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Using Okra (*Abelmoschus esculentus* L.: Malvacae) in the treatment of diabetic rats in Egypt

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Abstract

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to the secreted insulin. Recently, some medicinal plants have been reported to be useful in diabetes treatment as Okra (*Abelmoschus esculentus* L.). Many drugs decrease the hyperglycemic effects like Cidophage. The aim of the present study was to evaluate the possible antihyperglycemic property of Okra water extract and the Cidophage drug (1000 mg metformin) and their antioxidant mechanisms in Alloxan induced diabetic rats.

This study was performed on twenty males and twenty females' albino rats from the genus *Rattus norvigious* with an average body weight of 150-350g. Animals were divided into eight groups (five/cage), control untreated group, diabetic group, diabetic group treated with plant extract that was given orally and diabetic group treated with the stock solution of Cidophage drug that was given orally.

Results showed in diabetic groups marked decline in levels of body weight, organs weight, total proteins and albumin. These are accompanied with marked elevation in levels of fasting blood glucose, AST, ALT and ALP. These had risk factors in diabetic rats as compared to the corresponding controls. While the daily administration of diabetic rats treated with the Okra showed more significant amelioration in most of these parameters than that of treated with the Cidophage drug.

Histopathologically; normal samples showed apparent intact morphological features of hepatic tissue (liver), kidney, pancreas, testes and ovary.Diabetic samples showed moderate degeneration, vacuolar changes and many pyknotic changes. Diabetic/Okra and Diabetic/ Cidophage samples showed moderate protective efficacy with persistence of milder records of degenerative changes in tissues. Diabetic/ Okra samples also, more significant amelioration in tissues than that of Diabetic/Cidophage samples.

It could be concluded that Okra treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress and pancreatic β-cells' damage which may be attributed to its antioxidative potential and antidiabetic property more than the Cidophage drug.

Keywords: *Diabetes mellitus, Okra plant, Alloxan, Metformin, Liver and kidney functions.*

Introduction

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type- 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. Type-1 diabetes is a chronic condition in which the pancreas produces little or no insulin by itself. There is a globally agreed target to halt the rise in diabetes and obesity by 2025. About 422 million people worldwide have diabetes, particularly in low and middle-income countries **(WHO, 2019)**. The *Diabetes mellitus*disease remains incurable and can only be controlled with drugs, and in some cases exercise and dietary recommendations**(Macedo *et al.*, 2002).**

Okra (*Abelmoschus esculentus* L.) is a flowering plant of the Malvacaefamily, which is also known as lady's fingers, gumbo, bamya or bamia or bhindi **(Georgiadis, *et. al.*, 2011; Subrahmanyam *et al.*, 2011)**. There are four known domesticated species of the genus *Abelmoschus*. Among these, *A. esculentus* (common Okra) is the most widely cultivated in South and East Asia, Africa and southern USA **(Crossly and Hilditech, 1951)**.

Okra contains high fiber, which "helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract". Because of fiber along with other nutrition, Okra shows useful for minimizing blood sugar levels within the body, assisting along with diabetes **(Gemede *et al.*, 2015).** Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds **(El-Sheikh, 2012)**.

The edible part of the fruit contains 1.9gm protein, 0.2gm dietary fiber, 6.4gm starch, 66mg Calcium, 56mg Phosphorous, 0.35mg Iron, 6.98mg Sodium, 103mg Potassium, 0.19mg Copper, 30mg Sulphur, 0.7mg Thiamine, 0.1mg Riboflavin, 13mg of Vitamin C and 89.6gm moisture**(Suraksha, 2018).** Carbohydrates are mainly present in the form of mucilage. That of young fruits consists of long chain of sugar units and amino acids. The main components are: 25% galactose, 22% rhamnose, 27% galacturonic acid and 11% amino acids **(Kumar*et al.*, 2009).** The polysaccharide mucilage is soluble in water due to their ability to interact with water and swell **(Gemede *et. al.*, 2018).** Metformin, a biguanide anti-hyperglycemic agent, is widely used in the management of type 2 diabetes mellitus. It lowers the blood glucose concentration without causing hypoglycemia. **(Scheen, 1996; Choi*et al.*, 2008; Meng *et al.*, 2017).**

The aim of the study is to observe the anti-diabetic activity of the extract of okra and Cidophage drug and their antioxidant mechanisms in Alloxan induced diabetic rats.

MATERIAL AND METHODS

Experimental Animals

Wister rats (*Rattus norvegicus*) were used in this study. Forty Wister albino rats divided into males and females(20 rats each), weighing between 150 -350 gm were obtained from the animal house of department of toxicity of marine animals and mammals, Central Agric.

Pesticides Laboratory, Agricultural Research Center, Giza, Egypt. The animals were placed in standard cages. Standard food and water were available for animals *ad libitum*.

Drugs and Chemicals

Cidophage tablets (1000 mg of metformin HCl), Chemical Industries Development (CID), Giza, ARE and purified water (50 ml approx.), Pharmapack Pharmaceutical Industries, 6th October, Egypt; were brought from the pharmacy. Alloxan monohydrate (25 gm) was purchased from The Recent Scientific Company. Formalin, Diethyl ether, Glucometer (OK meter Match II Blood Glucose Monitoring System, Germany) and sterile Twist Top Lancets (28 G) were purchased from Al-Gomhouria Chemical Company.

