Potential protective Effects of okra aquatas extract against the complications of diabetes mellitus in rats

Amira A Sheteewy1, Neveen A Elwardany1, Ali A Abdel-Nabey.2
Home Economics Department, Faculty of Specific Education, Alexandria University1
Food Sciences and Technology Department, Faculty of Agriculture, Alexandria University2

Abstract
The current study aimed to use different concentrations of okra aquatas extract (OAE) (15, 30 and 45g/100ml), in addition to whole and residues mashed okrain feeding induced diabetes rat groups for six weeks. Such groups were biologically and histopathologically compared with another negative and positive group. The proximate chemical composition as well as fiber fractions (crude, neutral detergent and acid detergent) and some minerals (Ca, Mg and Fe) of okra pods were also studied. Organoleptic evaluation of OAE was also assessed by diabetic patients. The results indicated that significant differences (P ≤ 0.05) were noted between body and organs weight especially heart and spleen of rats fed on OAE diets in comparison with the other groups. In addition, OAE enhanced insulin secretion and lowered glucose level. Also, OAE lowered TG, LDL and VLDL while the HDL, liver and kidney functions improved. Pancreatic histopathological examination showed that groups fed on OAE (30, 45g/100ml) seemed to be close to the negative control group with no histopathological changes in relative to the other diabetic rat groups. The results also showed that diabetic patients preferred OAE (30g/100ml). In general addition of OAE in diets could be managed of diabetes through improve liver and kidney functions as welled pancreas histopathological.

Key words: okra aquatas extract, diabetes mellitus, organs weight, serum lipid profile, histopathological examination.
Introduction

Okra (*Abelmoschus esculentus*, L. Moench.) is known as lady’s finger in some countries and bamia in Egypt. It is a plant of the *Malvaceae* family originated in the subtropical and tropical regions of the world (Adetuyi et al., 2011). Recently, it is spread worldwide, while its planting and consumption are more common around the Mediterranean basin, and especially Egypt, Cyprus, Greece, and Turkey. It is the main ingredient in many local and traditional dishes (Çalisır et al., 2005 and Kumar et al., 2013). In addition, it supplies common nutrients like minerals, vitamins, dietary fibers and bioactive chemicals. Sabitha et al. (2011) reported that the peel and seed of okra have antidiabetic and antihyperlipidemic affects instreptozotocin-induced diabetic rats. Deters et al., (2005) reported that okra can reduce blood glucose level and lipid level in obese rats, also it may play an important role in the regulation of glucose and lipid metabolisms. Okra is rich in bioactive polysaccharides, which have many biological activities (Panagiotis 2008 and Wittschier et al., 2007). Apart from edible use, extracts from okra fruit have been used for many applications in the food and pharmaceutical industry as emulsifiers, drug tablet formulations or blood plasma replacement, due to their highly content of biopolymers, such as polysaccharides and bioactive compounds such as ascorbic acid and β-carotene (Adetuyi et al., 2011, Arlai et al., 2012 and Ghori et al., 2014). Fresh okra pods are the most important vegetable source of viscous fiber to lower cholesterol. Seven-days-old fresh okra pods have the highest concentration of nutrients (Gemede et al., 2014). The different in polysaccharides found in the mucilage are high in okra pods according to Hirose et al. (2004). Okra is reported to have hypolipidemic effect by lowering the absorption of cholesterol from the diet (Huynh et al., 2008). Type 2 diabetes mellitus (DM) is the most common disease of the endocrine system that arises as a result of impaired insulin sensitivity and decreased cellular uptake of glucose (Vetrichevaniot et al., 2001). Properties of DM is hyperglycemia and dysregulation of carbohydrate, fat and protein metabolism due to destruction or inactivation of pancreatic β- cells, and defective insulin secretion, leading to morbidity which affect more than 100 million people worldwide (Scherbaum 2002), and is gradually emerging as an important health problem in developing countries (Bahmaniet et al., 2014). Epidemiological data indicated that 2.8% of the world's population was diabetic in the year 2000 and it may progress to 4.4% of the world's population by 2030. It affects all age groups of
people and ethnic groups (Xing et al., 2009). There is a need to take urgent actions to counter the increase in diabetes through awareness, better detection, prevention continuously, knowledge of the prevalence of diabetes and prediabetes and risk factors could raise awareness of the disease and lead to new policies and strategies for prevention and management. Long-standing diabetes can lead to circulation, kidney and heart problems, including stroke. In traditional world, nutrition and health care have a connection for which many plants are consumed as food in order to benefit health (Pieroni and Price, 2005). The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables are regarded worldwide as an important area of the nutritional and phytotherapeutic research. Motivation of people towards herbal medicines is increasing to avoid side effects of drugs prepared from synthetic materials (El and Karakaya, 2004). Therefore, the current study was undertaken to investigate the role of fresh OAE in managing the biological parameters of normal and induced diabetic rats as well as the sensory attributes of these extracts by patients suffering from diabetes mellitus.

