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EVALUATION OF DIURETIC, SALURETIC AND HYPOGLYCEMIC ACTIVITY OF AQUEOUS EXTRACTS OF THE *ABELMOSCHUS ESCULENTUS* (LINN) MOENCH FRUITS IN RATS

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ABSTRACT

Objectives: The present study was undertaken to establish the diuretic, saluretic and hypoglycemic potential of aqueous extracts of *Abelmoschus esculentus* (L.) Moench fruits in rats.

Methods: The selected male Sprague-Dawley rats were divided into different groups (n=6) and for evaluation of diuretic and saluretic activity, animals loaded with normal saline (10 ml/kg). Animals received *Abelmoschus esculentus* at 100, 200 and 400 mg/kg, po respectively and estimated urine volume (in ml) and content of Na^+, K^+ & Cl^- were measured in the urine of rats after 5th hour. For evaluation of hypoglycemic activity, alloxan induced diabetic rats were selected and estimation of hypoglycemic activity, animals treated with aqueous extracts of the *Abelmoschus esculentus* received 100, and 200 mg/kg, po respectively for 10 days. The blood glucose levels were estimated on day 0, 3, 6 and 10 day.

Results: There were statistically a significant difference ($P<0.05$), in the urinary output shown by aqueous extracts of *Abelmoschus esculentus* at 200mg/kg and 400mg/kg and Furosemide (10mg/kg). Aqueous extracts have showed significant increments in urinary excretion of Na^+

($P<0.05$), K^+ ($P<0.05$), Cl^- ($P<0.05$) when compared with control group after 5 hours and animals have also shown significant decrease ($P<0.05$) in blood glucose levels with increased period of exposure to the extracts.

Conclusion: The study suggests that the aqueous extracts of *Abelmoschus esculentus* induce diuretic, saluretic and hypoglycemic response that will helpful in a various chronic diseases like hypertension, diabetes and metabolic syndrome.

Keywords: Diuretic, Diabetic Mellitus, Okra, Furosemide, and Metabolic Syndrome

INTRODUCTION

Rapid globalization & industrialization occurring in developing countries has resulted in considerable increase in life style related diseases like metabolic disorder. Indians are known to have a higher probability of suffering from increased central adiposity, oxidative stress, dyslipidemia and hyperglycemia and cardiovascular risk **[1]**. The currently-available drug regimens for multiple risk factors have certain drawbacks and therefore there is urgent need to develop new medications or strategies to counter the huge increase in prevalence and incidence of Metabolic Syndrome. Diuretics play a significant role in many life threatening disease conditions such as congestive heart failure, nephrotic syndrome, cirrhosis of liver, renal failure, hypertension, and pregnancy toxaemia **[2]**. Diabetes mellitus is a metabolic disorder with multiple causes marked by chronic hyperglycemia with disturbances of carbohydrates, fats and protein metabolism resulting from defects in secretion of insulin, action of insulin or both **[3]**.

Botanicals may serve as effective agents for the treatment or prevention of metabolic syndrome because they often contain diverse collections of biologically active compounds with multiple mechanisms of action **[4]**. Of the array of such plants with diuretic activity potentials are *Abelmoschus esculentus* which the increasing urine output ability is not scientifically validated and documented into a greater extent. *Abelmoschus esculentus* (Moench) or Okra (Synonym; Hibiscus esculentus, Family: Malvaceae, Lady's fingers) is a flowering plant and is cultivated throughout the tropical, sub-tropical and warm temperate regions around the world **[5, 6]**. Okra contains health-promoting phenolics and flavonoids such as quercetin glucoside, epigallocatechin, , myricetin, hibifolin, isoquercetin, hyperin, and rutin **[7]**. Okra seeds, pods, leaves, fruit, even bud, and

stem are employed in the treatment of human diseases throughout the world. Okra and its phytoconstituents have shown cardioprotective, renal protective, neuroprotective, hypocholesterolemic, hypoglycaemic, antioxidant, antimicrobial, anti-inflammatory, anti-constipation, anticancer, antiulcer, and antifatigue **[8, 9]**.

Literature review indicated that the okra plant extract has yet not been screened for evaluating its diuretic activity. Thus, the overall goal of our study is to comprehensively evaluate *Abelmoschus esculentus* in addressing the patho-physiological mechanisms that leads to the development of metabolic syndrome. Therefore, the present study was taken up to investigate the antioxidant, diuretic and hypoglycemic activity of *Abelmoschus esculentus*.

MATERIAL AND METHODS

Chemicals and reagents

Furosemide and Glibenclamide were used as the standard drugs supplied by Mariya Pharmaceutical Pvt. Ltd., Indore, India. The solvents and reagents, used in the research work were obtained commercially and were of analytical grade.

Collection of Plant Material

The species for the proposed study that is *Abelmoschus esculentus,* fruits were collected, from the local market of Indore was authenticated. The fruits/pods 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.

Experimental Animals

Animal ethical clearance was obtained from Ethics Committee (1627/PO/Re/2012/CPCSEA) of Acropolis institute of pharmaceutical education and research, Indore, India. Healthy male Sprague-Dawley 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 allowed free access to tap water and standard laboratory rat food.

Preparation of the Plant Extracts

The powdered fruit kept in 500ml of distilled water. Heated with stirred continuous at 60°C for approximately 4h. The mixture was filtered using a white muslin cloth to remove the insoluble matters and mucilaginous filtrate was collected and acetone was added to precipitate the extracted gum. Pressed mucilage was further dried to constant weight at 35–45°C in hot air oven. Hard mucilage cake was grinded and sieved

through sieve no. 22, stored in desiccator for further used.