Sourcing of plant material

1000 gm of fresh mature fruits of okra (*Abelmoschus esculentus*) were brought from the grocery market, washed and dried in an oven (100°C) and gave 0.124 gm of dried Okra, then ground it to convert it into powder.

Extraction of the active ingredient in okra (Mucilage)

The powdered material was soaked in the purified water for 5-6 h and boiled in water path for 30 min. Thick; mucus fluid was obtained which was the extracted mucilage **(Bindu and Fahsa, 2013)**.

Preparation of Cidophage drug solution

Tablets of the Cidophage drug contain 1000 mg of the active ingredient of metformin. These tablets ground to fine powder. This powder was dissolved in the purified water to give the stock solution.

Induction of diabetes

Animals were fasted overnight and diabetes was induced by a single intra-peritoneal injection of a freshly prepared solution of Alloxan (140 mg/kg body weight) in purified water into animals. After an hour; animals are re-feed with adding 5% sugar to the water just for the first day then, after that the ordinary water *ad libitum.*

Experimental design

The experimental animals were divided into eight groups, five for each and were used as follow:

- **T1** (control group) non-diabetic male rats
- **T2** (control group) non-diabetic female rats
- **T3** (diabetic group) male rats were injected intra-peritoneally with a single dose of Alloxan (140 mg/ kg)
- **T4** (diabetic group) female rats were injected intra-peritoneally with a single dose of Alloxan (140 mg/ kg)

- **T5** (treated group) diabetic male rats treated orally with the Okra (200 mg/ kg, twice/ day) for 28 days
- **T6** (treated group) diabetic female rats treated orally with the Okra (200 mg/ kg, twice/ day) for 28 days
- **T7** (treated group) diabetic male rats treated orally with the Cidophage drug (100 mg/ kg, once/ day) for 28 days
- **T8** (treated group) diabetic female rats treated orally with the Cidophage drug (100 mg/ kg, once/ day) for 28 days

Blood sample collection

Diabetes will be verified by evaluating blood glucose levels and diabetic rats are confirmed if blood glucose level greater than 300 mg/dl; as in the former studies.

In this study; after 72h of Alloxan injection, blood samples were taken from tail vein and the fasting blood glucose concentration was determined by means of one touch ultra-glucometer and compatible blood glucose strips. Animals with fasting blood glucose levels 480-500 mg/dl; were selected as diabetic rats for the experiment.

At the end of study, the animals were sacrificed under diethyl ether anesthesia. Measurements of the blood glucose and blood samples were taken from the orbital vein. The fasting blood glucose concentrations were determined by means of one touch ultra-glucometer and the blood glucose strips. Animals with fasting blood glucose levels > 400 mg/dl.

The blood samples centrifuged at 3000 rpm for 30min. The clear non- haemolysed supernatant sera were removed and immediately stored at-18°C till used for further analysis of biochemical parameters.

Biochemical analysis

1- Liver functions:-

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine phosphatase (ALP), total protein and albumin

All enzyme levels were determined by colorimetric method.

2- Complete Blood Count (CBC)

Technical Procedures

All invasive procedures on animals were performed under general anesthesia using diethyl ether. Blood samples were obtained from the orbital vein at the end of experiment. The entire liver were removed, rinsed, and weighed on an analytical balance after exsanguination. Segments representing the entire organ were randomly taken from the rats in each experimental group and cut, prefixed in buffered 10% formalin, processed in paraffin blocks, and sectioned at 3–5 μm for histological analyses. The tissue slides from each block were stained with Hematoxylin and Eosin (HE).

Results

Blood sugar

The results showed elevated blood sugar levels in either male and female diabetic rats after 72-hr., being 502.200 mg and 490.200 mg for each of male and female diabetic rats, respectively. After 28- days, these figures were 421.000 mg for male and 427.200 mg for female as compared with the control one (Table 1). This is due to the selective toxicity of Alloxan on β-cells of pancreas after the Alloxan injection resulting in reduced synthesis and release of insulin which leads to alteration of glucose metabolism and utilization thereby causing hyperglycemia.

Table (1) Mean blood glucose of male and female rats after 72-hr and 28-days of different treatments ($P \leq 0.05$).

Treatments	Male		Female	
	72-hr	28-days	72-hr	28-days
Control	107.400^{de}	107.400^{de}	87.600^{d}	87.600^{d}
Alloxan	502.200^{a}	421.000^{c}	490.200^{a}	427.200^{b}
Alloxan + Okra	493.800^{ab}	100.200^{e}	492.600^{a}	98.200*^{cd}
Alloxan + Cidophage	483.600^{b}	117.000^{d}	491.000^{a}	109.800^{c}
LSD	15.99		18.90	

After 28-days of treatment with Okra and Cidophage, a decrease in sugar level was detected either with diabetic male rats (100.200 mg) and 98.200 mg in diabetic female rats when treated with Okra. On the other hand, these were 117.000 mg and 109.800 mg for male and female rats, respectively when treated with Cidophage as compared with diabetic one. The treatment of Okra showed more decreasing levels as compared with that treatment of Cidophage (Table 1). The hypoglycemic effect of Okra fruit might bedue to the presence of a mixture of soluble dietary fibers, large amount of carbohydrates, the high viscosity of the mucilage of Okra, the inhibition of intestinal glucose diffusion and absorption as well as inhibition of some enteric intestinal enzymes such as α- glucosidase and α- amylase that are all associated with hypoglycemic effect.

