IJPSR (2019), Volume 10, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 June 2018; received in revised form, 04 December 2018; accepted, 22 February 2019; published 01 March 2019

ANTIDIABETIC POTENTIAL OF *ABELMOSCHUS ESCULENTUS* LINN. IN ALLOXAN-INDUCED DIABETIC RATS

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Keywords:

Abelmoschus esculentus Linn., Antidiabetic activity, Oral glucose tolerance test, Aqueous and ethanolic extracts, Insulin

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ABSTRACT: *Abelmoschus esculentus* Linn. (Malvaceae) is described in medicine for the treatment of diabetes mellitus. The monech seeds of *Abelmoschus esculentus* having potential in the development of a drug for diabetes due to their antidiabetic activity. The hypoglycemic effect of the extract was tested in normal, glucose loading and alloxan-induced diabetic rats. Aqueous and ethanolic extracts (250 and 500 mg/kg body weight), were administered orally to male Wistar albino rats. The parameters studied included oral glucose tolerance test in blood, fasting blood glucose, serum insulin, and glycated hemoglobin levels, liver glycogen content, serum lipid profile, and changes in body weights. The extracts produced a dose-dependent fall in fasting blood glucose (FBG). After 15 days of treatment with extracts, the maximum reduction in FBG (35.14%) was observed in diabetic rats treated with ethanolic extract 500 mg/kg dose. Serum lipid levels were reversed towards near normal, and control in the loss of body weight was observed in treated rats as compared to diabetic control. The extract treatment also showed a significant increase in the liver glycogen and a significant decrease in glycated hemoglobin levels.

INTRODUCTION: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion or insulin action. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are extensively prescribed even when their biologically active compounds are unknown [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(3).1180-85

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(3).1180-85

Synthetic hypoglycemic agents can produce serious side effects, and also, they are not suitable for use during pregnancy [2]. Therefore the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendations made by WHO on diabetes mellitus [3], investigations on hypoglycemic agents from medicinal plants have become more important.

Abelmoschus esculentus (Linn.) Moench (Family: Malvaceae) is an annual or perennial herb, growing to 2 m tall. The leaves are heart-shaped, 10-20 cm long and broad, palmately lobed with 5-7 lobes. The large, yellow, hibiscus-like flowers are 4-8 cm diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The seed pods are 3 to 10 inches long, tapering, usually with ribs down its length. These tender, unripe seed pods are used as a vegetable and have a unique texture and sweet flavor. The pods, when cut, exude a mucilaginous juice that is used to

thicken stews (gumbo), and have a flavor somewhat like a cross between asparagus and eggplant. The fruit is a capsule up to 18 cm long, containing numerous seeds. It's also known as Lady's Fingers, gombo, gumbo, ochro, bamia, bamie, quiabo. In Spanish okra is quibombo; the French word is gombo, bamia or bamya, in India it is bhindi, and in the eastern Mediterranean and Arab countries bamies [4, 5].

The fruit is highly proteinaceous. It is a good source of vitamin A and vitamin C. It is low in calories and is fat-free. The fruit is also containing considerable medicinal and industrial value[6]. A mucilaginous preparation from the pod can be used as a plasma replacement [7-9].

Okra mucilage has medicinal applications; when used as a plasma replacement or blood-volume expander. The mucilage of Okra not only binds cholesterol but the bile acid carrying toxins dumped into it by the filtering liver. It also has industrial applications; when added as size to glaze paper and used in confectionary [10-12].