Materials and Methods
Materials
Chemicals
Alloxan monohydrate was purchased from Sigma Chemicals Company, st Louis US, and all the other materials were purchased from EL-Goumhorya Company for the trades Drugs Chemicals, and Medical Instruments.
Okra selection
Okra (Abelmoschusesculentus) pods were collected from a local market at Alexandria, Egypt. The fruits were selected for uniformity of size and colour, and they were free from visible wounds and rottenness.
Methods
Analytical methods
Preparation of okra pods
The fresh okra was washed with tap water, and cut into small pieces, homogenized and used for analysis. Part of okra pieces was soaked in three concentrations (15, 30 and 45 g fresh pieces of okra /100 ml of potable water). They were soaked overnight. Okra pieces were removed and the liquid was kept in glass bottles and used for sensory evaluation and feeding rats.
Proximate chemical composition
Crude protein, crude fat and total ash were determined as described in AOAC (2000) procedures unless otherwise stated. Total carbohydrates were calculated by difference. Crude fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to the AOAC (2000) procedure via filter bags technology (Fiber analyzer, Ankon zoo) USA model no: Azzo. Minerals (Ca, Mg and Fe) were determined in ash solution using Atomic Absorption Spectrometer (AAS) 300Va-50-60H2-100-240V, UK as described by the AOAC (2000) procedure.

Experimental design
Forty-eight healthy adult male western rats weighing 190-200g were obtained from the Animal House, Home Economics Department, Faculty of Agriculture, Alexandria University. Animals were housed under laboratory conditions of temperature at 23 ± 3 °C with a 12 h light–dark cycle and were given standard diet and access to distilled water ad libitum according to the American Institute of Nutrition AIN, (1980). The protocol conforms to the guidelines of National Institutes of Health (NIH). Rats were acclimatized before the commencement of the experiment for a period of seven days, and then they were divided into eight groups (six animals in each group) as follows:

Group 1: negative control (healthy untreated rats).

Group 2: positive control (diabetic rat) that was treated by administering alloxan monohydrate (150 mg/ kg bodyweight) as described by Vanitha et al. (2013) and were considered diabetic when the fasting blood glucose levels was in the range of 150–200 mg/dL for 5 consecutive days.

Groups 3, 4 and 5: included diabetic rats which were orally given OAE at dose of 2.25, 4.5 and 6.75g / kg bw/day, respectively using an orogastric tube.

Group 6: diabetic rats which were orally given 10 g /kg bw /day of residues mashed okra after extraction using an orogastric tube.

Group 7: diabetic rats which were given suspended whole okra in distilled water with the dosage of 10g /kg bw /day using an orogastric tube.

Group 8: diabetic rats treated with 5 mg/kg bw /day glibenclamide as standard drug.

After the end of the treatment period (42 days), the rats were weighed, fasted overnight for 10 h and then sacrificed under ether anesthesia.
Blood samples were collected for biochemical analyses and organs such as liver, kidney, brain, lung, heart, pancreas, testes and spleen were removed; washed with cold saline solution and weighed. After that pancreas samples were stored in 10% formalin for histopathological examination.

**Biochemical analysis**

Serum samples were separated from blood by centrifugation at 3000 rpm for 15 min and analyzed for the following biochemical parameters: glucose levels, and insulin level were determined according to the methods of Trinder (1969) and Temple et al. (1992), respectively, serum lipid profile such as triglycerides, total cholesterol, high density lipoprotein (HDL) and low-density lipoprotein (LDL) were analyzed enzymatically according to Banchereau et al., Grundy et al., (1993) and Expert Panel on Detection, respectively. Serum urea, uric acid and creatinine were determined as kidney functions by Lumeij and Remple (1991), Young (1995), and Fossati et al. (1980), respectively. The activities of the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated according to International Federation of Clinical Chemistry as liver functions (Tietz et al., 1983 and Banchereau et al., 2000).