Khatun *et al*. (2011) reported that the effect of water-soluble fraction (WSF) of Okra in reducing the absorption of glucose is more stable due to the presence of a mixture of soluble fibers and large amount of carbohydrates in WSF of Okra. Water-soluble fraction of the fruits of Okra was studied to check the absorption of oral glucose as well as metformin from the gastrointestinal tract in the Long Evans rats. It showed significant reduction in absorption of glucose as studied in the 24 hours fasting rats.

Blood picture

After injection with Alloxan a decrease in Hemoglobin (Hb) was occur in both male and female's diabetic rats after 28-days. The values were 10.220 gm% and 9.080 gm%, respectively, as compared with control one (Table 2 and 3). This may be due to the decrease in protein

synthesis in all tissues, thus the synthesis of hemoglobin is also reduced due to relative deficiency of insulin.

Table (2) Male blood picture after 28-days of different treatments.
(LSD for the difference between different treatments at $P \leq 0.05$)

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
Hb	13.080[a]	10.220[c]	11.980[b]	11.740[b]	1.079
RBCs	5.126	5.054	4.904	4.280	1.021 (ns)
WBCs	6220.000[b]	12360.000[a]	6680.000[b]	7320.000[b]	2272
Heamatocrit	39.240[a]	30.840[c]	35.940[b]	35.220[b]	3.144
MCV	78.084[a]	61.868[b]	74.384[a]	83.022[a]	9.739
MCH	25.962[a]	20.620[b]	24.816[a]	27.672[a]	3.289

Table (3) Female blood picture after 28-days of different treatments.
(LSD for the difference between different treatments at $P \leq 0.05$)

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
Hb	11.660[a]	9.080[b]	10.540[a]	11.240[a]	1.343
RBC.s	4.632	4.560	4.590	4.684	0.8483 (ns)
WBC.s	5500.000[c]	13100.000[a]	4580.000[c]	8560.000[b]	1003
Heamatocrit	34.980[a]	27.240[b]	31.640[a]	33.720[a]	4.027
MCV	76.074[a]	59.528[b]	690252[ab]	73.098[a]	10.02
MCH	25.360[a]	19.842[b]	23.082[ab]	24.326[a]	3.303

In diabetes, the persistent and excess amount of glucose present in the blood reacts with hemoglobin to form glycated hemoglobin which may also induce the generation of oxygen derived free radicals and other diabetes-associated complications in prolonged diabetic condition **(Lucchesi *et al.* 2015).**

The treatment of Okra showed increasing levels reaching to the control ones, 11.980 gm% and 10.540 gm%, while in the treatment with Cidophage the values are 11.740 gm% and 11.240 gm%, for male and female, respectively, as shown in Tables (2 and 3). This is due to that, the Okra dietary fiber could bind to glucose and prevent or delay it absorption from the intestinal lumen. The antioxidant or free radical scavenging property in okra fruit may inhibit oxidative reactions associated with glycation.

RBCs count, Heamatocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) decreased in either males or females diabetic rats after 28-days from injection with Alloxan drug as compared with control one. Treatment with either Okra or Cidophage, show an increase in their levels and reach to the control ones (Tables 2 and 3). On the other hand, WBCs count increase in each of male and female diabetic rats after 28- days of treatment with Alloxan, these values decreased near the control levels, after treatment with Okra and Cidophage (Tables 2 and 3).

Liver functions

Results of each of Alanine aminotransferase enzyme (ALT), Aspartate aminotransferase enzyme (AST) and Alkaline phosphatase enzyme (ALP) of each of male and female diabetic rats

after 28-days of treatments are shown in Tables (4 and 5) for male and female diabetic rats, respectively. The level of each of ALT, AST and ALP are increased after treatment with Alloxan as compared with the control one. Significant decrease ($P \leq 0.05$) in the level of each of ALT, AST and ALP was occur when male (Table 4) and female (Table 5) diabetic rats are treated with Okra water extract and Cidophage drug.

Table (4) Male liver functions after 28-days of different treatments. (LSD for the difference between different treatments at $P \leq 0.05$)

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
ALT	6.200^{d}	20.000^{a}	11.200^{c}	14.800^{b}	2.804
AST	148.600^{c}	293.600^{a}	180.600^{b}	189.400^{b}	28.02
ALP	14.600^{c}	40.000^{a}	28.400^{b}	29.200^{b}	2.532
Total Protein	4.000^{a}	3.020^{b}	2.400^{b}	2.400^{b}	0.9734
Albumin	4.580^{a}	2.380^{c}	4.100^{a}	3.220^{b}	0.7266

Table (5) Female liver functions after 28-days of different treatments. (LSD for the difference between different treatments at $P \leq 0.05$)

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
ALT	5.800^{c}	20.200^{a}	15.000^{b}	17.000^{b}	2.912
AST	142.400^{c}	292.400^{a}	215.800^{b}	236.600^{b}	22.02
ALP	14.400^{c}	40.800^{a}	28.000^{b}	28.000^{b}	5.031
Total Protein	3.420^{a}	1.960^{b}	2.840^{a}	2.880^{a}	0.63
Albumin	3.900^{a}	2.420^{c}	2.980^{b}	2.800^{bc}	0.4673

This is may be as a result of metabolic changes in the liver due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of Alloxan. The elevation in the activities of AST & ALT enzymes in diabetic rats reflects a state of hepatocyte injury. The increased activity of ALP enzyme in serum may be a result of diabetes induced damage to the tissues. ALP is a potent anti-inflammatory mediator that can protect tissues from damage resulting from injury. *A. esculentus* fruits treatment brought down the elevated levels of ALP. This result could be due to certain compound present in the plant extract which undergo exchange reactions with titrable–SH groups of the enzyme and proteins in the body spontaneously and inhibit the enzyme activity.

