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Hibiscus esculentus seed and mucilage beneficial effects in reducing complications of diabetes in streptozotocin-induced diabetic rats

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Abstract

Introduction: Diabetes mellitus (DM) is the most common endocrine disease. *Hibiscus esculentus* (L.) with common name of okra is a rich source of biologically active molecules.
Objectives: This study aimed to examine the effects of mucilage and seeds of okra on various biochemical and histopathological features of streptozotocin (STZ)-induced diabetic rats.
Materials and Methods: Forty male Wistar rats were divided randomly into 4 groups of 10 mice each: Two groups received seed (2 g/kg bw/day, i.p.) and mucilage (2 g/kg bw/day, i.p.) of okra daily for 14 days. Diabetes were induced in rats by administration of STZ (60 mg/kg bw, i.p.). Treatment was started from 2 weeks prior to the induction of diabetes and continued for another 3 weeks thereafter.
Results: Serum glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and malondialdehyde (MDA) was increased and serum insulin decreased significantly in diabetic rats compared to other groups. Mucilage consumption decreased glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C in diabetic rats and increased insulin level. Seed consumption significantly decreased glucose, antioxidant capacity, C-reactive protein (CRP), cholesterol, HDL, LDL, triglyceride and increased insulin level in diabetic rats. In diabetic rats that were treated with mucilage and seed of okra, histomorphic examinations showed reduction of inflammation in pancreatic beta cells.
Conclusion: Dietary supplementation with okra is effective in reducing serum glucose, CRP and lipid levels whilst improving antioxidant capacity in STZ-induced diabetic rats, showing the promising antihyperglycemic, antihyperlipidemic, anti-inflammatory and antioxidant properties of okra seeds.

Introduction

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. DM is characterized by hyperglycemia and dysregulation of carbohydrate metabolism due to destruction or inactivation of pancreatic beta cells, and defective insulin secretion (1-3). The management of DM is a worldwide medical problem and research for finding effective agents still continues (4-6). Based on epidemiological findings, a healthy diet rich in fruits and vegetables is associated with lower risk of DM and a better glycemic control in diabetic subjects. In this context, the use of medicinal plants with hypoglycemic properties has been receiving increasing attention (7-9). During the past 2 decades, several phytochemicals have been isolated from plants with positive effects on insulin

Core tip
The management of diabetes mellitus (DM) is a worldwide medical problem and research for finding effective agents still continues. Based on epidemiological findings, a healthy diet rich in fruits and vegetables is associated with lower risk of DM and a better glycemic control in diabetic subjects. In this context, the use of medicinal plants with hypoglycemic properties has been receiving increasing attention.

sensitivity, insulin secretion or intestinal glucose absorption. Another promise with herbal remedies is their safety, availability, low-price compared to chemical or biological therapies (9-12). Genus *Hibiscus* (Malvaceae) is widely distributed all over the world and comprises around 220 species, most of which being rich sources of biologically active molecules such as phenolic compounds, triterpenes and phytosterols (13). *Hibiscus esculentus* L. (common names: gumbo, okra and lady finger) is a medicinal plant belonging to the genus *Hibiscus* and grows in the tropical and subtropical regions. Okra pod contains high amounts of fibers such as pectin, xylan, xyloglucan, cellulose and vitamins B_6, A, C and folic acid. Okra mucilage refers to the slimy and thick substance in pod walls (not in seed) which has been suggested for the treatment of inflammatory and cardiovascular diseases. Okra seeds contain polyphenolic compounds, oligomeric catechins, flavonol and zinc (14). Flavonoids such as quercetin, isoquercetin, kaempferol and gossypin are found in different species of the *Hibiscus* genus and possess antioxidant, anti-hyperlipidemic and anti-diabetic effects (15).

Objectives

Given the presence of diverse bioactive phytochemicals in Okra, the present study aimed to investigate the effects of mucilage and seeds of *H. esculentus* on some biochemical and histopathological features of streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Plant material

The plant was collected during August 2012 from Ahvaz and identified by the department of natural resource of Isfahan.

Extraction of mucilage

Following removal of seeds, fresh *Hibiscus esculentus* fruits were washed, crushed and soaked in water for 5–6 hours. Afterward, fruits were boiled for 30 minutes and left for 1 hour to allow complete release of the mucilage into the water. The mucilage was extracted using a multi-layer muslin fabric bag. The mucilage was precipitated by adding acetone (in the quantity of 3 times the volume of filtrate). The mucilage was then separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in desiccator at 30°C and 45% relative humidity till use (16).