**Organoleptic assessment of OAE**

The three liquids remained after soaking okra overnight (15, 30, 45g, /100 ml water) were subjected to sensory evaluation. Diabetic patients (20 members) were selected from clients with health insurance in Kafr El-Dawar city, Behera Governorate, Egypt. These patients were asked to assess the odour, texture, appearance, taste, colour and overall acceptability according to Wichchukit and O’Mahony (2015). This experiment was approved by the Ethics committee on human research, Alexandria University, Alexandria, Egypt.

**Histopathological examination of pancreas**

After fixation in 10% formalin, tissues were washed, and dehydrated in ascending concentration of alcohol. The dried organs were cut into thin slices (4 μm) then stained with hematoxylin and eosin according to Bancroft (2002). Tissue sections were examined and photographed using microscope (H&E stains, X400mag).

**Statistical Analysis**

The results were expressed as mean values and standard deviations (SD), and analyzed using one-way analysis of variance (ANOVA)
followed by Duncan's multiple range Test with $p \leq 0.05$ (Kirkpatrick and Feeney 2012).

Results and Discussion

Chemical composition, minerals and fiber content of fresh okra

The results in Table (1) showed that crude protein content of fresh okra was 39.60%. This value is in a good agreement with the value reported by Gopalan et al. (2007), Var mudy (2011), Benchacri (2012) and Falusiet al. (2012). As it can be noted from Table (1) the crude fat of fresh okra was very low being 2.04%. This value is not in agreement with the value reported by Adetuyiet al. (2011). The percentage of total ash was 13.45%, on the other hand carbohydrate content of fresh okra was 35.05%. The results in Table (1) also showed that fresh okra contained 53.17% neutral detergent fiber and 20.81% acid detergent fiber. Also, the results showed that okra contained 925, 447 and 1.97mg/100g for Ca, Mg and Fe, respectively. In accordance with the results obtained in the present study, Adetuyiet al. (2011), Dossantosiet al. (2013), Kumar et al. (2013), Gemedeeet al. (2016) and Petropoulos et al. (2018) found that okra fruits contained from 58.22-382 mg/100g Ca, from 1.08 to 101mg/100g Mg and from 0.29 to 36.68mg/100g Fe. These wide variations are mainly due to variety, origin where the okra was grown.

Table (1): Chemical composition, fiber contents of fresh okra (on dry weight basis)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>g/ 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>39.60±0.19</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.04±0.02</td>
</tr>
<tr>
<td>Total ash</td>
<td>13.45±0.55</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>9.88±0.13</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>35.03±0.34</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>53.17±1.02</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>20.81±0.65</td>
</tr>
<tr>
<td>Mineral (mg /100g)</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>925±11.0</td>
</tr>
<tr>
<td>Mg</td>
<td>447±7.0</td>
</tr>
<tr>
<td>Fe</td>
<td>1.97±0.30</td>
</tr>
</tbody>
</table>
Effect of okra and OAE on body weight and organ weight in alloxan–induced diabetic rats