Okra constitutes minerals, vitamins, proteins, carbohydrates, enzymes and very high quantities of mucilages [13]. *Abelmoschus esculentus* L. (or *Hibiscus esculentus* or Okra) – Malvaceae is used for a long time as an edible vegetable in many countries, and commonly eaten in Vietnam because of its nourishing components. Traditionally, it is believed that the plant is useful in the treatment of inflammatory disorders, constipation, retention of urine, On the other hand, a number of previous studies have reported that *Abelmoschus sp.* possessed hypoglycemic effect. However, there is a little study regarding its hypolipidemic effect. It has reported antidiabetic and antihyperlipidemic potential of okra peel and seed powder [14] and okra fruit extract [15] in streptozotocin (STZ)-induced diabetic 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 Long Evans rats. It showed a significant reduction in absorption of glucose as studied in the 24 h fasting rats [16]. It has reported, the presence of two major flavonol glucosides named isoquercetin (2) and quercetin-3-O-beta-glucopyranosyl- (1"→6")-glucoside (3) in okra seeds which are α-glucosidase inhibitors [17]. Ethanol extracts of okra (EO) and its major flavonoids isoquercitrin and quercetin 3-O-gentiobioside reduced blood glucose and serum insulin levels and improved glucose tolerance in obese mice [18].

Fresh fruits are cut into small pieces and then boiled in water, to obtain mucilaginous decoction. This decoction is prescribed for cough suppressant, throat infection, and bronchitis. Wehmer records that the fruit contains abundant pectin; mucilage; starch; some fat, 4 percent; water 80.7 percent; and ash, 1.41 percent. Popp analyzed the seeds and found nitrogen, 2.4 to 2.5 percent; their ash finding K_2O_{39} percent; MgO 12 percent, CaO 7.8 percent and P_2O_5 24.7 percent. Jamieson and Baugham analyzed the seeds of okra; their results are as follows: palmitic acid, 27.23 percent; stearic acid, 2.75 percent; arachidic acid, 0.05 percent; oleic acid, 43.74 percent; linolic acid, 26.62 percent; Unsaponifiable matter, 0.37 percent. Read reports that the roots contain gum, 16 percent; and the seeds, vitamin C [19-24].

MATERIAL AND METHOD:

Collection of Plant Material: The species for the proposed study that is *Abelmoschus esculentus* Linn., Fruits were collected, from the local market of Bhopal (M.P.) India was authenticated by Agriculture College (Plant authentification no. 11032), Indore (M.P.) with the help of forest department botanist.

Preparation of the Plant Extracts: The fruits were washed properly with water to remove the mud or dust if any. Initially, it was dried in the sun for an hour than dried under shade completely. The dried fruit was then powdered using the wood grinder and was sieved through sieve no. 40 and then stored in airtight containers. The powdered fruit (150 g) was extracted with ethanol (90% w/v) for 24 h using a Soxhlet extractor. The powdered fruits (30 g) were extracted with distilled water by cold maceration method.

This extract was concentrated to dryness under reduced pressure and controlled temperature (50-60 ºC) to yield solid masses that were completely free from solvents (yield: 18.92% w/w and 12.36% w/w for aqueous extract and ethanolic extract respectively). The animals treated with 250 and 500

mg/kg body weight of the ethanolic and aqueous extracts which were suspended in 0.5% tween 80 in saline (0.9% w/v) for oral administration.

Animals: Animal ethical clearance was obtained from Ethics Committee (1429/PO/a/11/CPCSEA) of Sagar Institute of Research & Technology-Pharmacy, Bhopal, India Healthy male Wistar albino rats (100-200 gm) were selected for the present investigation. The rats were housed in a well-ventilated, temperature-controlled (27 ± 2 ºC, RH 60-70%) animal room with 12-12 h light dark cycle for 7 days before the experimental period. The animals were provided with standard pellet diet and water *ad libitum* in polypropylene cages. Animals were periodically weighed before and after experiments.

