Research Article

Anti-diabetic properties of the seeds of fluted pumpkin (*Telfairia occidentalis* Hook f.) in a rat model of alloxan-induced diabetes mellitus

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**ABSTRACT:** Available treatment options for diabetes mellitus (a highly prevalent disease) are expensive and do not reverse the course of the disease such that affordable alternative treatment options are needed. The usefulness of boiled seeds of *Telfairia occidentalis* (Hook f.) in reducing the effects of diabetes in rats was studied. Diabetes was induced in 20 rats using alloxan monohydrate. The diabetic rats were grouped into four groups of 5 rats each, and treated with 0, 1250, 2500, and 5000 mg/kg bw of the seeds, respectively. After fourteen days, the mean blood glucose concentrations (BGC) of the test rats were significantly (*P* < 0.05) lower than those of the control group. There was a 42% and 73% reduction in the BGC of rats in the 1250 and 2500 mg/kg bw groups, respectively, compared to the negative control. The serum concentrations of triacylglycerol, total cholesterol and LDL-cholesterol of rats in the 5000 mg/kg bw group were significantly (*P* < 0.05) reduced, compared to the control. The seeds reduced the concentrations of aspartate aminotransferase by 11% and alkaline phosphatase by 15 in the 2500 mg/kg bw group, compared to the negative control group. The differences in the concentrations of these enzymes in the said group relative to the control were significant (*P* > 0.05). Histological observations of sections of the pancreas and livers of the studied rats corroborate these biochemical findings. Boiled seeds of *Telfairia occidentalis* may be useful in reducing the hyperglycemia, dyslipidemia and diabetic hepatocellular damage found in alloxan-induced diabetes in rats.

**KEYWORDS:** Diabetes mellitus, local foods, *Telfairia occidentalis*, treatment
INTRODUCTION

Diabetes mellitus, characterized by impairments in insulin secretion and/or action and the attendant dysregulation of carbohydrate metabolism, is currently a global health problem of alarming proportions (Ejike et al., 2015). The hyperglycemic state, which is the hallmark of diabetes mellitus, results in microvascular complications such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and diabetic cardiomyopathy. These conditions increase the morbidity due to diabetes mellitus and are responsible for the high mortality rates from the disease. At the end of 2013, as much as 382 million people around the world had diabetes mellitus and the number is expected to reach 592 million by 2035 (IDF, 2013). Painfully the burden of the disease is highest in low and middle-income countries (LMICs) where communicable diseases still take a good chunk of the (often) meagre healthcare budgets (Ejike et al., 2015).

The mainstay of the management of diabetes mellitus in its early stages is lifestyle modification (diet and exercise), and in advanced stages is pharmacological (hypoglycemic drugs, or exogenous insulin) treatments. The literature is however replete with reports of the failure of the above methods to reverse the course of the disease (Shori, 2015) and the deleterious side-effects that come with their use. The costs of these pharmacologic agents are often out of the reach of poor people in LMICs, where the use of traditional remedies is still widespread (Graf et al., 2010). Consequently, the World Health Organization (WHO) recommended the search for phytopharmacologic agents that can be used in the management of diabetes (WHO, 1980).

Sequel to the WHO recommendations, a lot of efforts has been geared towards the identification of novel botanicals with potential(s) in disease prevention and health promotion. The studies have however concentrated on extracts and fractions of plant parts, such that little attention is given to dietary botanicals that may have potentials in managing diseases such as diabetes mellitus. One of the dietary plants studied for possible diabetes-related properties is fluted pumpkin, *Telfairia occidentalis* Hook f. - an underutilized tropical oil- and protein-rich crop with many health benefits (Ejike et al., 2010a; Ejike and Ezeanyika, 2011). Extracts from the plant’s parts have been shown to possess antidiabetic, hypoglycemic and blood glucose lowering activities in different animal models through a variety of mechanisms (Aderbigbe et al., 1999; Nwozo et al., 2004; Oboh et al., 2012; Eseyin et al., 2007; Eseyin et al., 2010; Teugwa et al., 2013). Unfortunately, there is (to the authors’ knowledge) no study on the antidiabetic properties of the whole seed.