Purification of the mucilage

The crude mucilage (1%) was homogenized (Potter homogenizer, Sartorius AG, Germany) with cold dilute trichloroacetic acid solution (5%). The solution was then centrifuged (3500 rpm for 20 minutes), neutralized by drop wise addition of sodium hydroxide and then dialyzed for 30 hours against distilled water. The mucilage was precipitated by adding three volumes of 95% ethanol. The precipitate was washed successively with ethanol, acetone and diethyl ether. The seeds were separated and dried under shade. Both seed and mucilage were made as fine powder using mixer and were stored in an airtight container up to the completion of the study (17).

Experimental animals

Forty male Wistar rats weighing 220–250 g were used in this study. The animals were purchased from Shahrekord University of Medical Sciences and were kept in the animal lair at the department of biology, Isfahan University of Medical Sciences, under appropriate temperature, humidity and light conditions. The animals were allowed to acclimatize for 2 weeks and kept in plastic cages with free access to food and water (18-21). The study was approved by the Ethics Committee of the Isfahan Cardiovascular Research Center affiliated within the Isfahan University of Medical Sciences, Isfahan, Iran.

Induction of diabetes

After an overnight fasting, diabetes was induced by intraperitoneal injection of STZ (Sigma-aldrich), which was already dissolved in 0.1M cold sodium citrate buffer, pH 4.5. The dose of STZ was adjusted to 60 mg/kg body weight (bw) whilst the control rats received the vehicle alone. After 3 days of injection, blood glucose was measured using a digital glucose analyzer (Easygluco, USA) and the rats with blood glucose above 250 mg/dL were used for the experiments (22).

Grouping

In this study, 40 rats were randomly divided into 4 groups of 10 each:

Group 1: Non-diabetic control group which received normal saline intraperitoneally (the amount of injected saline was equal to the amount of injected extract). This group was included to serve as negative control and also to normalize the changes in blood glucose due to injection shock.

Group 2: Diabetic control group.

Group3: Seed-treated animals receiving *H. esculentus* seed powder at a daily dose of 2 g/kg bw for 2 weeks, followed by STZ injection. After induction of diabetes, supplementation with seed powder was continued through gavage for another 3 weeks.

Group 4: Mucilage-treated animals receiving *H. esculentus* mucilage at a daily dose of 2 g/kg bw for 2 weeks, followed by STZ injection. After induction of diabetes, supplementation with mucilage was continued through gavage for another 3 weeks.

Fasting blood glucose was collected on days 1 and 14 (prior to the injection of STZ) as well as days 3 and 17 post diabetes induction. Blood sampling was performed through retro-orbital plexus puncture after anesthesia with ketamine (10 mg/kg i.p.)/xylazine (15 mg/kg i.p.).

Biochemical parameters estimation

Fasted blood samples were collected at baseline, day 14 (prior to the diabetes induction), day 17 (3 days after diabetes induction) and at the endpoint (3 weeks after diabetes induction). Samples were collected from each rat and serum was separated from blood 2-3 hours after sampling

by centrifugation at 3500-4000 rpm for 10 minutes. Collected serum samples were stored at -80°C until analysis. Glucose concentrations were determined at all 4 time points described above. Other biochemical parameters were determined in the samples obtained at days 3 and 21 after induction of diabetes, and included lipid profile total cholesterol, low-density lipoprotein (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, hemoglobin A1c (HbA1c), insulin, C-reactive protein (C-RP), malondialdehyde (MDA) and total antioxidant capacity, all being measured by routine spectrophotometric or enzymatic methods on an Automatic Hitachi 902 auto-analyzer (23-27).

Histologic examination of pancreas

At the end of study, animals were sacrificed and their pancreas removed and rinsed with cold saline. After fixation in 10% formalin, paraffin–embedded tissues were cut into 4 μm sections and stained with hematoxylin and eosin. Stained samples were scored according to the degree of inflammation as described previously (25).

Statistical analysis

All the values were expressed as mean ± standard deviation (SD). Between- and within-group comparisons were made using Friedman and Wilcoxon signed-rank tests, respectively. Post hoc multiple comparisons were performed using Kruskal-Wallis and Mann-Whitney U tests. Differences between groups were considered significant at the α = 0.05 level.