Experimental Procedures*:*

Oral Glucose Tolerance Test: The oral glucose tolerance test (OGTT) was performed on overnight fasting (12 h) normal rats. Ethanolic and aqueous extract of *Abelmoschus esculentus* fruits (250 and 500 mg/kg, per os) and glibenclamide (2.5 mg/kg b.w. per os) were administered to six groups of rats, respectively. Glucose (4 g/kg, per os) was fed 90 min after pretreatment with the vehicle, ethanolic and aqueous extract of *Abelmoschus esculentus* or glibenclamide. Blood sample (0.3 ml) was withdrawn from the retro-orbital plexus under ether anesthesia at 0, 30, 60, 90 and 120 min of extract or glibenclamide administration. At the end of each experiment, polyvidone iodine solution (Betadine®) was applied at the site of the retro-orbital puncture to prevent infection. Blood glucose levels were determined using a glucometer [25, 26].

Study on Diabetic Rats (Non-Insulin Dependent Diabetes Model - NIDDM):

Induction of Diabetes: Diabetes was induced in rats by the single intraperitoneal (i.p.) injection of alloxan monohydrate at a dose of 150 mg/kg b.w. freshly prepared in distilled water (1 ml/kg b.w.). Seven days after the injection, the blood glucose levels were measured. Each animal with a blood glucose concentration level above 250 g/dl was considered to be diabetic and used in the experiments [27-29]. To prevent the hypoglycemia, which occurred during the first 24 h following the alloxan administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were under fasting for 16 h before administered alloxan monohydrate injection.

Forty-two animals were divided into seven groups of six animals each as follows:

Group 1: Served as normal control (0.9% w/v Saline 10 ml/kg b.w./day).

Group 2: Served as diabetic control (Alloxan induced).

Group 3: Received aqueous extract, 250 mg/kg b.w./day orally.

Group 4: Received ethanolic extract, 250 mg/kg b.w./day orally.

Group 5: Received aqueous extract, 500 mg/kg b.w./day orally.

Group 6: Received ethanolic extract, 500 mg/kg b.w./day orally.

Group 7: Served as reference standards (Glibenclamide, 2.5 mg/kg b.w./day).

Acute Anti-Diabetes Effect of Test Samples: The test samples (ethanolic extracts, aqueous extracts, and glibenclamide) were administered orally by using a gastric gavage needle. The blood sample was collected from rats by "retro-orbital plexus bleeding method," and blood glucose levels were determined at 0, 2, 4, 6 and 24 h after the single oral administration of the test samples by using glucometer [29].

Sub-acute Anti-Diabetes effect of Test Samples: The test samples (ethanolic extracts, aqueous extracts, and glibenclamide) were administered orally for 14 days consecutively. Blood glucose levels were determined at 10:00 a.m. on 1st, 3rd, 5th and 14th days after the single oral administration of the test samples by using glucometer [29].

Statistical Analysis: Values are presented as means ± S.E.M. Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the Dunnett test using the "SYSAT" statistic computer program. A difference in the mean values of $P<0.01$ or less was considered to be statistically significant.

RESULTS: The phytochemical screening of *Abelmoschus esculentus* revealed the presence of alkaloids, carbohydrates, terpenoids, and flavonoids.

Glucose Tolerance Test: The effect of the different doses of aqueous and ethanolic extract of *Abelmoschus esculentus* on the fasting blood glucose levels of both normal and treated rats are given in **Table 1**. The result showed the blood glucose level had decreased significantly by 60 min (concerning 30 min level) and this was maintained until 120 min. The ethanolic extract at the dose of 500 mg/kg showed a significant reduction (P<0.01) in blood glucose level within 120 min.

TABLE 1: BLOOD GLUCOSE LEVEL OF AQUEOUS AND ETHANOLIC FRUIT EXTRACTS OF *ABELMOSCHUS ESCULENTUS* DURING ORAL GLUCOSE TOLERANCE TEST