This study therefore aims to investigate the potentials of using the seeds of fluted pumpkin in managing diabetes mellitus and its complications. The results will be helpful in developing therapies (even if complementary) based on commonly available food materials.

![Figure 1: *Telfairia occidentalis* Hook f. fruit and leaves](image)

MATERIALS AND METHODS

Preparation of *Telfairia occidentalis* Hook f. seeds

Fresh fruits of *Telfairia occidentalis* (Fig. 1) were purchased from the Ogige Market, Nsukka. They were sliced open and the seeds harvested. The seeds were sorted to remove bad ones, and then extraneous materials around the seeds were removed. The seeds were thereafter washed thoroughly in running tap water. They were then boiled in tap water for 1 hr. Once cool, the seeds were shelled manually and the “meat” dried in an oven at 50°C for 72 hours. The dried samples were subsequently ground using an electric grinder (Starlight SL: 242), packed into labelled cellophane bags and refrigerated at 4°C until required for the experiments.

Animal handling and induction of diabetes

Adult male Wistar rats weighing 140-220 g were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatized to the animal house environment for four days during which period they were kept...
in humid tropical conditions, and fed normal rat chow and water, *ad libitum*. At the end of the acclimatization period, the rats were each given intraperitoneal injections of alloxan monohydrate (Sigma Aldrich, Hamburg) at a dose of 150 mg/kg body weight. Thereafter the rats were allowed to stabilize their blood glucose concentrations for two days, during which period they had free access to 5% glucose solution to avoid possible hypoglycemia. At the end of the second day, rats with a blood glucose concentration of 200 mg/dL or more were considered diabetic and thus used for the study.

**Experimental design**

The diabetic rats were randomized into four groups of five rats each. Group 1 served as the negative (diabetic) control group and were placed on normal rat chow only. Groups 2 to 4 served as the test groups and received 1250, 2500, and 5000 mg/kg body weight, respectively, of boiled, dried and ground *Telfairia occidentalis* Hook f. seeds. The experiment lasted for fourteen days and the rats had access to normal rat chow and tap water, *ad libitum*.

Blood glucose concentrations of the rats were determined at the beginning of the experiment, then at day 7 and at day 14. At the end of the experiment, the rats were dazed humanely and bled exhaustively from the retro-orbital plexus. Blood samples were collected into sterile properly labelled sample containers, and a drop used immediately for blood glucose determination. The rest were allowed to clot, and then centrifuged 3000 rpm for 5 minutes to separate the cells from the serum. The sera were then pipetted into duly labelled sterile containers and used for the relevant biochemical analyses. The livers and pancreas of the rats were immediately harvested for histological studies.

**Biochemical analyses**

Blood glucose concentrations were determined using a glucometer. Serum total cholesterol, high density lipoprotein (HDL) cholesterol and triacylglycerols were determined using standard enzymatic colorimetric techniques of Allain *et al.* (1974), Lopes-Virella *et al.*, (1977) and Tietz (1990) respectively; while low density lipoprotein cholesterol was estimated by difference (Friedewald *et al.*, 1972). The

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**Figure 2:** Blood glucose concentrations of diabetic rats treated with different doses of fluted pumpkin seeds for fourteen days *indicates significant difference (P < 0.05)* relative to the negative control group. Blood glucose concentrations were significantly reduced in the 1250 and 2500 mg/kg bw groups, relative to the negative control group on day 7; and in all test groups on day 14.
enzymatic colorimetric methods of Reitman and Frankel (1957) were used for the determinations of the serum concentrations of alanine and aspartate aminotransferases; while that of Rec (1972) was used for alkaline phosphatase determination.

