Effect of *Abelmoschus esculentus* (okra)-based diet on streptozotocin-induced diabetes mellitus in adult Wistar rats

Patrick O Uadia*1, Isaac O Imagbovomwan1, Kelly Oriakhi2, Ikechi G Eze3

1Department of Biochemistry, Faculty of Life Sciences, 2Department of Medical Biochemistry, 3Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

*For correspondence: Email: psouadia@uniben.edu; Tel: +234(80)27443746

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Abstract

**Purpose:** To evaluate the effects of an okra-based diet on streptozotocin-induced diabetes mellitus in adult Wistar rats and its mechanism of action.

**Methods:** Wistar rats (6) were administered streptozotocin (50 mg/kg ip) after an overnight fast. Upon confirmation of diabetes mellitus, the animals were fed ad libitum for 21 days with formulated okra-based test diet in place of normal diet. The rats treated similarly with streptozotocin and fed ad libitum with the normal diet served as diabetic control while rats fed on normal diet and not treated with streptozotocin served as the negative control. Thereafter, the rats were sacrificed, fasting blood collected and analysed for glucose concentration and biochemical parameters. Pancreas was also excised for histopathological studies.

**Results:** There was a significant increase in body weight, HDL-cholesterol (p < 0.05) but significant decrease in blood glucose (p < 0.05), serum total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol concentrations in the okra-fed diabetic treated rats when compared to the diabetic control group. Furthermore, superoxide dismutase activity (SOD) was significantly higher in the diabetic control, and reduced significantly when fed with okra-based diet (p < 0.05). Catalase (CAT) activity was significantly (p < 0.05) decreased in diabetic control and treated group, whereas it was significantly (p < 0.05) increased in normal control rats. There was a significant (p < 0.05) decrease in reduced glutathione levels. The significant (p < 0.05) increase in malondialdehyde in the diabetic group was significantly decrease in the diabetic rats fed with okra-diet. Also serum insulin level was significantly (p < 0.05) increased and serum α-amylase activity was significantly (p < 0.05) decrease in the diabetic treated rats. Histology results show that there was damage to the β cells of the pancreas in the diabetic control when compared to normal control, but rats fed okra diet was able to regenerate endocrine β cells.

**Conclusion:** Okra-based diet lowers hyperglycaemia as well as regenerate/repair endocrine β-cells and exocrine tissues of the pancreas damage by streptozotocin

**Keywords:** Abelmoschus esculentus, Diabetes mellitus, anti-diabetic, Insulin, α-amylase, Mechanism
INTRODUCTION

Folk or indigenous medicine which is also called conventional medicine comprises of medical aspects of traditional knowledge that evolved over generations within various societies before the age of modern medicine [1]. The use of traditional, complementary and unconventional medicine is extensive in many countries of the world, expressly amongst patients with chronic illnesses [1,2].

Conventional Western biomedicine is increasingly regarded as costly, inaccessible, depersonalized and not completely effectual especially for patients with chronic diseases [2]. Traditional healing rituals have existed in Africa long before conventional medicine [3].

Diabetes mellitus is a chronic disease associated with obesity, ageing, lack of physical activity, and genetic disposition [4]. Type II diabetes is in most cases caused by insulin insensitivity leading to health complications, such as cardiovascular disease, diabetic nephropathy, diabetic retinopathy, glaucoma and amputation of the limbs [4]. All over the world, approximately 1.3 million deaths were reported due to diabetes and its associated complications [5]. The major objective in managing diabetes is to maintain blood glucose level to prevent diabetic complications [4].

Pharmacological agents used to manage diabetes include those that stimulate insulin secretion (sulphonylureas), delay digestion, reduce hepatic glucose production (biguanides) and absorption of intestinal carbohydrate (α-glucosidase inhibitors) or improved insulin action (thiazolidinediones) [6]. However, pharmacologic agents may ultimately fail to alter the rate of progression of hyperglycemia [6], thus failing to prevent the progress of systemic complications arising from unregulated blood glucose levels [7].

