryer and the powder was kept in properly sealed container for further use.

Induction of diabetes with Streptozotocin (STZ)

Diabetic was induced in rats (fasted overnight but with free access to water) by intraperitoneal injection(single dose, using insulin syringe) of freshly prepared STZ, at a dose of 60 mg/kg body weight reconstituted with 0.9% saline (in a ratio of 10 mg/mL). Diabetes was confirmed on the 10th day post STZ treatment by the observation of fasting blood glucose (FGB) (mmol/L) that was three times greater than the basal values using glucometer (accu-check).[11]

Experimental design

Twenty-five (25) adults rat (Wistar strain) weighing between 170-210 g were used. The rats were weighed and grouped into five (5) groups of 5 rats/group according to range in body weight. Two groups served as normal and diabetic controls, and were maintained on normal diet and water daily. The other three groups served as diabetic rats treated with 10% (10 g fonio millet + 90 g animal feed), 20% (20 g fonio millet + 80 g animal feed) fonio millet supplemented diet and 500 mg aqueous extract of fonio millet, respectively. The treatment which was carried out daily lasted for a period of 14 days. 500 mg aqueous extract was administered orally using gavage. Blood glucose was monitored weekly while nitric oxide, MDA and some antioxidant enzymes (Catalase, GPX, and GSH) were assayed in heart homogenate after the 14th day.

Collection and preparation of sample for analyses

At the end of the experiment, on the 14th day, the rats were fasted overnight and sedated with chloroform by following all safety operation procedures for chloroform use. The organ (heart) was excised and washed in ice-cold saline. Subsequently, weighed portion of the organ, 200 mg was homogenized with 8 mL of 0.1 M phosphate buffer (pH 7.4) and was centrifuged at 3500 rpm for 15 min and the resultant supernatant was used for the biochemical analyses.

Biochemical analysis:

Glucometer (accu-check) was used to analyze fasting blood glucose. In this procedure, the tail of restrained rats were cleaned with cotton wool containing a disinfectant (methylated spirit).The tail was massage, and the tip was pricked with a syringe. A drop of blood was placed on the test strip already inserted in the glucometer. The result (mmol/L) displayed on the screen of the meter was recorded. Catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) and malondialdehyde (MDA) were assayed using standard methods of Cohen *et al.* (1970),[12] Chance and Maehly (1955),[13]Ellman (1959)[14]and Buege and Aust (1978),[15] respectively. Nitric oxide was assayed using Griess method[16] and its concentration extrapolated from nitrite standard curve (Figure 1).

Statistical analysis

Data were presented as means ± SEM (n=5). One-way ANOVA was used to compare the means followed by Duncan post-hoc test using the Statistical Package for the Social Sciences (SPSS) version 16. Statistical significant difference was set at $P<0.05$.

Results and Discussion

Insulin resistance is implicated in both type 1 and type 2 diabetes mellitus and peripheral insulin resistance plays a fundamental role in the pathophysiology of diabetes[17] as well as decreased insulin secretory capacity.[18] Diabetes is known to be associated with cardiovascular diseases. Although other factors may be responsible for the development of cardiovascular diseases, the disease is more prevalent in diabetic patients (type 1 and 2).[19] The pathophysiological mechanism for the development of cardiovascular disease in diabetes is yet to be fully elucidated; although hyperglycemia appears to be a major contributing factor.[20] In the present study, the results of the fasting blood glucose as presented in Figure 2 showed significant *($p<0.05$)* increases in fasting blood glucose in diabetic control animals from day 1-14 when compared with the basal values.

Groups treated with 10% and 20% fonio millet supplemented diet demonstrated significant *($p<0.05$)* fasting blood glucose reduction from day 7-14th. Oral administration of 500 mg/kg aqueous extract did not show any significant change in fasting blood glucose level (Figure 2)

The significant increase in fasting blood glucose of diabetic rats observed in this study may not be unconnected to the effects of streptozotocin on the rat's pancreatic β-cells. Streptozotocin action causes β-cells destruction by necrosis.[21] According to Damasceno *et al.,*[22] loss of pancreatic islet cells and diminished insulin-producing beta cell mass are contributing factors to the pathogenesis of diabetes. Hence the increased fasting blood glucose in streptozotocin-treated rats is understandable. However, while 10% and 20% *Digitaria exilis* exhibited fasting blood glucose reduction progressively from the 7-14th day in the diabetic-treated rats, diabetic rats treated with 500 mg/kg did not produce any significant effect on fasting blood glucose level. The improvement of blood glucose by 10% and 20% of *Digitaria exilis* may be because of the insoluble fibre content contained in them. According to Koréissi*et al.,*[7]fonio millet is known to contain high content of fibre and low fat. Caroline *et al.*[23] have reported improvement in glucose homeostasis in animals fed with cereal fibre. Numerous epidemiological investigations have documented dietary fibre as one of the most effective nutrient components for diabetes prevention.[24] Other researchers have also documented cereal fibres to possess the most preventive effect on diabetes.[25,26] The risk of developing long-term diabetes complications can be reduced with good glycemic control.[27] Although Weickert *et al.*[28] have attributed the mechanisms of insoluble cereal fibres to reduce blood glucose to their ability to improve insulin sensitivity; we believe, though not investigated, that the glucose lowering potential of *Digitaria exilis* in this study might have exploited additional mechanism owing to the mechanism of streptozotocin action, which selectively destroys the β-cells of the pancreas.

