Journal of Home Economics

Volume 28, Number (3), 2018

http://homeEcon.menofia.edu.eg

Journal of Home Economics

ISSN 1110-2578

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

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Abstract

The current study aimed to use different concentrations of okra aquatas extract(OAE) (15,30and 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 ($P \leq 0.05$) 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 preferredOAE (30g/100ml). In general addition of OAE in diets could be managed of diabetes through improve liver and kidney functions as welledpancreashistopathological.

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

Introduction

Okra (*Abelmoschusesculentus,* 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 **(Çalişi*r et al.,* 2005 and Kumar *et al.,* 2013).**In addition,itsupplies 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 andWittschier*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 ß-carotene **(Adetuyi*et al.,* 2011,Arlai*et al.,* 2012 andGhori*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 **(Vetrichelvan*et al.,* 2001)**. properties of DM is hyperglycemia and dysregulation of carbohydrate, fat and protein metabolism due to destruction or inactivation of pancreatic ß- 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 **(Bahmani*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 nutraueutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables are regarded worldwide as an important area of the nutritional and phytotherapic 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 othermaterials were purchased from EL-Goumhorya Company for 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 andfeeding 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 ℃ 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 OAEat 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 ratstreated 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*., (2000**), **Grundy *et al*., (1993)** and **Expert Panel on Detection,(2001)**, respectively. Serum urea, uric acid andcreatinine 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 andBanchereau*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.

Histophathological 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 Feeney2012)**.

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),varmudy (2011), Benchacri (2012) and Falusi*et 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 **Adetuyi*et al.* (2011)**. The percentage of total ash was 13.45%, on the other handcarbohydrate 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, **Adetuyi*et al.* (2011), Dossantos*et al.* (2013), Kumar *et al.* (2013),Gemede*et 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 freshokra(on dry weight basis)

Constituent	g/ 100g
Crude protein	39.60±0.19
Crudefat	2.04±0.02
Total ash	13.45±0.55
Crude fiber	9.88±0.13
Carbohydrate	35.03±0.34
Neutral detergent fiber	53.17±1.02
Acid detergent fiber	20.81±0.65
Mineral (mg /100g)	
Ca	925±11.0
Mg	447±7.0
Fe	1.97±0.30

Effect of okra and OAE on body weight and organ weightin 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 valuesof body weightby 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 6.75g / Kg bw/day (groups3, 4 and 5) caused a significant increased 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 (Table2).

Table (2): Effect of okra and OAE on body weight and organ weight in alloxan – induced diabetic rats