Statistically, no significant difference was observed in the mean initial body weights between all experimental groups (1–8) as shown in Table (2). Diabetic rats group (positive control) was significantly decreased in the final values of body weight by 26.77% compared to the negative control group. This may be due to injurious effects of alloxan which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. Oral administration of OAE at dose of 2.25, 4.5 and 6.75 g/Kg bw/day (groups 3, 4 and 5) caused a significant increase in body weight compared to the positive control, by 25.32%, 31.34%, and 34.41%, respectively as shown in Table (2). Groups of rats treated with residues mashed okra (6) had no significant decrease in the body weights compared to the positive control group. Also, no significant difference was observed in the final values of the body weights between diabetic rats treated with 5 mg/kg bw/day glibenclamide compared to the negative control group. These results are in agreement with Ahangarpour et al. (2017). This may be due to decrease or insufficient insulin which causes lipolysis and proteolysis that result in weight loss. Treatment male rats with OAE improved the level of insulin and explain the returning of weight gain to nearly the control values (Frier and Fisher, 2006). The results indicated that there were no significant changes in weight of liver, kidney, testes, brain, pancreas and spleen in all groups. However, OAE or okra administration did not cause statistical differences in organ weight of rat except a significant decrease was observed in lungs, when compared to the negative control group (Table 2).
Table 2: Effect of okra and OAE on body weight and organ weight in alloxan – induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testes</th>
<th>Brain</th>
<th>Heart</th>
<th>Lungs</th>
<th>Pancreas</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Final</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>(1) Negative control (ve)</td>
<td>195.3±4.4</td>
<td>249.2±6.9</td>
<td>1.39±0.2</td>
<td>2.31±0.3</td>
<td>1.56±0.3</td>
<td>.67±0.2</td>
<td>1.79±0.2</td>
<td>.39±0.2</td>
<td>.83±0.2</td>
</tr>
<tr>
<td>(2) Positive control (ve)</td>
<td>194.5±3.7</td>
<td>182.5±14.6</td>
<td>1.16±0.1</td>
<td>1.65±0.3</td>
<td>1.35±0.2</td>
<td>.49±0.2</td>
<td>1.93±0.2</td>
<td>1.27±0.2</td>
<td>.87±0.2</td>
</tr>
<tr>
<td>(3) ve + OAE 2.25g/kg bw/day</td>
<td>196.2±3.1</td>
<td>228.7±2.6</td>
<td>6.24±1.0</td>
<td>1.47±0.2</td>
<td>1.83±0.2</td>
<td>1.66±0.2</td>
<td>1.45±0.2</td>
<td>1.45±0.2</td>
<td>.89±0.2</td>
</tr>
<tr>
<td>(4) ve + OAE 4.5g/kg bw/day</td>
<td>193.1±4.9</td>
<td>239.7±5.4</td>
<td>5.90±1.0</td>
<td>1.13±0.2</td>
<td>2.02±0.2</td>
<td>1.21±0.2</td>
<td>1.61±0.2</td>
<td>1.38±0.2</td>
<td>.78±0.2</td>
</tr>
<tr>
<td>(5) ve + OAE 6.75g/kg bw/day</td>
<td>194.7±2.7</td>
<td>245.3±4.6</td>
<td>5.38±1.0</td>
<td>1.47±0.2</td>
<td>2.23±0.2</td>
<td>1.76±0.2</td>
<td>1.41±0.2</td>
<td>1.35±0.2</td>
<td>.79±0.2</td>
</tr>
<tr>
<td>(6) ve + residues mashed okra 10 g/kg bw/day</td>
<td>195.7±5.2</td>
<td>181.5±4.9</td>
<td>4.59±2.0</td>
<td>1.22±1.0</td>
<td>1.70±1.0</td>
<td>1.17±1.0</td>
<td>1.17±1.0</td>
<td>1.17±1.0</td>
<td>.17±1.0</td>
</tr>
<tr>
<td>(7) ve + whole okra pods 10 g/kg bw/day.</td>
<td>193.2±5.2</td>
<td>200.8±5.3</td>
<td>5.13±1.0</td>
<td>1.08±1.0</td>
<td>1.65±1.0</td>
<td>1.61±1.0</td>
<td>1.07±1.0</td>
<td>1.07±1.0</td>
<td>.54±1.0</td>
</tr>
<tr>
<td>(8)ve + drug.</td>
<td>196.5±3.5</td>
<td>246.8±4.3</td>
<td>6.34±1.0</td>
<td>1.35±1.0</td>
<td>1.74±1.0</td>
<td>1.51±1.0</td>
<td>1.41±1.0</td>
<td>1.41±1.0</td>
<td>.38±1.0</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, Means with different superscript letters on the same column were significantly different p ≤ 0.05

Comparison of serum glucose and insulin levels between groups.

Table 3 shows the results of fasting blood glucose and insulin level of the rats at the end of the experiment. Diabetic rats showed a significant increment in glucose level the serum (221mg/dl) as compared with the control group (106.6 mg/dl). The present data revealed that administration of OAE caused significant decrement in glucose level of the male rats comparing with the positive control group. The level of glucose was 193.66, 171.17 and 150 mg/dl for group 3, 4, and 5, respectively. However, the administration of whole okra or residues mashed okra to the diabetic rats did not cause any significant differences.
in the level of glucose (Table 3). Highly significant decrease in glucose level was detected in group 8 when compared with group 2. The lowering of glucose levels by OAE could be attributed to the high content of insulin which contributes to the control of hyperglycemia in diabetes. Additionally, it could promote glucose uptake in hepatocytes and increase the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase in the liver of STZ-induced diabetic rats (Nabila, et al., 2018). The present results showed that diabetic rat groups had significant decrement in insulin levels compared to the control group. Treatment with OAE significantly increased insulin level compared to the positive control group (Table 3). Also it can be noted that administration of residues mashed okra showed the lowest insulin value (23.67 U / ml). On the other hand, treatment with glibenclamide significantly decreased insulin level compared to the control group. The results of insulin is similar to that reported by Fan et al. (2014) who found that insulin levels were slightly increased in high-fat diet-induced obese mouse, while the treatment with okra extract (equivalent to 30-g fresh okra pod/kg/ day) reduced insulin levels. Okra polysaccharides possess anticomplementary and hypoglycemic activity in normal mice (Tomoda et al., 1989). Seeds and mucilage of H. esculentus (200 mg okra extract /kg/day, for 19 day) exerted positive effects such as increasing serum insulin, improving total antioxidant capacity, reducing MDA, and improving lipid profile by decreasing total cholesterol, LDL-C and triglyceride in pregnant rats (Tian et al., 2015).