On the other hand, Total Protein and Albumin; decreased in male (3.020) and female (2.38) in the serum of diabetic rats after 28- days, for Total Protein in compared with control one. For Albumin, there is a decrease of serum Albumin in males (1.96) and in females (2.42), as compared to the control. Levels of each of Total Protein and Albumin are restored in control rats by insulin treatment, which accelerates amino acid transport through cells and stimulates the protein manufacturing machinery of the cell. The treatment with either Okra or Cidophage show an increase in the levels of each of Total Protein and Albumin to reach the control ones. The increment with Okra gives results higher than that of Cidophage (Tables 4 and 5). This is may be due the fact that the aqueous extract of Okra plant can control the rate of gluconeogenesis in diabetes.

Body weight at different periods of time

Tables (6 and 7) show results of body weight of diabetic rats at three different times; initial, 72-hrs and 28-days of different treatments. The results show that the average of body weight in the control group was increased. At 72- hr. weights after Alloxan injection an increased in males and females diabetic rats by 239.200 gm and 218.800 gm, respectively, as compared with control one as shown in Tables (6 and 7). This is may be due to the increase in food and water intake.

Table (6): Male body weight at 3 different times; initial, after 72-hr and 28-days under different treatments (LSD for the difference between different treatments at *P*≤ 0.05).

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
Initial	167.000[d]	189.400[a]	180.600[b]	174.200[c]	4.749
After 72-hr	191.600[b]	239.200[a]	171.200[c]	164.200[c]	7.697
After 28-days	340.200[a]	159.800[c]	238.000[b]	234.000[b]	8.806

Table (7): Female body weight at 3 different times; initial, after 72-hr and 28-days under different treatments (LSD for the difference between different treatments at *P*≤ 0.05).

Body weight	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
Initial	163.200[b]	168.600[ab]	171.600[a]	148.400[c]	7.497
After 72-hr	179.200[b]	218.800[a]	164.400[c]	139.800[d]	8.238
After 28-days	327.000[a]	147.400[c]	245.000[b]	221.000[b]	25.56

A decrease in the weight of males and female diabetic rats after 28-days of treatments, by 159.800 gm and 147.400 gm, respectively, as compared with control one (Tables 6 and 7). The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Drastic decrease in body weight of the Alloxan- induced diabetic rats could be due to the selective destruction of pancreatic ß-cells of the islets of Langerhans (insulin producing cells) by Alloxan. This leads to insulin deficiency and leading to decrease in peripheral glucose uptake and utilization and increase gluconeogenesis. These cause increase degradation of structural proteins thereby affecting the body weight of the animals. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.

The treatment with Okra show increase in the weights reaching to the control ones. Same results was occur in of treatment with Cidophage drug (Tables 6 and 7). Okra fruit has the ability to reduce hyperglycemia and improve glucose metabolism. Also, may lead to improve peripheral glucose uptake and utilization, glycogen synthesis and decrease gluconeogenesis. These spare structural protein from degradation (muscle wasting) and help maintain the weight.

Liver weight

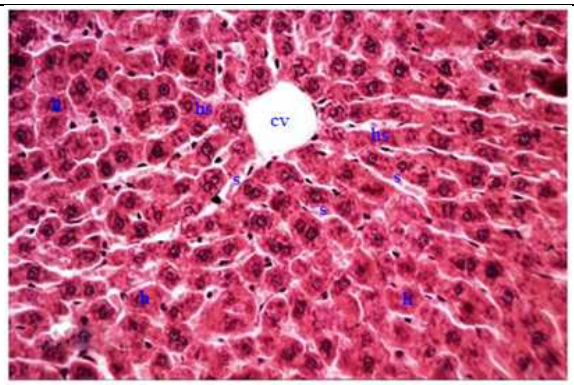
Liver weight decreased in male and female diabetic rats after 28- days. The values are 4.953 and 4.759 for males and females, respectively, as compared with control one 7.515 and 6.872. Treatment of Okra shows an increase in the weights reaching to the control one. On the

other hand, there is an increase in body weight with the application of Cidophage treatment, but this increase is lower than that of Okra.

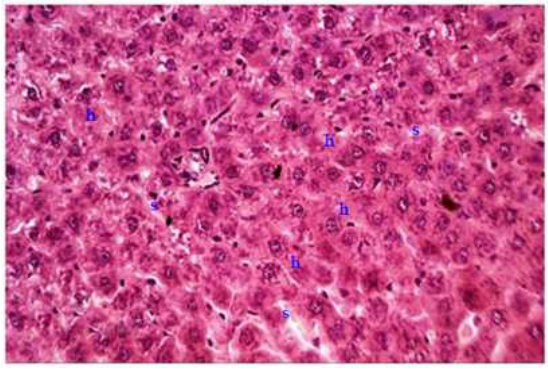
Table (8): Male and Female liver weight in gm. under different treatments (LSD for the difference between different treatments at $P \leq 0.05$).