Results

Baseline blood glucose levels were comparable in all study groups. None of the herbal preparations showed a preventive effect against STZ-induced diabetes, confirmed by the comparable blood glucose concentrations in the seed- and mucilage-treated groups with the diabetic control group at the third day following diabetes induction ($P>0.05$). At the end of study (day 21 after induction of diabetes), blood glucose levels were significantly decreased in the seed- ($P=0.012$) and mucilage- ($P=0.002$) treated groups whilst there was a significant elevation in the diabetic control group ($P=0.002$) (Table 1). Comparison of the magnitude of changes between the study groups revealed a significant anti-hyperglycemic effect for seed but not mucilage of the plant.

Before induction of diabetes all parameters in serum of 4 groups were not significantly different. Hence, treatment with seed and mucilage for 14 days did not have effect on blood variables ($P>0.05$). After induction of diabetes, MDA and insulin decrease significantly in diabetic rats compared to other groups ($P<0.05$) (Table 2). The results of comparison between 2 times before and after induction of diabetes showed that HbA1c and MDA increased and insulin decreased significantly in diabetic control rats ($P<0.05$). CRP and antioxidant capacity decreased significantly in diabetic rats treated with seed of okra ($P<0.05$) (Table 2).

There was no significant difference between the study groups regarding their baseline levels of insulin, HbA1c, MDA, antioxidant capacity and CRP ($P>0.05$). Treatment with *H. esculentus* seed and mucilage was associated with a significant increase in serum insulin and antioxidant capacity, and a decrease in serum MDA ($P<0.05$) compared to the diabetic control group. Notably, serum levels of insulin and MDA in both seed and mucilage groups were comparable with those of the non-diabetic control group ($P>0.05$). Serum levels of HbA1c and CRP in either of the seed- or mucilage-treated groups were not changed significantly compared to the diabetic control group ($P>0.05$). Serum levels of all lipid parameters were comparable between the groups at baseline ($P>0.05$). Supplementation with either *H. esculentus* seed or mucilage caused a significant reduction in total cholesterol, LDL-C, HDL-C and triglyceride compared to the control group ($P<0.05$). In the same manner, concentrations of total cholesterol, LDL-C and triglyceride at the end of study in both seed- and mucilage-treated groups were lower than those of the non-diabetic control group ($P<0.05$) (Table 3).

Histopathologic findings

Pancreatic sections were scored from 0 to 3 according to lymphocyte inflammation, whilst score zero was assigned to normal tissue without any observable inflammation (Figure 1A). Mild (Figure 1B), moderate (Figure 1C) and severe (Figure 1D) tissue inflammations were given scores of 1, 2 and 3, respectively. Score 1 was assigned to low inflammation (Figure 1B), score 2 to moderate inflammation (Figure 1C) and score 3 to high inflammation (Figure 1D). In diabetic group the percent of inflammation was significantly higher than other groups. The group treated with seed had more reduction in inflammation than mucilage group ($P<0.05$) (Table 4).

Discussion

The present study aimed to investigate the effectiveness of

Table 1. Effects of seed and mucilage of *H. esculentus* on blood glucose (mg/dl) level in STZ induced diabetic rats

Groups	Ist day before induced diabetes	14th day before induced diabetes	3rd day after induced diabetes	21st day after induced diabetes	*P* value
Diabetic control	60.33±7.33	63.40±5.33	278.33±97.19[a]	338.0±134.42[a]	0.002
Non diabetic control	59.5±8.73	57.10±68.27	62.30±4.52[b]	57.10±6.74[b]	0.241
Seed treatment	59.33±6.56	56.50±7.57	218.80±103.86[a]	155.30±108.12[c]	0.012
Mucilage treatment	57.16±6.61	56.66±4.74	255±137.75[a]	248.71±144.31[a]	0.002
P value	0.811	0.06	<0.001	0.01	

Values are presented as mean ± SEM (n = 10). Similar letter meant no significant difference ($P<0.05$).

