**Anti-diabetic effects of okra \[Abelmoschus esculentus (L.) Moench\] fruits in alloxan-induced diabetic rats**

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**ABSTRACT:** The development of affordable treatment options for diabetes mellitus is critical to reversing the rising prevalence of the disease. Therefore, the usefulness of the whole fruits of *Abelmoschus esculentus*, a popularly consumed plant food, in treating diabetes and its comorbid conditions was studied in alloxan-induced diabetic rats. Twenty diabetic rats were assigned to four groups of five rats each. They were treated with 0, 1250, 2500, and 5000 mg/kg bw of the ground fruits, respectively. After 14 days of treatment, the blood glucose concentration (BGC) of the test rats decreased by 44%, 67% and 78% in the 1250, 2500 and 5000 mg/kg bw groups, respectively, compared to the negative control group. At high concentrations, the fruits reversed the dyslipidemia that is associated with diabetes mellitus. For instance, in the 5000 mg/kg bw group, total cholesterol and triacylglycerol concentrations were 27% and 39%, respectively, lower; while high density lipoprotein cholesterol concentration was 21% higher, than the control group. At 2500 mg/kg bw or above, the fruits significantly (\(P < 0.05\)) reduced the concentrations of serum alanine and aspartate amino transferases and alkaline phosphatase, relative to the control group. All the biochemical data are corroborated by histological findings of the pancreas and livers of the studied rats. The whole fruits of *Abelmoschus esculentus* possess noteworthy antidiabetic properties and significantly reversed diabetes induced dyslipidemia and liver damage in rats.

**KEYWORDS:** *Abelmoschus esculentus*, diabetes mellitus, local foods, treatment
INTRODUCTION

Diabetes mellitus is a chronic disease attributed to gene-environment interactions. It is currently one of the leading chronic diseases globally, and particularly in low and middle income countries (LMICs) where communicable diseases still take a large portion of the (often) meagre healthcare budgets (Ejike et al., 2015). It is estimated that about 382 million people around the world had diabetes mellitus at the end of 2013. The number is expected to reach 592 million by 2035 (IDF, 2013). The disease is characterised by impaired insulin secretion and/or action and clinically presents as sustained hyperglycaemia in affected individuals. Treatment for diabetes includes the use of pharmacological agents, exogenous insulin administration, and lifestyle modification. The most widely used pharmacological agents are drugs such as sulfonylureas, meglitinides, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, biguanides, sodium glucose transporter 2 inhibitors, α-glucosidase inhibitors, amylin analogues and glucagon-like peptide-1 (GLP-1) receptor agonists (Pelikanova, 2009; Bos and Agyemang, 2013).

However, there are reports in the literature that the existing treatment options do not reverse the course of the disease (Pelikanova, 2009; Shori, 2015). It is reported that 50% to 60% of diabetic patients treated with conventional means have achieved their glycemic goals (Tavafi, 2016). Furthermore, deleterious side-effects are associated with the use of the often expensive antidiabetic drugs (Graf et al., 2010; Tavafi, 2016). The World Health Organization (WHO) has recommended the search for cheap and easy-to-access phytopharmacologic agents that can be used in the management of diabetes (WHO, 1980). Consequently, efforts are geared towards the identification of novel botanicals with potential(s) in diabetes prevention and treatment.

*Abelmoschus esculentus*, (L.) Moench, commonly known as Okra or Lady Finger, is a flowering plant of the mallow family that is native to Southern Europe, Asia and Africa. It has a wide range of medicinal applications owing to its rich milieu of bioactive constituents. Its usefulness in diabetes control has been reported (Sabitha et al., 2011; Gemede et al., 2015; Akbari et al., 2016; Mishra et al., 2016; Hajian et al., 2016; Prabhune et al., 2017). It is reported that when oOkra is taken regularly as a part of diet, it confers protective effect against diabetes on the subject (Moise et al., 2012). Though there have been a lot of work done on the usefulness of Okra in the management of diabetes (Prabhune et al., 2017), there has however been no report, to the authors’ knowledge, on the usefulness of the whole fruits of the plant in treating diabetes and its comorbid conditions. This study therefore investigated the subject in alloxan-induced diabetic rats.

**Materials and Methods**

Fresh fruits of *Abelmoschus esculentus* (Fig. 1) were obtained from the Ogige Market, Nsukka. They were sorted to remove extraneous materials and bad fruits. The sorted fruits were thoroughly washed with clean running tap water to remove adhering dirt. Thereafter they were cut into small circular discs and then ground to a consistent paste using an electric blender (Starlight SL: 242). The okra paste was packaged in plastic containers and stored in a freezer at -4°C until used.

**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 achieve stable blood glucose concentrations for two days. In this period the animals 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 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 ground *Abelmoschus esculentus* fruits. 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 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 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. Thereafter, the samples were
cells of the pancreas through GLUT-2 glucose transporters (Elsner et al., 2002; Lenzen, 2008), it exerts its toxicity by oxidatively destroying the cells (Ashok et al., 2011). Therefore, 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). It is consequently logical that any antidiabetic agent should cause the reduction in blood glucose concentrations of the subject(s). On this rests the promise of *Abelmoschus esculentus* fruits used for this study.