Item	Control	Alloxan	Alloxan + Okra	Alloxan + Cidophage	LSD
Male	7.515[a]	4.953[c]	7.540[a]	6.066[b]	0.8672
Female	6.872[a]	4.759[b]	6.417[a]	5.271[b]	0.7292

Histopathology

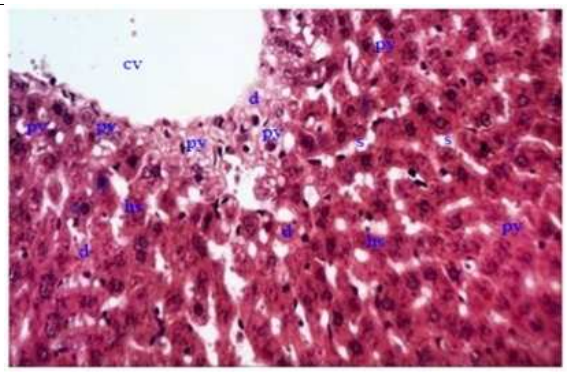


Slide (1) Normal male rat liver

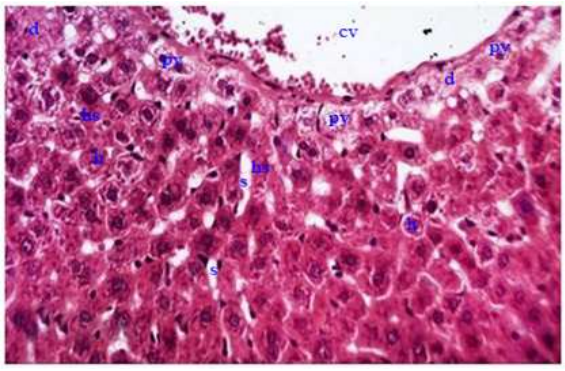


Slide (2) Normal female rat liver

Slides (1 and 2) Male and female normal liver samples showed apparent intact morphological features of hepatic tissue with many intact hepatocytes with large vesicular nuclei, intact hepatic sinusoids as well as vasculatures.

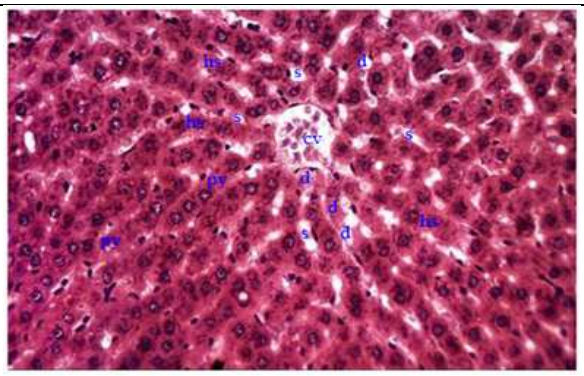


Slide (3) Diabetic male rat liver

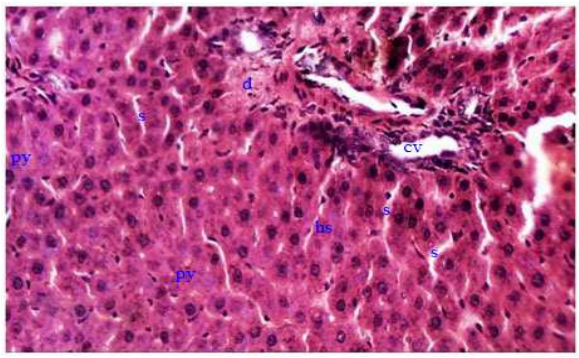


Slide (4) Diabetic female rat liver

Slides (3 and 4) Male and female liver samples of diabetic rats after the injection with Alloxan, showed moderate pre-central hepatocellular degenerative and vacuolar changes with moderate dilation of hepatic blood vessels and occasional records of periportal inflammatory cells infiltrates.

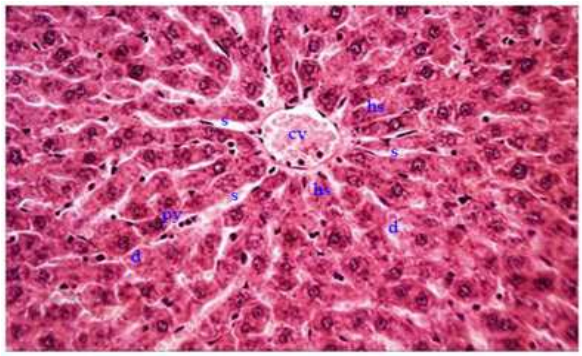


Slide (5) Diabetic male rat liver treated with Okra

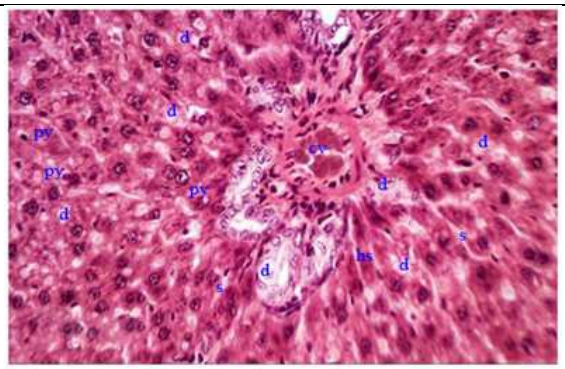


Slide (6) Diabetic female rat liver treated with Okra

Slides (5 and 6) Male and female liver of diabetic rat samples after injection with Alloxan and treatment with Okra showed slight area of cellular restoration with marked cellular degeneration, pyknotic nuclei and vascular congestion.