**Histological studies**

The harvested livers and pancreas were carefully freed of external fasciae, rinsed in normal saline, blotted with filter paper, and fixed immediately in formal saline. The tissues were then dehydrated in grades of ethanol, cleared in xylene, then infiltrated with paraffin. This was followed by embedding in paraffin. A microtome was used to section the tissues at 5 μm. The sections were then stained with hematoxylin and eosin. They were subsequently mounted on a microscope, viewed and their photomicrographs taken (Magnification: x 400).

**Statistical analysis**

The data generated were analysed statistically. Means and standard deviations for each parameter per group were calculated. The one way ANOVA method followed by post hoc multiple comparisons were used to separate difference between means. A P value less than 0.05 was considered significant. Data analyses was done using the statistical software IBM-SPSS version 21.0 (IBM Corp. Atlanta, GA).

**RESULTS AND DISCUSSION**

The blood glucose concentration of the test rats (except those treated with 5000 mg/kg bw TO) reduced after seven days, to values that were significantly ($P < 0.05$) lower than that of the negative control. After fourteen days, all test concentrations of *Telfaria occidentalis* seeds resulted in significant reductions in the blood glucose concentrations of the test rats, relative to the negative control (Fig. 2). The effect of the seeds on blood glucose reductions in the studied diabetic rats were therefore dose-dependent up to the 2500 mg/kg bw dose. Figure 3 shows photomicrographs of the pancreas of the rats. It is observed that the pancreatic islets surrounded by exocrine cells are shrunken and damaged (and surrounded by a few cells) in all the groups except the 5000 mg/kg bw group where they were apparently normal.

Alloxan induces diabetes mellitus and the attendant insulin deficiency in a manner that is considerably similar to human insulin-dependent diabetes mellitus (Jin et al., 2013). Its active diabetogenic agent, a hydrophilic and chemically unstable pyrimidine derivative, that has close structural similarity with glucose, is taken up by the beta cells of the pancreas through GLUT-2 glucose transporters (Elsner et al., 2002; Lenzen, 2008). Within the beta cell, it exerts its toxicity, thereby destroying the cells (Ashok et al., 2011). However, there are reports of the possibility that a few beta cells survive and remain amenable to regeneration if appropriately stimulated. This appears to be responsible for the observation of anti-hyperglycemic activity with oral hypoglycemic agents in alloxan-induced diabetic rats (Subramoniam et al., 1996; Prince et al., 1999). Therefore, it is plausible that the seeds of *Telfaria occidentalis* may have stimulated the restoration and regeneration of the beta cells of the islets of Langerhans. It may have also acted as an insulin sensitizer thereby ensuring that even minimal insulin production by the re-growing cells resulted in maximal insulin action by priming the sensitivity of target tissues such as the liver, muscle and adipose tissue. The lack of significant reductions in the blood glucose concentrations of the test rats in the highest dose group at seven days is difficult to explain. It may however reflect a reduced adaptation of the rats to the test diet. The photomicrographs (Figure 3) corroborate the data on blood glucose concentrations and support the restoration of secretory activity of the pancreatic beta cells.

The antidiabetic property of the seeds reported here comes after reports that the leaves possess hypoglycemic properties (Nwozo et al., 2004; Eseyin et al. 2007). The ethanol extract of leaves, seeds and whole fruits were also shown to have hypoglycemic activity (Eseyin et al., 2010). Furthermore, more recently, Cucurbitaceae seeds (including those of
Figure 4: Lipid profile of diabetic rats treated with different doses of fluted pumpkin seeds for fourteen days. * indicates significant difference ($P < 0.05$) relative to the negative control group. Significant reductions (relative to the negative control) in serum triacylglycerols and low density lipoprotein cholesterol is observed especially in the 2500 mg/kg bw and 5000 mg/kg bw groups. Serum total cholesterol concentrations are also significantly reduced relative to the negative control, due to the treatment.