At the end of the study, the blood glucose concentrations (mg/dL) of the test rats were 163.0 ± 49.5, 98.8 ± 22.6, 65.0 ± 22.9 for the 1250, 2500 and 5000 mg/kg bw groups, respectively, as against 293.3 ± 43.4 mg/dL for the negative control group. The blood glucose concentrations of rats in all the test groups dropped to significantly lower (*P* < 0.05) concentrations relative to the negative control group both on the seventh and fourteenth days (Fig. 2). Figure 3 shows the improvement in the architecture of the pancreatic islets as seen in the 1250 and 5000 mg/kg bw (AE-G1 and AE-G3, respectively) groups. The islets are also seen surrounded by more exocrine cells, compared to what is seen in the photomicrographs of the negative control group. Clearly the test agent showed significant antidiabetic activity by lowering blood glucose concentrations and improving the architecture of the cells of the islets of Langerhans.

There are reports of the possibility that a few beta cells survive the destruction of islet cells by alloxan, and remain amenable to regeneration if appropriately stimulated. This appears to be responsible for the observation of antihyperglycemic activity with oral hypoglycemic agents in alloxan-induced diabetic rats (Subramoniam et al., 1996; Prince et al., 1999). It is therefore plausible that the bioactive principles in *Abelmoschus esculentus* fruits induced a regeneration of the islet cells, improved insulin secretion and therefore reduced blood glucose concentrations. Dayal et al. (2012) earlier reported that okra seed extracts protected against oxidation-induced inflammation and destruction of pancreatic β-cells. In fact, several authors have reported the antidiabetic properties of the plant (Sabitha et al., 2011; Gemede et al., 2015; Akbari et al., 2016; Mishra et al., 2016; Hajian et al., 2016). Some of the hitherto identified antidiabetic compounds found in *Abelmoschus esculentus* are oleanolic acid, β-sitosterol, myricetin, and kaempferol (Prabhune et al., 2017). Additionally, polysaccharides in the plant have been shown to maintain blood glucose levels in the normal range by modulating the control of glucose absorption from the lumen of the small intestine (Xia et al., 2015). It appears evident that okra is useful in the management of diabetes mellitus.
Figure 4: Lipid profile of diabetic rats treated with different doses of okra fruits for fourteen days. * indicates significant difference ($P < 0.05$) relative to the negative control group. Serum total cholesterol concentrations were reduced significantly in all the test groups and serum triacylglycerols were significantly reduced in the 2500 mg/kg bw and 5000 mg/kg bw groups, relative to the negative control. HDL-Cholesterol concentrations were significantly increased only in the highest dose group, relative to the negative control group.

Figure 5: Enzyme markers of diabetic liver damage in rats treated with different doses okra fruits for fourteen days. * indicates significant difference ($P < 0.05$) relative to the negative control group. Serum Aspartate aminotransferase concentrations were dose-dependently reduced, and significantly reduced in the 2500 mg/kg bw and 5000 mg/kg bw groups, relative to the control group. Though serum alkaline phosphatase concentrations were does-dependently reduced, only the 5000 mg/kg bw group reached statistical significance.
not only reduced hyperglycemia, but also reversed dyslipidemia in streptozotocin-induced diabetic rats. This implies that the fruits are useful, irrespective of the agent used in inducing the disease.

The enzyme markers of hepatocellular integrity show that the treatments significantly \( P < 0.05 \) reduced the alanine aminotransferase (ALT) concentrations of the test rats only in the 2500 mg/kg bw group relative to the control group. The serum aspartate aminotransferase (AST) concentrations were significantly \( P < 0.05 \) reduced in the 2500 and 5000 mg/kg bw groups, compared to the control group. Significant \( P < 0.05 \) reductions (compared to the control) in serum alkaline phosphatase (ALP) concentrations were seen only in the highest dose group (Fig. 5). From Figure 6 it is seen that the degeneration and necrosis seen in the hepatocytes in the negative control (CONT-G) and the low dose (1250 mg/kg bw; AE-G1) groups, are absent in the higher dose treatment groups [2500 mg/kg bw (AE-G2) and 5000 mg/kg bw (AE-G3)]. The restoration of the architecture of the hepatocytes suggests an amelioration of toxicity due to diabetes and indicates that the \textit{Abelmoschus esculentus} fruits used in the study were not hepatotoxic. This effect however appears more prominent at higher doses of the fruits. It is not surprising that there is no toxicity attributable to the fruits as the fruits are used in local delicacies and are even eaten raw. Gemede \textit{et al.} (2015) noted the absence of any reports of toxicity due to the use of the fruits.

In conclusion, the whole fruits of okra, \textit{Abelmoschus esculentus}, were studied for their antidiabetic properties and capacity to modulate diabetes induced complications in alloxan-induced diabetic rats. The results show that the whole fruits were capable of significantly reducing the blood glucose concentrations of diabetic rats. They also positively modulated the blood lipids and thereby reversed considerably the dyslipidemia associated with diabetes. Finally the whole fruits of \textit{Abelmoschus esculentus} reversed the liver damage induced by alloxan-induced diabetes and did not show any toxicity in the studied rats. The whole fruits of \textit{Abelmoschus esculentus} therefore are useful in the management of diabetes mellitus induced by alloxan monohydrate in rats.

\textbf{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.

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REFERENCES