Slide (7) Diabetic male rat liver treated with Cidophage



Slide (8) Diabetic female rat liver treated with Cidophage

Slides (7 and 8) Male and female liver of diabetic rat samples after injection with Alloxan and treatment with Cidophage showed almost intact hepatocytes allover different hepatic zones with milder records of periportal inflammatory cells infiltrates and mild hyperplasia of bile ducts.

Discussion

Diabetes mellitus is a disease initially characterized by a hyperglycaemia. The disease is progressive and possibly the world's fastest growing metabolic disorder. Oxidative stress a condition in which excessive formation of free oxygen radicals is greater than the ability of the organism to eliminate them, plays an important role in the aetiology of *Diabetes mellitus*. Reactive oxygen species (ROS) generated in diabetes causes oxidative damage to all of the entire organs. One of the organs most susceptible to oxidative stress in diabetes is liver, which plays a major role in the metabolism of carbohydrates and lipids and others. Since, under the pressure of oxidation-reduction imbalance, the body needs help, it is desirable to bring additional quantities of antioxidants into the diet **(Orsolic *et al.*, 2008; Leopoldini *et al.*, 2011; Sirovina *et al.*, 2013).**

The three main proposed mechanisms through which the antioxidants may realize their protective role are the hydrogen atom transfer, single electron transfer, and metal chelation **(Heim *et al.*, 2002).**

In alloxan induced diabetic rats, there was marked hyperglycaemia groups due to the selective destruction of the insulin-producing pancreatic beta-islets. The groups treated with an anti-hyperglycemic agent and aqueous and dried Okra extract showed steady decrease in glucose levels significantly ($P \leq 0.05$) in the first and second week. Alloxan induces a multiphasic blood glucose response when injected into an experimental animal and accompanied by corresponding inverse changes in the plasma insulin concentration, followed by sequential beta cell changes leading to necrotic cell death **(Kliber *et al.*, 1996)**. The first phase that comes into view within the first minutes after Alloxan injection is transient hypoglycemic phase that lasts maximally for 30 minutes. The 2nd phase appearing one hour after administration of Alloxan leads to rise in blood glucose concentration. Moreover, the plasma insulin concentration has been noted to decrease at the same time. This is the first hyperglycemic phase after the first contact of the pancreatic beta cells with the toxin. The 3rd phase is again a hypoglycemic phase that is noted 4-8 hours after the Alloxan injection, which lasts for several hours.

The last and the 4th phase of the blood glucose response is the final permanent diabetic hyperglycemic phase during which complete degranulation and loss of the integrity of the beta cells within 24-48 h after administration of the Alloxan takes place (**Kliber *et al.*,1996; Ankur and Shahjad, 2015)**.

Plants are natural resources for human ailment. Folk medical uses are still implemented in this modern civilized era for the remedy of various complications. Okra, *Abelmoschus esculentus* revealed statistically significant hypoglycemic activity because it contains secondary metabolites to reflect their hypoglycemic activity. Aqueous extract was showing maximum effect than the dried powdered form of *A. esculentus*(**Ben-Chioma *et al.*, 2015)**. Although many anti-diabetic drugs have been used for the treatment of diabetes, potent ones with high efficiency and low side effects are necessary; one of them is metformin**(Meng *et al.*, 2017)**.

Induction of *D. mellitus* was evident in the diabetic rats based on significantly higher blood glucose level, significantly larger 24-h urine output and significantly smaller body weight gain than the controls. Smaller body weight gain was related to the phosphorus depletion of soft tissues in Alloxan-induced diabetes (**Locatto*et al.*, 1997; Choi *et al.*, 2006**). **Choi *et al.* (2006and 2008)** said that the hematocrit was significantly greater than the controls.

Ramachandran *et al*. (2010) reported that the potential of Okra as anti-diabetic activity on Alloxan-induced diabetic rats. **Sabita *et al*. (2013)** has reported anti-diabetic potential of Okra peel and seed powder in streptozotocin (STZ)-induced diabetic rats. Administration of this powder at 100 and 200 mg/kg dose in diabetic rats showed significant reduction in blood glucose level and increase in body weight than diabetic control rats. Water soluble fraction of the fruits of Okra was studied to check the absorption of oral glucose as well as metformin from the gastrointestinal tract in the rats. It showed significant reduction in absorption of glucose as studies in the 24 hours fasting rats.

Subrahmanyam *et al*. (2011) has reported anti-diabetic activity of Okra fruit extract and used metformin drug as standard. After 24 hours of injecting Alloxan, an increase in the blood glucose levels was observed. Okra fruit extract was given along with feed to group1. The second

group was given metformin as anti-diabetic drug. A gradual decrease in the blood glucose levels of both groups.