Abelmoschus esculentus generally known as Okra or Lady Finger is in demand all over the world as a vegetable for its health and nutritional benefits [8]. Traditionally, it is used as an alternative remedy for diabetes [9].

Previous study has shown that extract from okra fruit possess antidiabetic activities and there is dearth of literature on the use of whole okra in diabetic studies and this research therefore, seeks to evaluate for the first time the effect of a diet compounded with whole okra fruit on streptozotocin-induced diabetic rats as well as the determination of its mechanism of action.

EXPERIMENTAL

Chemicals and reagents

Streptozotocin (STZ) of analytical grade, citric acid, tri-sodium citrate, triglyceride kit, total cholesterol kit, and high-density lipoprotein-cholesterol kit were purchased from Sigma-Aldrich (USA). Amylase kit was the product of Agappe Diagnostics, Switzerland and insulin ELISA kit was obtained from Cal biotech, USA.

Collection and identification of okra fruit

Okra was purchased from a vegetable market in Benin City, Edo State, Nigeria. It was identified and authenticated by Dr. Akinibosun Henry A. (FLS) a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Plant specimen (voucher number UBH-A442) was deposited at the Herbarium of the University of Benin.

Thereafter, they were sorted, washed and stumps trimmed off, sliced and air dried for seven days and ground into fine powder. The dried ground powdered A. esculentus fruit was stored in an air-tight container until used for the study”.

Diet formulation

Diets were formulated (Table 1) in powdered form into pelleted to minimize waste and reduce segregation of the feed.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal Diet(G)</th>
<th>Test Diet(G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>62.5</td>
<td>31.25</td>
</tr>
<tr>
<td>Casein</td>
<td>14.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Fibre (ground filter paper)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Premix vitamin</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Okra powder</td>
<td>-</td>
<td>31.25</td>
</tr>
</tbody>
</table>

Animals

Eighteen (18) adult male Wistar rats were obtained from the Animal House in the Department of Biochemistry, University of Benin, Benin City, Nigeria. The animals were provided access to food and clean water ad libitum under controlled environmental temperature (28 ± 2°C), and 12-hour light/dark cycle. They were equally allowed to acclimatize to the formulated normal diet for two weeks.
Experimental design

The rats were divided into three groups of six animals each and treated as follows:

Group I (normal control): Rats fed the normal formulated diet and allowed access to distilled water ad libitum.

Group II (diabetic control): Diabetic rats were fed the normal formulated diet, as in group one ad libitum.

Group III (diabetic treated): Diabetic rats were fed the okra-based diet (test diet) and allowed access to distilled water ad libitum.

Diabetes mellitus was induced in rats following an overnight fast, by a single intraperitoneal injection of streptozotocin (0.09g of STZ in 2.93 ml of cold citrate buffer at pH 4.5) at a dose 50mg/kg b.wt. Then 10% glucose solution was administered via oral gavage after 6h. Hyperglycaemia was monitored for 72 hr after injection by measuring the blood glucose level using a glucometer (Accu-check, Roche Diabetes Care Inc. USA). Under this condition, only the streptozotocin-treated rats with fasting glucose levels ≥200mg/dL were considered diabetic and used for the study.

Fasting blood glucose levels obtained from the tail vein of the animals was checked before the start of the experiment (day 0 or the day streptozotocin injection was administered) and after administration of streptozotocin injection (day 3). Thereafter, the blood glucose was monitored on days 7 and 14 with a glucometer (Accu-check). The animals were weighed at the beginning and at the end of the feeding period. On the 21st day, the animals after an overnight fast were anaesthetized and blood was collected via cardiac puncture into fluoride oxalate tube and plain tube respectively. Blood collected in the fluoride oxalate tube was centrifuged immediately and plasma glucose assayed using the glucose oxidase method. Blood collected in the plain tubes was allowed to stand for 2h at room temperature before centrifuging at 3000 rpm for 10min. The serum was used for the estimation of various parameters and keep in a deep freezer when not in use. Furthermore, the pancreas of each rat was excised and preserved in formalin for histopathology studies.