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Possible mechanisms may be the exploitation of notable health promoting phytochemicals/minerals reported to be present in the plant. For example, saponin has been reported to contribute to glucose reduction in diabetics by its potential to regenerate beta cells of the pancreas and the ability to activate enzymes required for glucose utilization.[29]

It is important to note that oxidative stress (imbalance between free radicals and anti-oxidants in the body), apart from its involvement in eliciting specific cardiac endpoints; is implicated with the risk of developing cardiovascular diseases. Several pathways exist by which oxidative stress elicits or exacerbates cardiovascular outcomes including endothelial dysfunction.[30]Whichever pathway that is adopted, for apparent cardiovascular outcome, cellular oxidative imbalances is implicated.[31] Nitric oxide availability- simply put; endothelial dysfunction is the loss of endothelium-dependent vasodilation which results to failure of anticoagulation, vasoactive and anti-inflammatory effects of functional endothelium.[32] Nitric oxide (NO) plays an important role in the protection against the onset and progression of cardiovascular diseases. It plays a fundamental role in the regulation of endothelial dysfunction. Hyperglycemia causes reduction in endothelial nitric oxide synthase (eNOS) by increased production of superoxide anion, an activator of protein kinase C (PKC) pathway, thus reducing NO synthesis.[3] In this study, NO and antioxidants levels were not significantly ($P \geq 0.05$) altered in all the test groups when compared with the respective controls as presented in Figure 3 and Table 1, respectively. This may be ascribed to the duration of the study. According to ADA,[33] occurrence of structural damage in vascular endothelium and nervous tissues with a resultant dysfunction of the endothelium or failure of different organs and tissues is dependent on the degree and duration of exposure to hyperglycemia. Similarly, increasing evidence demonstrates excessive production of reactive oxygen species (ROS) to be implicated in the initiation and progression of cardiovascular outcomes.[34,35] Thus the non-significant effect observed in these parameters is not surprising.