A. esculentus fruits significantly decreased serum glucose levels, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities on diabetic albino rats **(Uraku *et al*., 2011).** Okra acts in improving insulin resistance and therefore preventing it from being lost in diabetics. As the vegetable is known to have zero fat and high fibre **(Suraksha, 2018).**

Uraku *et al*. (2011) said that there was a significant ($P \leq 0.05$) decrease in weight gain in the untreated diabetic group when compare to the control group. This observation may be attributed to the reduction in utilization of food and fluid. However, inducement of hyperglycemia has been linked to weight loss **(Ugochukwu and Babady, 2003)**. Administration of different doses (200, 400 and 800 mg/kg) of *A. esculentus* aqueous–ethanolic extract to diabetic rats group recorded significantly higher ($P \leq 0.05$) body weight gain. There was a significant ($P \leq 0.05$) increase in serum glucose of the untreated diabetic group compare to the control group. This increase indicates uncontrolled hyperglycemia in the Alloxan induced animals. Okra fruits treatment brought down such elevated levels of ALP significantly ($P \leq 0.05$) in diabetic animals on administration of varying dosages (200, 400 and 800 mg/kg) body weight of the aqueous–ethanolic extracts. The activity of AST and ALT were enormously elevated ($P < 0.05$) from that of normal group, indicative of enhanced gluconeogenesis in uncontrolled diabetes. Okra fruits treatment significantly ($P \leq 0.05$) decreased the activities of AST. The same results reported by **Balamash *et al*. (2018)**when examined the effect of metformin on diabetic rats the activity of AST, ALT ALP serum levels were significantly higher than that in non-diabetic control and diabetic control groups.

Stained slides were examined under a light microscope. Liver sections were examined for vacuolization, lymphocyte infiltrations, necrosis, and apoptosis. **Balamash *et al.* (2018)**showed theliver histopathology. Observation of the hepatic tissue of the untreated non-diabetic control rats showed histological features like radially arranged hepatocytes around the central vein. The cells have an acidophilic cytoplasm and rounded central vesicular euchromatic nuclei with well-defined nucleoli. The hepatocyte plates are separated by thin-walled blood sinusoids lined by flat endothelial cells. Occasionally, the prominent nuclei of von Kupffer cells were observed. The section of the hepatic tissue of untreated diabetic control rats showed an increase in apoptotic hepatocytes (shrunken and dark-stained cells with small degenerated nuclei). The liver tissue sectioned from the diabetic rats treated with metformin showed nearly normal radially arranged hepatocytes around the central vein. Blood sinusoidal spaces and their von Kupffer cells are similar to those of the non-diabetic control group. The liver tissue taken from diabetic rats treated with metformin exhibited almost normal hepatic structure with radially arranged hepatocytes around the central vein with the absence of any degenerated apoptotic cells. The hepatic sinusoidal spaces and von Kupffer cells are similar to those of the non-diabetic control group.

Conclusion

OKRA is a natural product and it has anti diabetic activity so the usage of Okra is not harmful to human health. It could be concluded that Okra (*Abelmoschus esculentus*) treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress and pancreatic β-cells' damage which may be attributed to its antioxidative potential and antidiabetic property more than the Cidophage drug. Therefore the Okra fruit can be used to treat/manage *Diabetes mellitus*.

References

Ankur, R. and Shahjad, A. (2015). Alloxan Induced Diabetes: Mechanisms and Effects. *Ijrpbs,*3(2): 819-823.

Balamash, K. S.; Alkreathy, H. M.; Al Gahdali, E. H.; Khoja, S. O. and Ahmad, A. (2018). Comparative Biochemical and Histopathological Studies on the Efficacy of Metformin and Virgin Olive Oil against Streptozotocin- Induced Diabetes in Sprague-Dawley Rats. *Journal of Diabetes Research,* Hindawi, Volume 2018, Article ID 4692197, 10 pages

Ben-Chioma, A. E.; Tamuno-Emine, D. G. and Dan, D. B. (2015).: The Effect of Abelmoschus esculentus in Alloxan- Induced Diabetic Wistar Rat. *IJSR,* 4(11): 540-543.

Bindu, R. N. and Fahsa, K. S. (2013). Isolation and characterization of mucilage from selected species of Abelmoschus *medic* (Malvaceae) and their application in pharmaceutical suspension preparation, *Int J Pharm Pharm Sci,* 5(1): 398-402.

Choi, Y. H.; Kim, S .G. and Lee, M. G. (2006). Dose independent pharmacokinetics of metformin after intravenous and oral administration in rats: hepatic and gastrointestinal first-pass effects. *J. Pharm. Sci.,* 95: 2543–2552.

Choi, Y. H.; Lee, M. G. and Lee, I. (2008). Effects of Diabetes Mellitus Induced by Alloxan on the Pharmacokinetics of Metformin in Rats: Restoration of Pharmacokinetic Parameters to the Control State by Insulin Treatment. *J Pharm Pharmaceut,*11 (1): 88-103.

Crossly, A. and Hilditech, T. P. (1951). The fatty acids and glycerides of okra seed oil. *I. Sci. Food Agric.,* 2: 252-255

El-Sheikh, N. M. (2012). Mangifera Indica leaves extract modulates serum leptin. Asymmetric Dimethylarginine and Endothelin-1 leaves in experimental Diabetes mellitus. *The Egptian J Bio & Mol Bio.,* 30(2): 229-244.