Table (3): Serum glucose and insulin levels in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Serum glucose (mg / dl)</th>
<th>Serum Insulin (µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Negative control (ve')</td>
<td>106.6± 0.87 a</td>
<td>46.67± 2.9 d</td>
</tr>
<tr>
<td>(2) Positive control (ve')</td>
<td>221± 1.04 cd</td>
<td>27.01± 2.1 a</td>
</tr>
<tr>
<td>(3) ve' + OAE 2.25g/kg bw/day</td>
<td>193.66± 4.3 cba</td>
<td>34.16± 4.2 b</td>
</tr>
<tr>
<td>(4) ve' + OAE 4.5g/kg bw/day</td>
<td>171.17± 5.6bc</td>
<td>36.33 ± 2.2 bc</td>
</tr>
<tr>
<td>( 5) ve' + OAE 6.75g/kg bw/day</td>
<td>150± 3.7 ab</td>
<td>38.17±2.6</td>
</tr>
<tr>
<td>( 6)ve' + residues mashed okra 10 g /kg bw /day</td>
<td>235.16± 3.18 a</td>
<td>23.67± 3.3 a</td>
</tr>
<tr>
<td>( 7) ve' + whole okra pods 10 g /kg bw /day.</td>
<td>225.8± 1.16 cd</td>
<td>25.33±1.99</td>
</tr>
<tr>
<td>( 8)ve' + drug</td>
<td>108.33± 3.1 b</td>
<td>46.5± 2.4</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, Means with different superscript letters on the same column were significantly different p ≤ 0.05
Effect of OAE on serum lipid profile of diabetic rats

The present results showed that the positive group had a significant increase in triglycerides (TG) by 67.44% and total cholesterol (TC) by 20.76% compared to the negative group as recorded in Table (4). Diabetic groups treated with OAE at three dosage levels showed improvement in lipid profile levels. Administration of OAE caused a significant reduction in TC, and TG compared to the positive group and significantly increased HDL compared to the negative group. The results revealed that the positive group had a significant increment in serum level of VLDL when compared to the negative group. Administration of OAE at 6.75 g/kg bw/day produced significant decrement in serum levels of VLDL by 22.07%, while the dose of 2.25 and 4.5 g/kg bw/day showed no significant change when compared to the positive group as shown in Table (4). The decline in VLDL levels in treated group could be directly correlated to decline in TG levels of these groups, as it is well established that VLDL particles are the main transporters of TG in plasma (Hertoget al., 1993). These results are in agreement with Tianet al. (2015) who reported that the okra extract had effect on serum lipid profile. Also, Alqasoumi (2012) found that administration of okra seed extract at a daily dose of 250 and 500 mg/kg bw decreased TC, LDL and TG levels in rats. Moreover, Hajianet al. (2016) reported that the supplementation with either H. esculentus seed at a daily dose of 2 g/kg bw for 2 weeks or mucilage at a daily dose of 2 g/kg bw for 2 weeks caused a significant reduction in TC, LDL-C, HDL-C and TG compared to the control group.