Table 2. Effect of seed and mucilage of *H. esculentus* on insulin, HbA1c, MDA, antioxidant capacity, CRP in STZ induced diabetic rats

Variable	Groups	Before induced diabetes	After induced diabetes	*P* value
Insulin ng/dl	Diabetic control	0.50±0.22	0.21±0.09[a]	0.026
	Non diabetic control	0.51±0.17	0.42±0.50[b]	0.221
	Seed treatment	0.55±0.09	0.55±0.20[b]	0.210
	Mucilage treatment	0.46±0.16	0.50±0.34[b]	0.343
	P value	0.181	0.05	
HbA1c %	Diabetic control	2.98±0.23	6.317±2.64	0.012
	Non diabetic control	3.03±0.22	5.48±2.91	0.057
	Seed treatment	3.02±36	6.43±0.82	0.051
	Mucilage treatment	2.07±0.27	6.82±1.43	0.059
	P value	0.941	0.074	
MDA u/l	Diabetic control	2.89±2.23	4.61±0.38[a]	0.012
	Non diabetic control	2.46±6.59	1.21±0.47[b]	0.594
	Seed treatment	1.88±1.21	2.84±0.45[b]	0.333
	Mucilage treatment	1.92±0.08	3.35±0.51[b]	0.338
	P value	<0.001	0.023	
Antioxidant capacity %	Diabetic control	78.41±2.23	85.83±0.38[a]	0.176
	Non diabetic control	44.63±6.59	72.64±0.47[a]	0.345
	Seed treatment	30.89±1.21	69.54±0.45[b]	0.049
	Mucilage treatment	82.39±0.08	50.04±0.51[b]	0.333
	P value	0.192	0.001	
CRP mg/dl	Diabetic control	40.50±2.50	42.87±3.09	0.173
	Non diabetic control	40.33±1.22	38.55±1.13	0.55
	Seed treatment	39.70±2.49	35.60±4.81	0.041
	Mucilage treatment	38.00±3.19	36.85±3.68	0.34
	P value	0.268	0.297	

Abbreviations: MDA, malondialdehyde; CRP, C-reactive protein; STZ, streptozotocin; HbA1c, hemoglobin A1c.
Values are presented as mean ± SEM (n = 10). Similar letter meant no significant difference ($P < 0.05$).

Table 3. Effect of seed and mucilage of *H. esculentus* on lipid profile in STZ induced diabetic rats

Variable	Groups	Before induced diabetes	After induced diabetes	*P* value
Cholesterol mg/dl	Diabetic control	74.80±7.74	83.0±9.68[a]	0.093
	Non diabetic control	73.70±7.81	74.20±10.9[b]	0.953
	Seed treatment	71.90±6.27	41.60±7.3[c]	0.005
	Mucilage treatment	76.60±6.51	52.0±9.18[c]	0.027
	P value	0.598	<0.001	
LDL-C mg/dl	Diabetic control	12.20±2.28	22.25±2.12[a]	0.085
	Non diabetic control	11.00±1.00	9.70±1.76[b]	0.286
	Seed treatment	10.42±0.97	9.70±2.71[b]	0.165
	Mucilage treatment	13.33±3.96	7.83±0.98[b]	0.043
	P value	0.294	0.002	
HDL-C mg/dl	Diabetic control	41.88±4.78	35.50±4.65[a]	0.108
	Non diabetic control	33.80±13.17	39.20±6.55[a]	0.506
	Seed treatment	36.0±9.46	21.00±7.00[b]	0.038
	Mucilage treatment	40.40±4.11	31.80±7.11[b]	0.047
	P value	0.278	<0.001	
Triglyceride mg/dl	Diabetic control	86.70±31.97	99.12±33.10[a]	0.208
	Non diabetic control	79.20±34.49	78.40±15.30[b]	0.065
	Seed treatment	76.00±12.44	38.90±19.30[c]	0.005
	Mucilage treatment	85.90±940.00	47.16±10.14[c]	0.028
	P value	0.47	0.002	

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; STZ, streptozotocin.
Values are presented as mean ± SEM (n = 10). Similar letter meant no significant difference ($P < 0.05$).

supplementation with *H. esculentus* seed and mucilage in counterbalancing the biochemical abnormalities induced by STZ. The results indicated that whilst none of the herbal preparations prevented the development of diabetes following STZ injection, they could exert hypoglycemic effects in diabetic rats. Seeds and mucilage of *H. esculentus* exerted positive effects such as increasing serum insulin, improving total antioxidant capacity, reducing MDA, and