Biochemical analyses

Blood glucose concentration, serum α-amylase, serum total cholesterol, high density lipoprotein (HDL) cholesterol and triacylglycerol levels were determined using Randox kits (Sigma-Aldrich, USA), while low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesters were estimated by the method described by Friedewald et al [10]. Serum insulin level was evaluated using insulin ELISA kit (Calbiotech, USA). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) activities were determined as earlier described [11-13], while reduced glutathione and malondialdehyde (MDA) were determined by a slightly modified method of Burge and Aust [14] and Tietz [15] respectively.

Histopathology Studies

The harvested pancreas carefully freed of external fasciae was rinsed in normal saline (0.9% NaCl), blotted with filter paper, and fixed immediately in formalin for 24 hours. Thereafter, the tissues were dehydrated in ethanol, cleared in xylene, and then infiltrated in paraffin wax. A microtome was then used to section the tissues at 5μm after which they were de-paraffinized in xylene twice for 5 minutes and then rehydrated with ethanol and stained with haematoxylin and eosin (H & E) dye. The photomicrographs of the stained sections were subsequently taken (magnification: ×100).

Statistical analysis

Data analysis of the results was done by one-way analysis of variance (ANOVA) using IBM-SPSS version 21.0. Duncan’s comparison test was employed to determine the significant difference between means [16]. Significance was set at p < 0.05.

RESULTS

Effect of formulated okra-based diet on body weight in STZ-induced diabetes mellitus in rats

The effect of the formulated okra-based diet on body weight in STZ-induced diabetes mellitus in rats is shown in Table 2. There was a reduction in the weight of the diabetic control rats on day 21 while the diabetic test rats fed the okra-based diet gained weight.

Effect of formulated okra-based diet on blood glucose concentration in STZ-induced diabetes mellitus in rats

The level of glucose in the blood of the diabetic control group was significantly elevated (p<0.05) as compared to the normal control (Table 3). However, there was a significant reduction
(p<0.05) in blood glucose level in rats fed okra-based diet as compared to that of the diabetic control group. The glucose in the blood of the rats fed the okra-based diet was comparable with that of the normal control group at the end of the experimental period.

Table 2: Effect of okra-based diet on body weight changes (g) in streptozotocin-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial-body weight (g)</th>
<th>Final-body weight (g)</th>
<th>Weight gain/loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>132.66±4.41a</td>
<td>164.66±8.82bc</td>
<td>24.50±6.78c</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>178.33±5.46c</td>
<td>150.66±2.74b</td>
<td>-14.20±2.77a</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>160.33±3.83b</td>
<td>163.50±2.63bc</td>
<td>1.78±1.12b</td>
</tr>
</tbody>
</table>

Data were stated as mean ± SEM, n=6. Values with same superscripts are not statistically significant but values with different superscript letters are significant (p<0.05)

Effect of formulated okra-based diet on serum lipid profile in STZ-induced diabetes mellitus in rats

There was significant increase (p<0.05) in the levels of serum total cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol in the diabetic control rats compared to the normal control rats (Table 4). However, rats fed okra-based diet had a significant decrease (p<0.05) of these lipids as compared to those of the diabetic control. HDL-cholesterol level was significantly (p<0.05) reduced in the diabetic control rats but was increased appreciably upon feeding with the okra-based diet.

Table 3: Effect of Okra-based diet on blood glucose (mmol/L) in streptozotocin-induced diabetic Wistar rats

<table>
<thead>
<tr>
<th>Glucose (mmol/L)</th>
<th>Group</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.39±0.31a</td>
<td>4.24±0.31a</td>
<td>4.03±0.29a</td>
<td>4.04±0.19a</td>
<td>4.98±0.19ab</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>4.57±0.35a</td>
<td>14.18±0.79c</td>
<td>15.23±0.62d</td>
<td>16.31±0.58e</td>
<td>18.11±0.47f</td>
<td></td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>3.59±0.39a</td>
<td>1.11±0.14a</td>
<td>1.03±0.01b</td>
<td>2.05±0.43a</td>
<td>0.51±0.06a</td>
<td></td>
</tr>
</tbody>
</table>