Table (4): Effect of OAE on serum lipid profile of diabetic rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Lipid profile</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(TG) (mg/dl)</td>
<td>(TC) (mg/dl)</td>
<td>HDL (mg/dl)</td>
<td>LDL (mg/dl)</td>
<td>VLDL (mg/dl)</td>
</tr>
<tr>
<td>(1) Negative control (ve+)</td>
<td>43.3±4.4a</td>
<td>70.8±2.5b</td>
<td>33.3±2.6a</td>
<td>28.8±2.7b</td>
<td>8.7±.88a</td>
</tr>
<tr>
<td>(2) Positive control (ve+)</td>
<td>72.5± 3.1d</td>
<td>85.5± 4.1d</td>
<td>44.5±3.6d</td>
<td>26.5±7.5d</td>
<td>14.5± 1.7d</td>
</tr>
<tr>
<td>(3) ve+ + OAE 2.25g/kg bw/day</td>
<td>48.3±2.6d</td>
<td>77.2±3.9d</td>
<td>42.5±2.1d</td>
<td>25.0±5.9d</td>
<td>13.9±1.4cd</td>
</tr>
<tr>
<td>(4) ve+ + OAE 4.5g/kg bw/day</td>
<td>47.4±2.1d</td>
<td>63.3±3.4d</td>
<td>38.7±1.1d</td>
<td>15.2±4.1d</td>
<td>12.0±3.3d</td>
</tr>
<tr>
<td>(5) ve+ + OAE 6.75g/kg bw/day</td>
<td>47.1±1.1d</td>
<td>60.7±2.9d</td>
<td>36.2±2.3d</td>
<td>15.1±2.1d</td>
<td>11.3±1.9bcd</td>
</tr>
<tr>
<td>(6) ve+ + residues mashed okra 10 g/kg bw / day</td>
<td>75.6± 2.7d</td>
<td>94.7±3.7d</td>
<td>44.3±2.2d</td>
<td>24.2±3.7d</td>
<td>11.7±3.4bcd</td>
</tr>
<tr>
<td>(7) ve+ + whole okra pods 10 g/kg bw / day.</td>
<td>64.3±1.6c</td>
<td>90.8±5.1c</td>
<td>43.7±2.6c</td>
<td>24.4±2.4c</td>
<td>11.7 ±2.8bcd</td>
</tr>
<tr>
<td>(8) ve+ + drug</td>
<td>45.6±9.2b</td>
<td>72.7± 3.1b</td>
<td>34.3±2.2b</td>
<td>28.1±2.1b</td>
<td>8.9±.71b</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, Means with different superscript letters on the same column were significantly different p ≤ 0.05
Effect of OAE on Liver functions of diabetic rats

The present results showed that the positive group had significant increment in serum levels of ALP, AST and ALT enzymes in serum when compared to the negative group by 63.38, 59.5 and 92.0 %, respectively. Rats given OAE at three dosage levels showed remarkably amelioration in enzymes level and the reduction in AST, ALP and ALT and the increasing dosages of okra water extract produced increase of reduction in enzymes level when compared with those of diabetic rat. Treatment with OAE 6.75g /kg bw/day to rats caused significant decrement in the levels of ALP, AST and ALT enzymes by 35.81, 36.69 and 23.15%, respectively, when compared to the positive control group. This may be due to the OAE stabilize the cell membrane and prevent the leakage of AST and ALT to the blood stream. In this content Alqasoumi (2012) found that ethanolic extract of okra administration(250 and 500 mg/kg bw) affect serum liver functions. On the other hand, glibenclamide treatment showed a mild improvement in the liver function.

Effect of OAE on Kidney functions of diabetic rats

The results of kidney functions are shown in Table (5). Groups of diabetic rats treated with okra (3 to 5) had no significant increment in the level of uric acid, urea and creatinine when compared with the positive control, whereas significant differences were observed when group 2 was compared with group 1 for the same parameters. Treatment with alloxan monohydrate (150 mg/kg bw) caused significant increment in the levels of uric acid, urea and creatinine by 56.91, 36 and 26.2 %, respectively when compared to the negative group. These results are in agreement with the results obtained by Hajianet al. (2016) who found that treating rats with okra extract led to insignificant reduction in uric acid, urea and creatinine compared to the positive group.
Table (5): Effect of OAE on Liver and Kidney functions of diabetic rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Liver functions</th>
<th>Kidney functions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALP (U/L)</td>
<td>AST (U/ml)</td>
</tr>
<tr>
<td>(1) Negative control (ve-)</td>
<td>82.2± 2.5a</td>
<td>36.8±2.3a</td>
</tr>
<tr>
<td>(2) Positive control (ve+)</td>
<td>134.3±3.7b</td>
<td>60.5±3.2c</td>
</tr>
<tr>
<td>(3) ve+ OAE 2.25g/kg bw /day</td>
<td>93.7± 3.7c</td>
<td>41.7±3.1bc</td>
</tr>
<tr>
<td>(4) ve+ OAE 4.5g/kg bw /day</td>
<td>90.2± 2.6b</td>
<td>38.3±3.0ab</td>
</tr>
<tr>
<td>(5) ve+ OAE 6.75g/kg bw /day</td>
<td>86.2± 1.7b</td>
<td>37.0±2.4c</td>
</tr>
<tr>
<td>(6)ve+ residues mashed okra 10 g /kg bw / day</td>
<td>136.3±3.8a</td>
<td>45.2±3.1d</td>
</tr>
<tr>
<td>(7) ve+ whole okra pods 10 g /kg bw / day</td>
<td>103.5±3.7c</td>
<td>43.2±3.2c</td>
</tr>
<tr>
<td>(8)ve+ drug</td>
<td>85.7±2.2a</td>
<td>38.3±3.5a</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, Means with different superscript letters on the same column were significantly different p ≤ 0.05