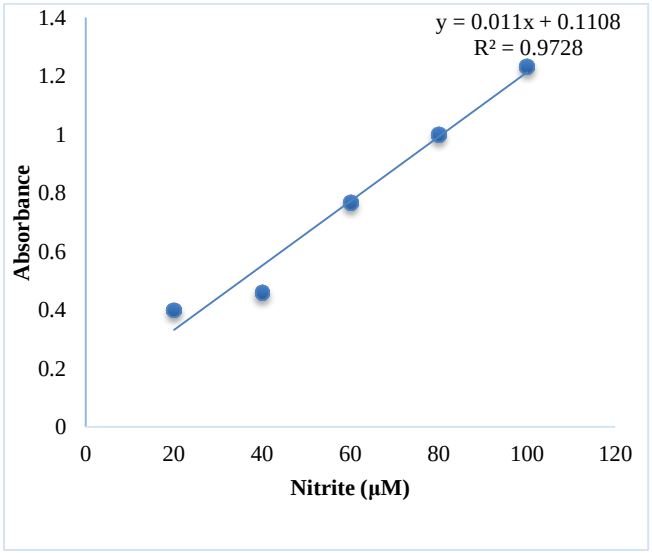

Figure 1: Nitrite standard curve with concentration ranging from 20 to 100 µM

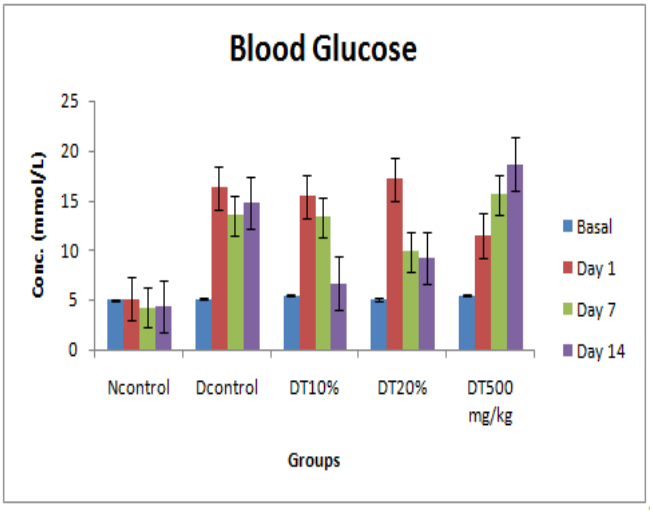

Figure 2: Mean fasting blood glucose concentrations (mmol/L) of normal and streptozotocin induceddiabetic rats treated with 500 mg/kg aqueous extract of fonio millet, 10% and 20% fonio millet supplemented diets respectively for a period of 14 days.
Values represent mean ± SEM (n=5). *p<0.05* compared with basal (before diabetes induction). (Ncontrol= Normal control, Dcontrol=Diabetic control, DT10%= Diabetic treated with 10% fonio millet supplemented diet, DT20%= Diabetic treated with 20% fonio millet supplemented diet, DT500 mg/kg= Diabetic treated with 500 mg/kg aqueous extract).

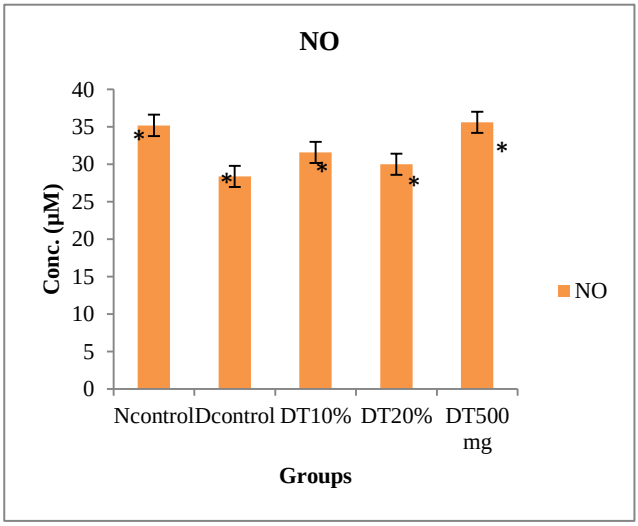

Figure 3: Nitric oxide(NO) concentrations (µM) of normal and streptozotocin induced diabetic rats treated with 500 mg/kg aqueous extract of fonio millet, 10% and 20% fonio millet supplemented diets respectively for a period of 14 days.
Values represent mean ± SEM (n=5). *p<0.05* compared with normal and diabetic controls. (Ncontrol= Normal control, Dcontrol=Diabetic control, DT10%= Diabetic treated with 10% fonio millet supplemented diet, DT20%= Diabetic treated with 20% fonio millet supplemented diet, DT500 mg= Diabetic treated with 500 mg/kg aqueous extract).

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Table 1: Mean cardiac antioxidant enzymes and MDA levels of normal and diabetic treated rats

	Antioxidant enzymes and MDA level			
Group	**Catalase (unit/mg protein)**	**GPX (unit/mg protein)**	**GSH (unit/ml)**	**MDA (nmol/mg protein)**
Ncontrol	0.06 ± 0.03^a	0.34 ± 0.07^a	0.12 ± 0.04^a	$3.8 \times 10^{-3} \pm 0.73^a$
Dcontrol	0.13 ± 0.02^a	0.35 ± 0.12^a	0.07 ± 0.03^a	$4.00 \times 10^{-3} \pm 0.78^a$
DT10%	0.12 ± 0.03^a	0.23 ± 0.07^a	0.11 ± 0.03^a	$4.40\times 10^{-3} \pm 0.81^a$
DT20%	0.15 ± 0.04^a	0.35 ± 0.12^a	0.07 ± 0.01^a	$4.40 \times 10^{-3} \pm 0.51^a$
DT500 mg	0.09 ± 0.03^a	0.17 ± 0.03^a	0.08 ± 0.01^a	$3.80 \times 10^{-3} \pm 0.49^a$

Values are expressed as activities of antioxidant enzymes and are means ±SEM (n=5). Values in the same column with the same superscript represent no significant difference *(p>0.05)* with the respective controls. (Ncontrol= normal control, Dcontrol= diabetic control, DT10%= dabetic treated with 10% fonio millet supplemented diet, DT20%= diabetic treated with 20% fonio millet supplemented diet, DT500 mg= diabetic treated with 500 mg

Conclusion

We conclude that 10% and 20% feed supplementation of fonio millet possesses anti-diabetic capacity. Nevertheless, its effects on cardiovascular events could not be ascertained by the results from this study and therefore requires further investigation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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