Groups	Weight		Liver (g)	Kidney (g)	Testes (g)	Brain (g)	Heart (g)	Lungs (g)	Pancreas (g)	Spleen (g)
	Initial (g)	Final (g)								
(1) Negative control (ve^-)	195.3 ±4.4^{a}	249.2 ±4.3^{e}	6.94 ±.346^{b}	1.39 ±.245^{a}	2.31 ±.373^{a}	1.56 ±.204^{ab}	.67 ±.072^{a}	1.79 ±.164^{bc}	.39 ±.073^{b}	.83 ±.305^{a}
(2) Positive control (ve^+)	194.5 ±3.7^{a}	182.5 ±5.4^{a}	4.65 ±1.14^{ab}	1.16 ±.563^{b}	1.65 ±.542^{a}	1.35 ±.706^{ab}	.49 ±.231^{e}	.93 ±.373^{ab}	.27 ±.043^{ab}	.87 ±.653^{a}
(3) ve^+ + OAE 2.25g/kg bw /day	196.2 ±3.1^{a}	228.7 ±2.6^{c}	6.24 ±1.06^{a}	1.47 ±.344^{a}	1.83 ±.716^{a}	1.66 ±.418^{ab}	.40 ±.130^{cd}	1.45 ±.045^{ab}	.28 ±.070^{ab}	.89 ±.299^{a}
(4) ve^+ + OAE 4.5g/kg bw /day	193.1 ±4.9^{a}	239.7 ±4.5^{d}	5.90 ±1.91^{a}	1.13 ±.442^{a}	2.02 ±.760^{a}	1.21 ±.302^{a}	.62 ±.148de	1.61 ±.406^{abc}	.38 ±.179^{b}	.78 ±.185^{a}
(5) ve^+ + OAE 6.75g/kg bw /day	194.7 ±2.7^{a}	245.3 ±4.6^{e}	5.38 ±.784^{a}	1.47 ±.074^{a}	2.23 ±.254^{a}	1.76 ±.321^{a}	.86 ±.536^{bc}	1.41 ±.156^{c}	.35 ±.083^{ab}	.79 ±.171^{a}
(6)ve^+ + residues mashed okra 10 g /kg bw / day	195.7 ± 5.2^{a}	181.5 ± 4.9^{a}	4.59 ± .595^{ab}	1.22 ± .323^{b}	1.70 ± .437^{a}	1.17 ± .159^{b}	.45 ± .067^{b}	1.17 ± .176^{ab}	.25 ± .071^{a}	.66 ± .242^{a}
(7) ve^+ + whole okra pods 10 g /kg bw / day.	193.2 ±6.2^{a}	200.8 ±5.3^{b}	5.13 ±1.70^{a}	1.08 ±.236^{ab}	1.65 ±.577^{a}	1.61 ±.320^{ab}	.31 ±.191^{ab}	1.07 ±.426^{a}	.23 ±.016^{a}	.54 ±.154^{a}
(8)ve^+ + drug	196.5 ±3.5^{a}	246.8 ±4.3^{e}	6.34 ±1.98^{a}	1.35 ±.206^{a}	1.74 ±.522^{a}	1.51 ±.266^{ab}	.56 ±.170^{a}	1.41 ±.054^{ab}	.38 ±.143^{b}	.96 ±.350^{a}

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

Comparison of serum glucose and insulin levelsbetween groups.

Table (3) shows the results of fasting blood glucoseand 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 OAEcaused significant decrement in glucose level of the male rats comparing with the positive control group.The level of glucose was 193.66, 171.17and 150 mg/dl for group 3, 4, and 5, respectively.However, the administration of whole okra or residues mashed okra to the diabetic ratsdid 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 group2.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 glibenclamideinsignificantly 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 19day)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 triglyceridein pregnant rats **(Tian*et al.*, 2015).**

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

Experimental Groups	Serum glucose (mg / dL)	Serum Insulin (µIU/mL)
(1) Negative control (ve^-)	106.6± 0.87 ^{a}	46.67± 2.9 ^{d}
(2) Positive control (ve^+)	221± 1.04^{cd}	27.01± 2.1^{a}
(3) ve^+ + OAE 2.25g/kg bw /day	193.66± 4.3^{bcd}	34.16± 4.2^{b}
(4) ve^+ + OAE 4.5g/kg bw /day	171.17± 5.6^{bc}	36.33 ± 2.2^{bc}
(5) ve^+ + OAE 6.75g/kg bw /day	150± 3.7^{ab}	38.17±2.6^{c}
(6)ve^+ + residues mashed okra 10 g /kg bw / day	235.16± 3.18^{d}	23.67± 3.3^{a}
(7) ve^+ + whole okra pods 10 g /kg bw / day.	225.8± 1.16^{cd}	25.33±1.9^{a}
(8)ve^+ + drug	108.33±.31^{a}	46.5± 2.4^{d}

Values represent mean ± SD, Means with different superscript letters on the same column were significantlydifferent $p \leq 0.05$