Figure 5: Enzyme markers of diabetic hepatocellular damage in rats treated with different doses of fluted pumpkin seeds for fourteen days. * indicates significant difference ($P < 0.05$) relative to the negative control group. Serum aspartate aminotransferase concentrations were significantly lower in the 1250 mg/kg bw and 5000 kg/mg bw groups, relative to the negative control.
Telfairia occidentalis have been reported to contain globulins with significant anti-hyperglycemic activity (Teugwa et al., 2013). We however do not currently have enough data to suggest which constituent(s) of the seeds is responsible for the observed activity. Whereas it may be the globulins mentioned by Teugwa et al. (2013), we are mindful that a variety of active principles may in fact work in concert to give the observed effect.

Figure 6. Photomicrographs of sections of the liver of diabetic rats treated with different doses of fluted pumpkin seeds for fourteen days. H and E stain; Magnification x 400. Note the high degeneration of hepatocytes (possibly fatty degeneration) in the Negative Control (arrow) and vascular congestion in the 1250 mg/kg bw group (white arrows) while hepatocytes in 2500 mg/kg bw and 5000 mg/kg bw groups are apparently normal (white arrows).

Figure 4 shows that the total cholesterol concentration of the test rats were significantly ($P < 0.05$) reduced in the 1250 mg/kg bw and 5000 mg/kg bw groups, relative to the negative control. The 2500 mg/kg bw group had statistically similar ($P > 0.05$) values compared to the control group. The triacylglycerol concentrations of all the test groups were significantly ($P < 0.05$) lower than that of the control group. Only the groups that received 2500 mg/kg bw and 5000 mg/kg bw of the Telfairia occidentalis seeds had significantly ($P < 0.05$) lower serum LDL concentrations compared to the control group. The 1250 mg/kg bw group had statistically similar ($P > 0.05$) values compared to the control group.

Serum HDL concentrations of all the test groups were statistically similar ($P > 0.05$) to the control group.

Clearly, whereas the boiled seeds reduced significantly the concentrations of triacylglycerol, total cholesterol and LDL cholesterol concentrations of test rats, relative to the negative control group, the HDL concentrations were not altered significantly ($P > 0.05$). It is nonetheless evident that the lipid abnormalities that accompany diabetes were considerably overcome as a result of treatment with the boiled seeds. Ejike et al. (2010b) had reported that diets incorporated with boiled Telfairia occidentalis seeds had a negative effect on blood glucose and lipids of normal rats only at concentrations in excess of 15 % of the diet. This study further shows that the seeds are beneficial in modulating lipid abnormalities arising from the induction of diabetes with alloxan monohydrate in rats.

Administration of 2500 mg/kg bw of Telfairia occidentalis seeds significantly ($P < 0.05$) reduced the serum concentration of ALT, relative to the control group. Serum AST concentrations were significantly ($P < 0.05$) lower in the 1250 mg/kg bw and 5000 mg/kg bw groups, relative to the control. Only treatment with 1250 mg/kg bw of the seeds reduced the serum ALP concentrations significantly ($P < 0.05$), compared to the control. The others had values that were statistically similar ($P > 0.05$) to the control group (Fig. 5). From the photomicrographs of the livers of the studied rats (Fig. 6), it is seen that there is a high degeneration of hepatocytes and vascular congestion in the control group compared to the test groups. These factors are observed to improve with increasing concentrations of Telfairia occidentalis seeds, such that they appear normal in the 2500 mg/kg bw and 5000 mg/kg bw groups. These data suggest that the seeds were able to ameliorate the impact of diabetic liver damage in the test rats. It also suggests consuming the boiled seeds may not lead to hepatotoxicity, and therefore corroborates the findings of Ejike et al. (2010a).

In conclusion, this study shows that the boiled, dried and ground seeds of Telfairia occidentalis (Hook f.) reduced the blood glucose concentrations of diabetic rats significantly. The seeds also resulted in a better lipid profile in the diabetic rats, and minimized the liver damage associated with sustained hyperglycemia typical of diabetes. Additional studies are required to illumine the exact mechanism of action of the active constituents of the seeds.

Declaration: The design and protocol for this study were approved by the Department of Home Science, Nutrition and Dietetics, University of Nigeria. The authors have no real or potential conflicts of interest to declare.
REFERENCES