Gemede, H. F.; Ratta, N.; Haki, G. D,; Woldegiorgis, A. Z. and Beyene, F. (2015). Nutritional Quality and Health Benefits of Okra (Abelmoschus esculentus): A Review. *J Food Process Technol,* 6(6): 6 pages.

Gemede, H. F.; Haki, G. D.; Beyene, F; Rakshit, S. K. and Woldegiorgis, A. Z. (2018). Indigenous Ethiopian okra (Abelmoschus esculentus) mucilage: A novel ingredient with functional and antioxidant properties. *Food Sci Nutr.,*6:563–571.

Georgiadis, N.; Ritzoulis, C.; Sioura, G.; Kornezou, P.; Vasiliadou, C. and Tsioptsias, C. (2011). Contribution of okra extracts to the stability and rheology of oil-in-water emulsions. *Food Hydrocolloids,* 25 (5): 991-999.

Heim, K. E.; Tagliaferro, A. R. and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem,*13: 572–584.

Kliber, A.; Skuldebeki, T. and Chichlowska J. (1996). Stimulation and subsequent inhibition of Insulin Release from Imide Performed Rat Pancrease. *Journal of Physiological Pharmacology,* 47: 321–328.

Kumar, R.; Patil, M. B.; Patil, S. R. and Paschapur, M. S. (2009). Evaluation of Abelmoschus esculentus mucilage as paracetamol suspension. Intl. *J Pharm Tech Res,* 1:658-665.

Leopoldini, M.; Russo, N. and Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem,*125: 288–306.

Locatto, M. E.; Di Loreto, V.: Fernandez, M. C.; Caferra, D. and Puche, R. C. (1997). Relative weight of glucose, insulin, and parathyroid hormone in urinary loss of phosphate by chronically diabetic rats. *Acta Diabetol,* 34: 211–216.

Lucchesi, A. N.; Cassettari, L. L. and Spadella, C. T. (2015). Alloxan-Induced Diabetes Causes Morphological and Ultrastructural Changes in Rat Liver that Resemble the Natural History of Chronic Fatty Liver Disease in Humans. *J Diabetes Res.*, 2015: 494578. doi: 10.1155/ 2015/494578.

Macedo, C. S.; Capellettism, M.C; Padovani, C. R. and Spadella, C.T. (2002). Role of Metabolic control on Diabetic Nephropathy. *Acta Bras.*,17(6): 37–45.

Meng, X. M.; Ma, X. X.; Tian, Y. L. ; Jiang, Q.; Wang, L. ; Shir, L. Ding, L. and Pang, S. G. (2017). Metformin improves the glucose and lipid metabolism via influencing the level of serum total bile acids in rats with streptozotocin-induced type 2 diabetes mellitus. *European Review for Medical and Pharmacological Sciences,* 21: 2232-2237.

Orsolic, N. and Basic, I. (2008). Honey bee products and their polyphenolic compounds in tretment of diabetes. In: Govil JN and Singh VK (eds) Recent Progress in Medical Plants 22, Phytopharmacology and Therapetutic Values. IV. LLC, USA: Studium Press, pp. 455–471.

Ramachandran, S.; Sandeep V. S.; Srinivas, N. K. and Dhanaraju, M. D. (2010). Anti-diabetic activity of Abelmoschus esculentus Linn on alloxan-induced diabetic rats. *Research & Reviews in BioSciences,* 4(3) : 121-123.

Sabitha, V; Ramachandran, S.; Naveen, K. R. and Panneerselvam, K. (2013). Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench. In streptozotocin induced diabetic rats. *Journal of Pharmacy and Bioallied Sciences,* 3(3): 397-402.

Scheen, A. J. (1996). Clinical pharmacokinetics of metformin. *ClinPharmacokinet,* 30: 359–371.

Sirovina, D.; Orsolic, N.; Koncic, M. Z.; Kovacevic, G.; Benkovic, V. and Gregorovic, G. (2013). Quercetin vs chrysin: Effect on liver histopathology in diabetic mice. *Human and Experimental Toxicology,* 32(10) 1058–1066.

Subrahmanyam, G.V.; Sushma, M.; Alekya, A.; Neeraja, Ch.; Sai Sri Harsha, H. and Ravindra, J. (2011). Antidiabetic activity of *Abelmoschus esculentus* fruit extract. *International Journal of Research in Pharmacy and Chemistry,* 1(1): 1-4.

Suraksha, M. (2018). A Review on Okra as an Antidiabetic, Antioxidant and An Excellent Energy Source. *Organic & Medicinal Chem. I. J.*, 6(1): 18-27.

Ugochukwu, N. H. and Babady, N. E. (2003). Antihyperglycemic effect of aqueous and ethanolic extracts of Gongronema latifolium Gongronema latifolium leaves on glucose and glycogen metabolism in liver of normal and streptozotocin – induced diabetic rats. *Life science,* 73: 1925 – 1938.

Uraku, A. J.; Onuoha, S. C.; Offor, C. E.; Ogbanshi, M. E. and Ndidi, U. S. (2011). The effects of Abelmoschus esculentus fruits on ALP, AST and ALT of diabetic albino rats. *International Journal of Science and Nature,* 2: 582-586.

WHO (2019). Classification of diabetes mellitus. World Health Organisation, 40 pp.