Effect of OAE on serumlipid 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 withOAE at three dosage levels showedimprovement in lipid profile levels. Administration of OAEcaused asignificant reduction inTC, and TG compared to the positive groupand significantly increasedHDLcompared 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 6.75g /kg bw/day produced significant decrement in serum levels of VLDL by 22.07%, while the dose of 2.25 and 4.5g /kg bw/dayshowed 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 (**Hertog*et al.*, 1993)**. These results are in agreement with**Tian*et al.* (2015)** who reported that the okra extract had effect on serum lipid profile. Also, **Alqasoumi (2012)** found that administrationethanolic extract of okra (250 and 500 mg/kg bw) decreased TC, LDL and TG levels in rats.Moreover, **Hajian*et 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

Experimental Groups	Lipid profile*				
	(TG) (mg/dl)	(TC) (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
(1) Negative control (ve^-)	43.3±4.4^{a}	70.8±2.5^{b}	33.3±2.6^{a}	28.8±2.7^{b}	8.7±.88^{a}
(2) Positive control (ve^+)	72.5± 3.1^{d}	85.5± 4.1^{d}	44.5±3.6^{c}	26.5±7.5^{b}	14.5± 1.7^{d}
(3) ve^+ + OAE 2.25g/kg bw /day	48.3± 2.6^{b}	77.2±3.9^{c}	42.5 ±2.1^{c}	25.0±5.9^{b}	13.9 ±1.4^{cd}
(4) ve^+ + OAE 4.5g/kg bw /day	47.4±2.1^{b}	63.33±3.4^{a}	38.7±1.1^{b}	15.2±4.1^{a}	12.0±3.3^{cd}
(5) ve^+ + OAE 6.75g/kg bw /day	47.1± 1.1^{b}	60.7± 2.9^{a}	36.2±2.3^{ab}	15.1±2.1^{a}	11.3±1.9^{abc}
(6)ve^+ + residues mashed okra 10 g /kg bw / day	75.6± 2.7^{e}	94.7±3.7^{e}	44.3±2.2^{c}	24.2±3.7^{b}	11.7±3.4^{bcd}
(7) ve^+ + whole okra pods 10 g /kg bw / day.	64.3±1.6^{c}	90.8±5.1^{e}	43.7±2.6^{c}	24.4±2.4^{b}	11.7 ±2.8^{bcd}
(8)ve^+ + drug	45.6±.92^{ab}	72.7± 3.1^{b}	34.3±2.2^{a}	28.1±2.1^{b}	8.9±.71^{ab}

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

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, 59.5and 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 and23.15%, 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 ofdiabetic rats treated withokra (3 to 5)had no significantincrement in the level of uric acid, urea and createninewhen 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 createnine by 56.91,36 and 26.2 %, respectively when compared to the negative group. These results are in agreement with the results obtained by **Hajian*et al.*(2016)** who found that treating rats with okra extract led to insignificant reduction in uric acid, urea and createninecompared to the positive group.