Data were stated as mean ± SEM, n=6. Values with same superscripts across the row are not statistically significant but values with different superscript letters are significant (p<0.05)

Table 4: Effect of Okra-based diet on serum lipid profile (mmol/L) in streptozotocin-induced diabetic Wistar rats

<table>
<thead>
<tr>
<th>Lipid profile (mmol/L)</th>
<th>Groups</th>
<th>Total Cholesterol</th>
<th>Triacylglycerol</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>2.21±0.08a</td>
<td>1.21±0.08a</td>
<td>1.09±0.01c</td>
<td>0.57±0.10a</td>
<td>0.55±0.04a</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>11.30±0.96c</td>
<td>2.56±0.26b</td>
<td>0.74±0.02a</td>
<td>10.01±0.97b</td>
<td>1.17±0.12c</td>
<td></td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>3.59±0.39a</td>
<td>1.11±0.14a</td>
<td>1.03±0.01b</td>
<td>2.05±0.43a</td>
<td>0.51±0.06a</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM, n=6. Values with same superscripts are not statistically significant but values with different superscript letters are significant (p<0.05)

Table 5: Effect of Okra-based diet on serum antioxidants and lipid peroxidation in streptozotocin-induced diabetic Wistar rats

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Group</th>
<th>SOD (Units/mL)</th>
<th>CAT (Units/mL)</th>
<th>GSH (µmol/L)</th>
<th>GPx (Units/mL)</th>
<th>MDA (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.0022±0.0001a</td>
<td>0.44±0.03c</td>
<td>0.05±0.01b</td>
<td>0.0025±0.0001a</td>
<td>0.0081±0.0016e</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.0028±0.0001b</td>
<td>0.32±0.01b</td>
<td>0.02±0.00a</td>
<td>0.0023±0.0000a</td>
<td>0.0117±0.0002c</td>
<td></td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>0.0023±0.0001a</td>
<td>0.12±0.01a</td>
<td>0.02±0.00a</td>
<td>0.0023±0.0001b</td>
<td>0.0109±0.0001b</td>
<td></td>
</tr>
</tbody>
</table>

Data were stated as mean ± SEM, n=6. Values with same superscripts across the column are not statistically significant but values with different superscript letters are significant (p<0.05)
Table 6: Effect of *Abelmoschos esculentus*-based diet on serum alpha amylase activity (U/L) and insulin level (µlU/mL) in streptozotocin-induced diabetic Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>α-Amylase(U/L)</th>
<th>Insulin(µlU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.84±1.95a</td>
<td>8.00±0.00c</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>41.31±2.84b</td>
<td>7.05±0.02a</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>38.14±3.38ab</td>
<td>7.60±0.04b</td>
</tr>
</tbody>
</table>

Data were stated as mean ± SEM, n=6. Values with same superscripts across the column are not statistically significant but values with different superscript letters are significant (p<0.05).

Effect of formulated okra-based diet on the activity of serum alpha amylase (U/L) and insulin Level (µlU/ml) in STZ-induced diabetes mellitus in rats

A significant (p< 0.05) increase in serum α-amylase activity was observed in the diabetic control rats but a decrease in insulin level when compared with the normal control rats (Table 6). However, upon treatment with okra-based diet, there was significant (p< 0.05) decrease in serum alpha amylase activity and a significant increase in the diabetic treated rats when compared with the diabetic control rats.

Histopathology

The photomicrographs (Figure 1) of the pancreas show the relationship between STZ-induced diabetic rats and rats feed with the formulated okra-based diet.