Groups (n=6)	Treatment and dose	Fasting blood glucose (mg/dl) (mean ± SEM)			
		0 min	30 min	60 min	120 min
Group 1	Normal control 10 ml/kg	89.33 ± 0.83	133.66 ± 0.56	117.33 ± 0.26	112.66 ± 1.38
Group 2	Standard 2.5 [a]	91.33 ± 0.80**	109.6 ± 0.50**	84.03 ± 0.16**	78.0 ± 0.97**
Group 3	Ethanolic extract 250 [a]	104.6 ± 0.46**	133.00 ± 0.63	116.66 ± 0.56	105.33 ± 0.35**
Group 4	Aqueous extract250 [a]	99.33 ± 0.79**	127.0 ± 0.36**	114 ± 0.35**	101.66 ± 0.55**
Group 5	Ethanolic extract500 [a]	101.6 ± 0.76**	129.0 ± 0.63**	116.33 ± 0.92	96.33 ± 0.91**
Group 6	Aqueous extract500 [a]	104.0 ± 0.86**	125.6 ± 0.56**	115.0 ± 0.34*	102.66 ± 0.56**

a = mg/kg b.w, Values ± SEM, n = no. of animal / group, [One way ANOVA followed by Dunnett test] ** P<0.01, * P<0.05 *vs.* normal control

Anti-Diabetic Activity: The single administration of (acute study) ethanolic extract (500 mg/kg b.w.) has more significantly reduced the blood glucose level at 4th h from 130.14 ± 1.4 to 113.23 ± 0.03, and significant hypoglycemia was maintained up to 24th h. Glibenclamide (2.5 mg/kg) has also significantly reduced the blood glucose level at 4th h 131.00 ± 1.61 to 106.33 ± 0.92, and significant hypoglycemia was maintained up to 24th h **Table 2**. On repeated administration (sub-acute study) vehicle, glibenclamide, aqueous and ethanolic extract of *Abelmoschus esculentus* for 14 days, a significant (P<0.01) decrease in blood glucose level of the diabetic rats were seen at a dose of (250, 500 mg/kg b.w.), as compared to the diabetic control group. On the other hand, glibenclamide showed a significant (P<0.01) decrease in blood glucose level at a dose of 2.5 mg/kg (25.34% decreases) as compared to diabetic control group **Table 3**.

TABLE 2: FASTING BLOOD GLUCOSE LEVEL OF ALLOXAN - INDUCED DIABETIC RATS IN ACUTE STUDY

Groups (n=6)	Treatment and dose	Fasting blood glucose (mg/dl)				
		0 hr	2 h	4 h	6 h	24 h
Group 1	Normal control 10 ml/kg	100.09 ± 0.43**	99.15 ± 1.06**	99.77 ± 1.04**	99.32 ± 1.07**	100.09 ± 1.12**
Group 2	Diabetic control 10 ml/kg	132.67 ± 1.27	135.33 ± 0.95	137.67 ± 2.5	140.0 ± 3.03	142.5 ± 3.33
Group 3	Standard 2.5 [a]	131.00 ± 1.61	114.17 ± 1.7**	106.33 ± 0.92**	100.83 ± 0.91**	97.0 ± 0.37**
Group 4	Ethanolic extract 250 [a]	131.05 ± 0.54	117.9 ± 0.24**	115.23 ± 0.71**	109.42 ± 0.91**	105.73 ± 0.26**
Group 5	Aqueous extract 250 [a]	129.45 ± 1.91	117.5 ±1.56**	116.1 ± 0.11**	113.15 ± 0.23**	108.12 ± .81**
Group 6	Ethanolic extract 500 [a]	130.14 ± 1.4	117.0 ± 0.2**	113.23 ± 0.03**	113.13 ± 1.10**	100.33 ± 1.08**
Group 7	Aqueous extract 500 [a]	131.42 ± 1.81	121.0 ± 1.02**	115.7 ± 0.23**	112.12 ± 0.41**	104.12 ± 0.18**

a = mg/kg b.w, Values ± SEM, n = no. of animal / group, [One way ANOVA followed by Dunnett test] ** P<0.01 *vs.* diabetic control