Table (5):Effect of OAE on Liver and Kidney functions of diabetic rats

Experimental Groups	Liver functions			Kidney functions		
	ALP (U/L)	AST (U/ml)	ALT (U/ml)	Uric acid (mg/dl)	Urea (mg/dl)	Createnine (mg/dl)
(1) Negative control (ve^-)	82.2± 2.5^{a}	36.8±2.3^{a}	22.5±1.9^{a}	34.0±3.6^{a}	0.77±0.18^{a}	0.67± 0.78^{a}
(2) Positive control (ve^+)	134.3±3.7^{d}	60.5±3.2^{d}	43.2±3.1^{f}	57.5±3.9^{e}	1.1± 0.39^{b}	0.89±0.13^{bc}
(3) ve^+ + OAE 2.25g/kg bw /day	93.7± 3.7^{c}	41.7±3.1^{bc}	35.7±2.2^{cd}	46.7±3.5^{cd}	1.2± 0.17^{b}	0.83±0.15^{abc}
(4) ve^+ + OAE 4.5g/kg bw /day	90.2± 2.6^{c}	38.3±3.0^{ab}	33.2±2.6^{c}	42.2±3.7^{bc}	1.2± 0.07^{b}	0.73±0.20^{abc}
(5) ve^+ + OAE 6.75g/kg bw /day	86.2± 1.7^{b}	37.0±2.4^{a}	28.4±2.9^{b}	40.2±5.5^{b}	1.1± 0.13^{b}	0.85±0.11^{abc}
(6)ve^+ + residues mashed okra 10 g /kg bw / day	136.3±3.8^{d}	45.2±3.1^{c}	40.5±1.9^{ef}	56.3±3.1^{e}	1.2± 0.11^{b}	0.79±0.10^{abc}
(7) ve^+ + whole okra pods 10 g /kg bw / day	103.5±3.7^{d}	43.2±3.2^{c}	37.8±1.7^{de}	49.5±2.9^{d}	0.85±0.13^{a}	0.91±0.13^{c}
(8)ve^+ + drug	85.7±2.2^{ab}	38.3±3.5^{ab}	25.2±3.5^{a}	37.5±6.3^{ab}	0.97±0.13^{ab}	0.71±0.16^{ab}

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

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 ofview.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 andKarakaya, 2004).**

Table (6):Organoleptic propertiesof OAE

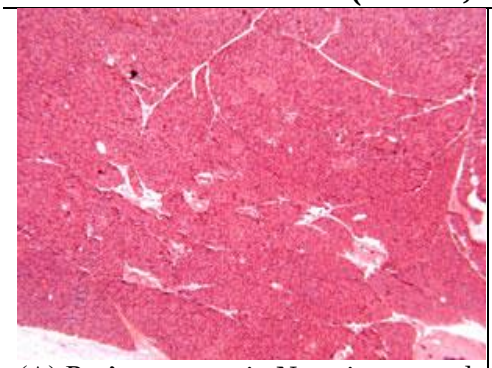
Sample	Organoleptic attributes					
	Appearance	Taste	Texture	Colour	Odour	Overall acceptability
15g/100 ml	6.95± 1.09^{b}	7.10±1.20^{b}	6.60±1.42^{ab}	7.15±.933^{a}	7.85±1.03^{a}	7.15±1.22^{a}
30g/100ml	6.90±1.4^{b}	7.45±.944^{b}	7.10±1.16^{b}	7.20±1.54^{a}	7.25±1.55^{a}	7.65±1.13^{a}
45g/100ml	5.25±1.48^{a}	5.95±1.53^{a}	6.05±1.3^{a}	6.85±1.34^{a}	6.90±1.63^{a}	6.80±1.64^{a}

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

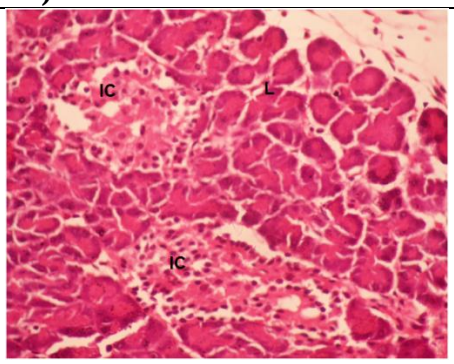
Histopathologicalexamination of diabetic rats pancreas treated with OAE

Histopathological examination of pancreas sections in the negative control rats showed normal arrangement of the largely islet cells with normal prominent ß-cells which have cytoplasm and rounded dark nuclei and no histopathological changes in the area of eosinophilicacini 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 ß- 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 withOAE at dose 6.75 g/kg bw/day showed the acinar cells which stained pale eosinophilicand 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,eosinophilicacini 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 ß-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 ß -cells, embedded within the acinar cells and surrounded by a fine capsule. There is eosinophilic material deposit in the gland with