Organoleptic assessment of OAE

Table (6) shows sensory evaluation of OAE. It can be noted that there were significant differences between the three OAE from the organoleptic point of view. The scores of the organoleptic attributes given for the three extracts decreased with increasing the amount of okra dissolved in water. No poor or rejected organoleptic attributes were obtained even at the higher amount of okra (30g/100ml water). As a conclusion, the diabetic patients accepted the three samples, but sample (15g/100ml) was well accepted compared with the other samples (30 and 45g/100ml). It has been reported that motivation of people towards herbal medicines is increasing to avoid the side effects of drug prepared from synthetic materials (El and Karakaya, 2004).

Table (6): Organoleptic properties of OAE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Taste</th>
<th>Texture</th>
<th>Colour</th>
<th>Odour</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>15g/100 ml</td>
<td>6.95±1.09a</td>
<td>7.10±1.20a</td>
<td>6.60±1.42a</td>
<td>7.15±9.33a</td>
<td>7.85±1.03a</td>
<td>7.15±1.22a</td>
</tr>
<tr>
<td>30g/100ml</td>
<td>6.90±1.4a</td>
<td>7.45±9.44a</td>
<td>7.10±1.16a</td>
<td>7.20±1.54a</td>
<td>7.25±1.55a</td>
<td>7.65±1.13a</td>
</tr>
<tr>
<td>45g/100ml</td>
<td>5.25±1.48a</td>
<td>5.95±1.53a</td>
<td>6.05±1.3a</td>
<td>6.85±1.34a</td>
<td>6.90±1.63a</td>
<td>6.80±1.64a</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, Means with different superscript letters on the same column were significantly different p ≤ 0.05
Histopathological examination of diabetic rats pancreas treated with OAE

Histopathological examination of pancreas sections in the negative control rats showed normal arrangement of the largely islet cells with normal prominent β-cells which have cytoplasm and rounded dark nuclei and no histopathological changes in the area of eosinophilic acini cells with hyperchromatic nuclei (Fig1, A). While, pancreas of alloxan-induced diabetic rats revealed marked dilation of interlobular and enzyme duct surrounded by infiltrating lymphocyte cells and more necrotic cells were apparent (Fig1, B). The pancreas of the treated rats with OAE at dose 2.25 g/kg bw/day revealed that the islet cells appeared embedded within the acinar cells and surrounded by a fine capsule, normal proportions with eosinophilic deposits in the gland, the islets smaller in volume, more uniform eosinophilic cytoplasm, and round nuclei (Fig1, C). Rat pancreas which was diabetic and treated with OAE at dose 4.5 g/kg bw/day showed the acinar cells which stained strongly and are arranged in lobules with prominent nuclei in their normal proportions. The large vacuolated islet cells with reduction of the β-cells are seen embedded within this acinar cell. There are area necrotic acinar cells (Fig1, D). Section of the rat pancreas which was diabetic and treated with OAE at dose 6.75 g/kg bw/day showed the acinar cells which stained pale eosinophilic and are arranged in lobules with pale nuclei (necrotic acinar cells). The enlarged or widely islet cells are seen embedded within the necrotic acinar cells (Fig1, E). Fig (1, F) shows the rat pancreas which was diabetic and fed on the okra residues after water extraction. The cells showed atrophied islet with mild dilation of the capsule space, eosinophilic acini cells with hyperchromatic nuclei and necrotic other cells surrounded the islet and mild dilation of the interlobular duct. Fig (1, G) showed that the rat pancreas which was diabetic and fed whole okra, showed the islets smaller in volumes with more atrophied β-cells are seen embedded within the eosinophilic cytoplasm acinar cells and surrounded by a fine capsule. Fig (1, H) shows the section of the diabetic rat pancreas treated with 5 mg/kg bw/day glibenclamide. An eosinophilic cytoplasm and necrotic nuclei of the acinar cells are arranged in lobules. The islets were atrophied with few atrophied β-cells, embedded within the acinar cells and surrounded by a fine capsule. There is eosinophilic material deposit in the gland with
mild dilation of blood vessels with no infiltrating lymphocytes. The present results are in agreement with Majdet et al. (2018) who demonstrated that okra may improve glucose homeostasis which are associated with reduced pancreatic tissue damage. The okra administrated showed that the reduction size of islets and population of insulin-producing β-cells were reduced in the pancreas of HFD/STZ-induced diabetic rats and these results were in accordance with previous studies that demonstrated that hyperglycemia leads to a progressive decline in β-cells function (Bonora, 2008).