TABLE 3: FASTING BLOOD GLUCOSE LEVEL OF ALLOXAN - INDUCED DIABETIC RATS AFTER 14 DAYS SUBACUTE TREATMENT

Groups (n=6)	Treatment and dose	Fasting Blood Glucose (mg/dl)				
		1st day	3rd day	5th day	10th day	14th day
Group 1	Normal control 10 ml/kg	101.0 ± 0.9**	102.6 ± 0.92**	101.6 ± 0.76**	102.3 ± 0.56**	100.7 ± 0.76**
Group 2	Diabetic control 10 ml/kg	133.17 ± 0.79	136.0 ± 1.34	139.67 ± 1.56	141.67 ± 1.41	145.33 ± 0.56
Group 3	Standard 2.5 [a]	130.83 ± 1.40	121.3 ± 1.65**	117.0 ± 1.93**	100.8 ± 1.40**	97.67 ± 0.56**
Group 4	Ethanolic extract 250 [a]	131.00 ± 1.34	128.0 ± 1.37**	122.7 ± 0.84**	121.3 ± 1.12**	114.6 ± 0.61**
Group 5	Aqueous extract 250 [a]	129.67 ± 1.38	127.3 ± 0.84**	125.3 ± 0.56**	122.0 ± 0.73**	115.7 ± 0.56**
Group 6	Ethanolic extract 500 [a]	132.33 ± 2.14	126.0 ± 0.73**	122.0 ± 0.73**	116.0 ± 1.77**	107.3 ± 3.16**
Group 7	Aqueous extract 500 [a]	130.67 ± 1.84	126.5 ± 1.12**	120 ± 0.73**	118.7 ± 0.56**	110.5 ± 1.23**

a = mg/kg b.w, Values ± SEM, n = no. of animal / group, [One way ANOVA followed by Dunnett test] ** P<0.01, * P<0.05 *vs.* Diabetic control

DISCUSSION: In the present study, ethanolic extract of fruits of *Abelmoschus esculentus* at a dose of 500 mg/kg b.w. could produce a significant fall in blood glucose levels by about 76% in diabetic rats, after 5 h of treatment. But none of these extracts could produce any hypoglycemic effect in normal rats. The aqueous extracts of fruits of *Abelmoschus esculentus* have not shown significant antihyperglycemic activity; hence the ethanolic extracts may be considered to have good antihyperglycemic active principles without causing any hypoglycemic effect unlike insulin and other synthetic drugs.

The phytochemical screening of fruits of *Abelmoschus esculentus* revealed the presence of alkaloids, carbohydrates, terpenoids, tannins, flavonoids, and phenolic compounds and volatile oil. Flavonoids, alkaloids, and phenolics are known to be bioactive anti-diabetic principles. The anti-diabetic effect of ethanolic extract of *Abelmoschus esculentus* fruits may be due to the presence of more than one antihyperglycemic principle and their synergistic properties.

CONCLUSION: In this study, the anti-hyperglycemic activity caused by glibenclamide in alloxan-induced diabetic rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells. The ethanolic extract of *Abelmoschus esculentus* fruits may have a stimulating effect on the remnant beta cells.

However, further experiments are required to elucidate the exact mechanism of action. Further studies will be focused on the determination of the mechanism(s) of action, as well as on the isolation of bioactive principles.

ACKNOWLEDGEMENT: The authors are grateful to Dr. Surendra Kumar Jain, Director, Sagar Institute of Research and Technology, Bhopal, India, for providing me the facility to carry out the pharmacological studies. We would like to thank Veterinary College, Mhow, Indore; his help in carrying out the histopathological parameters were studied.

CONFLICT OF INTEREST: The authors declare no conflicts of interest.

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How to cite this article:
Jain N, Jain R and Jain SK: Antidiabetic potential of *Abelmoschus esculentus* Linn. In alloxan- induced diabetic rats. Int J Pharm Sci & Res 2019; 10(3): 1180-85. doi: 10.13040/ IJPSR.0975-8232.10(3).1180-85.