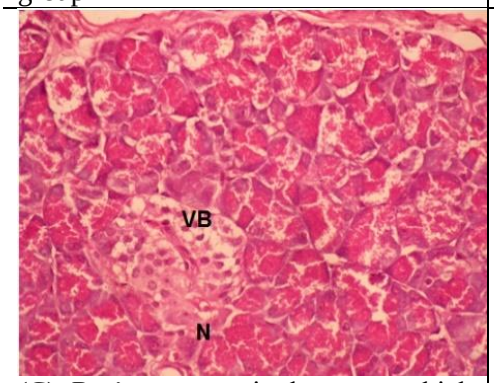
mild dilation of blood vessels with no infiltrating lymphocytes. The present results are in agreement with**Majd*et al.*** **(2018)** whodemonstrated that okra may improve glucose homeostasis which are associated with reduced pancreatic tissue damage. The okra administrated showed that the reduction size of islets and population of insulin-producing ß-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 ß-cells function**(Bonora, 2008).**



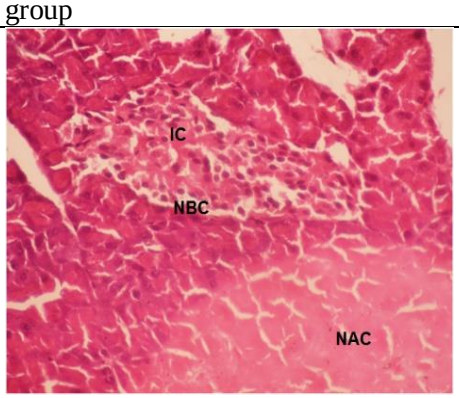
(A) Rat's pancreas in Negative control group



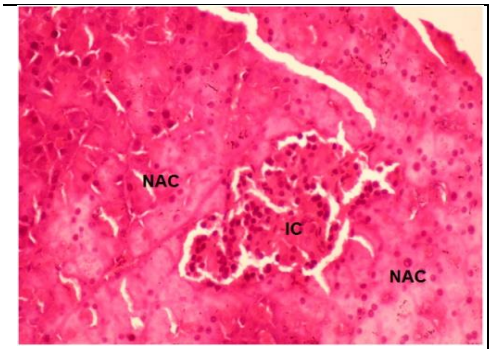
(B) Rat's pancreas in Positive control group



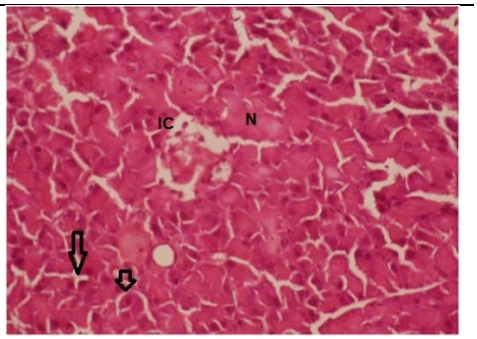
(C): Rat's pancreas in the group which was ve^{+} + OAE 2.25g/kg bw /day



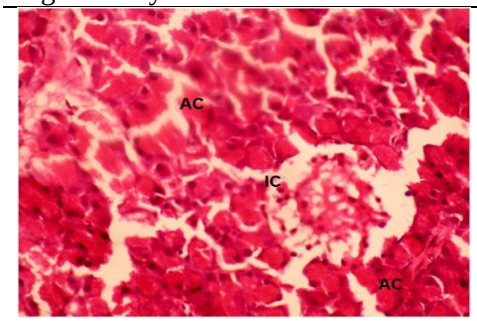
(D) Rat's pancreas in the group which wasve^{+} +OAE 4.5g/kg bw /day



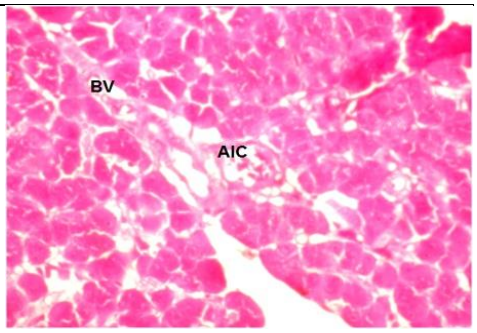
(E): Rat's pancreas in the group which was ve^{+} + residues mashed okra 10 g /kg bw / day