(A) Rat’s pancreas in Negative control group

(B) Rat’s pancreas in Positive control group

(C): Rat’s pancreas in the group which was ve’ + OAE 2.25g/kg bw /day

(D) Rat’s pancreas in the group which wasve’ +OAE 4.5g/kg bw /day
Fig (1): Histopathological examination of diabetic rats pancreas treated with OAE

**Conclusion**

In general, it could be concluded that OAE at different concentrations showed a beneficial role in the management of many biological factors in diabetic rats. This is because these extracts improved mainly liver and kidney functions. So, we recommended to okra aquatas extracts on our daily dishes.

**Acknowledgement**

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References


التأثيرات الوقائية المحتملة للمستخلص المائي للبيامي تجاه مضاعفات مرض السكر في الفران

أmirah שטיوي.1 نفين الورداني.2 علي عبد النبي
1قسم الاقتصاد المنزلي، كلية التربية النوعية، جامعة الإسكندرية
2قسم علم وتقنية اللغة، كلية الزراعة، جامعة الإسكندرية

الملخص:

هدف الدراسة الحالية إلى استخدام تركيزات مختلفة من المستخلص المائي للبيامي (15، 30، 45 جرام/100مل) بالإضافة إلى البياميك الكالسيوم وتلك الناتجة بعد الاستخلاص المائي وذلك لتغذية الفران المصابة بالداء السكري والمغذاة لفترة ستة أسابيع. هذه الجماعات تم مقارنتها من الناحية البيولوجية والهستوبولوجية مع مجموعتين أخريات تمثل المجموعة الضابطة الموجبة والأخرى تمثل المجموعة الضابطة السالبة. هذا وقد تم دراسة التركيب الترطيب للمياه بالإضافة إلى تدقيق نسبة الأليل الخام وكل من الألياف الذاتية الحامضية والمتعادلة وبعض المعادن مثل الكالسيوم والمغنيسيوم والخ. تم اجراء التقييم الحسي للمستخلصات المائية الثلاث باستخدام أفراد مصابون بداء السكري. وأظهرت النتائج أن هناك اختلافات معنوية (P ≤ 0.05) بين أوزان الجسم والأعضاء الداخلية خصوصا القلب والطحال بالنسبة للفران التي تم تغذيتها على المستخلصات المائية للبيامي بالمقارنة بالمجموعات الأخرى. بالإضافة إلى ذلك وجد أن المستخلصات المائية تشيع من افراد الأساليب ومن ثم تقل من مستوى الجلوکوز. أيضا أثبتت الدراسة أن المستخلص المائي أدى إلى تحسن صورة دهون الدم وذلك لخفض مستوى الجليکوزيدات الثلاثية والكولسترول مخفض الكثافة والكولسترول المنخفض جدا للكثافة في حين حدث تحسن ملحوظ في نسبة الكولسترول عال الكثافة وكل الوظائف الخاصة بالكبد والكلي. أثبت الفحص البلازمولوجي للكريات ان المجتمعات التي تم تغذيتها على المستخلص المائي الليمون (30، 45 جرام/100مل) كانت متشابهة الى حد كبير من المجموعة السالبة مع عدم وجود اي تغيرات هستوبولوجية بالمقارنة مع مجامع الفران الأخرى المصابة بداء السكري. أوضحت الدراسة أيضا أن مرض الداء السكري كان تعيين للمستخلص المائي (30 جرام/100مل) أحسن وأفضل من التركيزات الأخرى. وصقفة عامة يمكن القول بأن أضافة المستخلص المائي للبيامي الليمون الوجبات الغذائية ممكن أن يؤدي الى التحكم في مرض الداء السكري وتحسين وظائف الكبد والكلية.

الكلمات المفتاحية: المستخلص المائي للبيامي - مرض الداء السكري - صورة دهون الدم - وزن الأعضاء - الفحص البيولوجي.