DISCUSSION

In this study, *Abelmoschos esculentus*-based diet possess antidiabetic and anti-hyperlipidemic activities. It was also observed that the formulated test diet has anti-oxidant activity against oxidative stress as well as regulates lipid peroxidation. Unlike the diabetic control rats that lost weight, those fed the okra-based diet gained weight (Table 2). The loss in weight of the diabetic control rats was probably due to uncontrolled lipolysis especially in the peripheral tissues such as the skeletal muscle [16]. Also, okra contains abundant array of carbohydrate, minerals, vitamins, tryptophan, lysine and linoleic acid which are important in maintaining good health [17] and could be attributed for the weight gain observed in diabetic rats fed the okra-based diet.

This study revealed that blood glucose and lipid levels were significantly reduced (p < 0.05) in rats fed the okra-based diet compared with the diabetic rats fed normal diet (Tables 3 and 4). In fact, the blood glucose concentration for the rats fed the okra-based diet was the same as those of the control rats fed the normal diet. It has been reported that myricetin is the major glucose reducing agent in okra. Others components include oleanolic acid, β- sitosterol, and kaempferol. These agents also have anti-lipidaemia effects [18]. Besides, okra is rich in fibre and mucilage [19] and these are capable of forming viscous gels which bind glucose and lipids, retarding their absorption from the intestinal mucosa into the blood. This study also
revealed that the diabetic rats fed the okra-based diet significantly secreted more insulin (p < 0.05) into the blood compared to the diabetic rats fed the normal diet (Table 7). Thus, the combination of the anti-diabetic agents such as myricetin present in okra as well as its gel forming viscous fibres which delay absorption of glucose and lipid from the intestine including their rapid faecal excretion and increased secretion of insulin make okra a potent anti-diabetic agent.

The photomicrograph of the pancreas of the diabetic rats fed normal diet shows deposition of proteinaceous material in the duct lumen, hypoplasic islets and vascular congestion (Figure 2). These changes were caused by the streptozotocin used to induce diabetes in the animals. In contrast, the photomicrograph of the pancreas of diabetic rats fed the okra-based diet shows the reversal of the damage caused by streptozotocin, including the regeneration of islets (Figure 3). Therefore, the reason for the increased secretion of insulin by diabetic rats fed okra-based diet is due to the ability of okra to regenerate damaged islets. This observation is substantiated by the fact that the level of serum α- amylase in diabetic control rats fed normal diet was significantly elevated (p < 0.05) as compared to the level of serum α-amylose in diabetic rats fed the okra-based diet (Table 6). The concomitant damage to the exocrine tissues of the pancreas in the diabetic rats caused increased leakage of α- amylase into the whereas, repair or regeneration of these exocrine tissues by okra led to reduced leakage of α- amylase into the blood of the diabetic rats fed the okra-based diet. This report is therefore showing that okra could regenerate damaged pancreatic cells with consequent increased secretion of insulin by pancreatic β-cells.

Oxidative stress is one of the hallmarks of diabetes mellitus. Hence, agents that inhibit oxidative reactions could reduce complications caused by diabetes [20]. This study shows that diabetic control rats fed normal diet significantly elevated (p < 0.05) serum SOD activities and MDA levels compared to diabetic rats fed okra-based diet. The okra-based diet had no effect on serum catalase and glutathione peroxidase activities. There was also a non-significant difference in reduced glutathione levels. However, diabetic rats fed with okra-based diet showed improved SOD activities and reduced MDA levels. This could be attributed to the inherent antioxidant compounds present in the okra.

**CONCLUSION**

The findings of our study have shown that okra-based diet increase insulin secretion lowers hyperglycaemia and hyperlipidaemia as well as regenerate/repair endocrine β-cells and exocrine tissues of the pancreas damaged by streptozotocin. Consequently, okra fruit possess active phytoconstituents which may be responsible for its anti-diabetic activities.

**DECLARATIONS**

**Conflict of interest**

No conflict of interest is associated with this work.

**Contribution of authors**

We declare that this work was done by the authors(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The research was designed and manuscript drafted by Patrick Uadia, Isaac Imagbovomwan carried out the bench work and wrote manuscript while Kelly Oriakhi supervised and proof read the manuscript. Ikechi Eze prepared and read the histology slides.

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