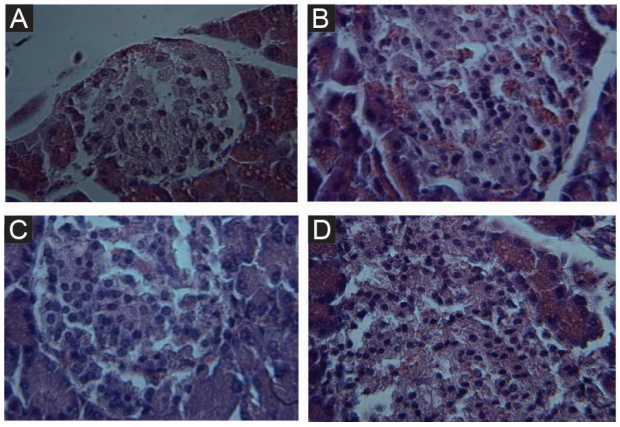

Figure 1. Effect of seed and mucilage of *H. esculentus* on pancreas tissue in STZ induced diabetic rats (H&E, 40×). A)Normal tissue without inflammation. B) Low inflammation. C)Moderate inflammation. D) High inflammation.

Table 4. Effect of seed and mucilage of *H. esculentus* on pancreas tissue in STZ induced diabetic rats

Groups	Score 3	Score 2	Score 1	Score 0
Diabetic control	57%	40%	3%	0
Non diabetic control	0	2%	10%	88%
Seed treatment	6%	45%	29%	20%
Mucilage treatment	26%	43%	28%	7%

improving lipid profile by decreasing total cholesterol, LDL-C and triglyceride. Overall, seeds of the plant were found to have a superior efficacy compared to mucilage in terms of the above mentioned effects. STZ is metabolized inside pancreatic beta cells, leading to the generation of nitric oxide which subsequently impairs insulin synthesis and secretion. Also STZ increases glycosylated hemoglobin and adversely affects other indices of glycemic control (28). Polyphenolic compounds have hypoglycemic and antidiabetic effect. Okra seeds have high concentrations of quercetin and phenolic molecules. Quercetin potentially has vasodilator and antioxidant effect (29) which protects and enhances insulin secretion of pancreatic beta cells (17) Quercetin, luteolin and epigallocatechin gallate are flavonoids have cellular action to glucose homeostasis and regulation of insulin action. Myricetin is an another flavonoid that has been reported to exert anti-hyperglycemic effects in STZ-induced diabetic rats (30). Oxidative stress is critically implicated in the pathogenesis of diabetes as well as some other complications. STZ-induced diabetes is also accompanied by a heightened state of reactive oxygen species (ROS) generation and lipid peroxidation along with significant depletion of antioxidant status. *H. esculentus* is a rich source of several antioxidants such as quercetin, epigallocatechins and vitamin C. Quercetin is a flavonoid antioxidant and protects against pancreatic beta cell damage in STZ-induced diabetic rats. Quercetin has also documented anti-inflammatory properties and inhibits nitric oxide formation whilst decreasing CRP concentrations in STZ-induced diabetic rats. There are a lot of medicinal plants which the same as *H. esculentus* have crucial roles in scavenging several types of ROS. The present results indicated a significant effect of *H. esculentus* seed in improving serum total antioxidant capacity and reduction of MDA levels. The hypoglycemic activity of okra is mainly due to the presence of polysaccharides such as pectin, gum, cellulose and hemicellulose. Polysaccharides present in okra can bind to the bile acids in the intestine and accelerate their excretion through inhibition of bile acid recycling. The next effect would be the flux of hepatic cholesterol reserve to the bile acid biosynthesis pathway, thereby leading to reduced levels of serum as well as hepatic cholesterol. Besides, *H. esculentus* contains other bioactive constituents such as phytosterols, flavonoids, tannins, phenolic compounds, volatile oil, linoleic and α-linolenic fatty acids, which are known to possess cardioprotective properties. The exact mechanisms of *H. esculentus* are not clear. Antioxidants have been shown to possess hypoglycemic and hypolipidemic properties (31-36). *H. esculentus* has antioxidant activity, too. So, its effects seem to be, at least in part, attributed to its antioxidant activity.

Conclusion

Based on the present experimental evidence, dietary supplementation with *H. esculentus*, particularly in the seed for, is effective in reducing serum glucose, CRP and lipid levels whilst improving antioxidant capacity in STZ-induced diabetic rats. Given the promising anti-hyperglycemic, anti-hyperlipidemic, anti-inflammatory and antioxidant properties of *H. esculentus* seeds, future clinical studies are warranted to confirm the present findings in diapetic patients and also the value of addition of *H. esculentus* to standard hypoglycemic agents.

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Authors' contribution

All authors contributed equally to the paper.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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