(F): Rat's pancreas in the group which was ve^{+} + whole okra pods 10 g /kg bw / day



(G): Rat's pancreas in the group which was ve^{+} +okra pods 10 g /kg bw / day



(H): Rat's pancreas in the group which was ve^{+} + wholedrug

Fig (1):Histopathologicalexamination 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

The authors wish to thank Prof. Dr. Mona I. MassoudHead of Crops Research Institute, Agriculture Research Center, El-Sahahia, Alex. Egypt for her valuable support

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Journal of Home Economics, Volume 28, Number (1,2,3), 2018

التأثيرات الوقائية المحتملة للمستخلص المائى للباميه تجاه مضاعفات مرض السكر في الفئران

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الملخص :

هدفت الدراسة الحالية إلى استخدام تركيزات مختلفة من المستخلص المائى للباميه (15، 30، 45 جرام /100مل) بالإضافة الى الباميه الكاملة وتلك الناتجة بعد الاستخلاص المائى وذلك لتغذية الفئران المصابة بالداء السكرى والمغذاة لفترة ستة اسابيع. هذه المجاميع تم مقارنتها من الناحية البيولوجية والهستوباثولوجية مع مجموعتين أحدهما تمثل االمجموعة الضابطة الموجبة والآخرى تمثل المجموعة الضابطة السالبة. هذا وقد تم دراسة التركيب التقريبى للباميه بالإضافه إلى تقدير نسب الألياف الخام وكل من الالياف الذاتية الحامضية والمتعادلة وبعض المعادن مثل الكالسيوم والمغنسيوم والحديد. تم اجراء التقييم الحسى للمستخلصات المائية الثلاث باستخدام أفراد مصابة بداء السكرى. وأوضحت النتائج ان هناك اختلافات معنوية ($P \leq 0.05$) بين أوزان الجسم والأعضاء الداخلية خصوصا القلب والطحال بالنسبة للفئران التى تم تغذيتها على المستخلصات المائية للباميه بالمقارنة بالمجاميع الآخرى. بالاضافة الى ذلك وجد ان المستخلصات المائية تشجع من افراز الأنسولين ومن ثم تقلل من مستوى الجلوكوز. أيضا أثبتت الدراسة ان المستخلص المائى آدى إلى تحسن صورة دهون الدم وذلك لخفض مستوى الجليسريدات الثلاثية والكوليسترول مخفض الكثافة والكلولسترول المنخفض جدا للكثافة فى حين حدث تحسن ملحوظ فى نسبة الكوليسترول عالى الكثافة وكل الوظائف الخاصة بالكبد والكلى. أثبت الفحص الهستوباثولوجى للبنكرياس ان المجاميع التى تم تغذيتها على المستخلص المائى للبامية (30، 45جرام/100مل) كانت متشابهه الى حد كبير من المجموعة السالبة مع عدم وجود اى تغيرات هستوباثولوجية بالمقارنة مع مجاميع الفئران الآخرى المصابة بالداء السكرى . أوضحت الدراسة أيضا ان مرض الداء السكرى كان تقبلهم للمستخلص المائى (30جرام/100مل) احسن وأفضل من التركيزات الاخرى. وبصفة عامة يمكن القول بان أضافة المستخلص المائى للباميهإلى الوجبات الغذائية ممكن ان يؤدى الى التحكم فى مرض الداء السكرى وتحسين وظائف الكبد والكلى.

الكلمات المفتاحية: المستخلص المائى للباميه - مرض الداء السكرى- صورة دهون الدم – وزن الأعضاء – الفحص الهستولوجى.