Acute Oral Toxicity

Test Female rats were used for the acute oral toxicity test of the aqueous extract of *Abelmoschus esculentus* as per the OECD guidelines.[10] They fasted overnight before and 4 h after administration of the extract. First, a sighting study was performed to determine the starting dose, and a female rat was given 2000 mg/kg of the aqueous extract as a single dose by oral gavage. Since no death was observed within 24 h, an additional four rats were used and dosed as mentioned above.

Assessment of Diuretic and saluretic activity

Diuretic activity was determined following the methods used by Lahlou *et al*., with slight modification **[11]**. After an acclimatization period of one week, animals fasted overnight with free access to water. Animals were randomly assigned into five groups (n=6) male rats for diuretic activity. Group 1 (Control) received Normal saline, 10 ml/kg, *p.o*., group 2 (Standard) received Furosemide, 10 mg/kg, *i.p.,* groups 3, 4 and 5 received Aqueous extracts of the *Abelmoschus esculentus* of the 100, 200, and 400 mg/kg, *p.o.* respectively. The animals were pretreated with physiological saline at an oral dose of 10 ml/kg body weight, to impose a uniform water and salt load. Immediately after administration the animals were placed in metabolic cages, specially designed to separate urine and feces, kept at room temperature 25±0.5º throughout the experiment. Urine was collected and measured at 1, 2, 3 and 5 hours after the dose. During this period, no food or water was made available to animals. The urine was then filtered and finally stored at −20°C for electrolyte analyses. The following parameters were calculated in order to compare the effects of the extracts with vehicle and standard on urine excretion.

Formulae

Urinary Excretion = (Total urinary output /Total liquid administered) x100%

Diuretic Action = (Urinary excretion of treatment groups/ Urinary excretion of control group)

Diuretic Activity = (Diuretic action of test drug/Diuretic action of standard drug)

Electrolyte Level Analysis

The parameters taken for individual rat were total concentration of Na^+, K^+ and Cl^- in the urine. The concentrations of Na^+ and K^+ were determined by using a high-precision flame photometer (SYSTRONICS, Flame Photometer 130) whereas, the Cl^- concentration was estimated by titration with

silver nitrate solution. The ratios of electrolytes (test/control), Na^+/K^+, and $Cl^-/[K^+ + Na^+]$ were calculated to evaluate the saluretic, natriuretic, and carbonic anhydrase inhibitory activity of the aqueous extracts of *Abelmoschus esculentus*.

Assessment of Hypoglycemic activity

Diabetes was induced in rats by administered the single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg b.w., freshly prepared in distilled water **[11]**. After 6 h of alloxan administration, rats in their cages were then allowed 10 % glucose solution for the next 24 hours in order to prevent alloxan- induced hypoglycemia. After 72 h, the fasting blood glucose level of the rats was checked to confirm that they were diabetic. The diabetic rats were grouped into five groups (n=6). Group 1 (normal control) received normal saline, group 2 (Diabetic control) received normal saline, group 3 (Standard) received the standard drug, Glibenclamide 2.5 mg/kg, *i.p.,* groups 4 and 5 received Aqueous extracts of the 100, and 200 mg/kg, *p.o.,* respectively for 10 days. Blood was collected in capillary tubes from rats by retro-orbital plexus bleeding method using anesthesia. Plasma was obtained by centrifugation (600rpm at 4 ◦C), and stored at −20 ◦C until analyzed and the fasting blood sugar level was checked at an interval of three days afterwards, using Accu-Chek active glucometer and strips.

Phytochemical screening

Qualitative phytochemical investigation of the aqueous extract was carried out to determine the presence of secondary metabolites like alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins, steroids, tannins, and terpenoids using standard methods **[11]**.

Statistical Analysis

All data were expressed as means ± SEM. Dunnett's test and one-way ANOVA test was used and then compared with the standard and control groups (Graph Pad Prism version 5.00, USA). A difference in the mean values of $P < 0.05$ or less was considered to be statistically significant.

RESULTS

Acute Oral Toxicity Test

The acute oral toxicity test of the aqueous extract of the pods of *Abelmoschus esculentus* showed no gross behavioral changes and mortality within 24 h as well as in the next 14 days, referring that the median lethal oral dose of the aqueous extract was greater than 2000 mg/kg in rats.

Diuretic Activity of Aqueous Extracts

Effect on Urine Output

The effect of aqueous extracts of fruit of the *Abelmoschus esculentus* on urinary output is

shown in **Table 1**. The aqueous extract produced diuresis which appeared to be a function of dose and time. *A. esculentus* (100) did not produce any detectable difference in urine volume within the first 4 hours after dosing, but it produced a significant increase in urine volume after 5 hours as compared to the negative control ($p<0.05$). *A. esculentus* (200) and *A. esculentus* (400), however, produced a significant increase in urine volume with maximum diuresis of 103% ($p<0.05$) and 142% ($p<0.05$), respectively. *A. esculentus* (200) and *A. esculentus* (400) had a comparable diuretic effect with F10. *A. esculentus* (200) and *A. esculentus* (400) exhibited a diuretic action of 2.03 and 2.14, respectively **(Table 1)**. The percent urinary excretion of *A. esculentus* (200) 95% and *A. esculentus* (400) 100% was higher as compared to the negative control 21%.

Effect on Cumulative Urine Output

The cumulative urine output of rats treated with aqueous extracts of *Abelmoschus esculentus* were presented in **Figure 2**. A significantly ($p <0.05$) increased cumulative urine output were observed in rats treated with the F10 and aqueous extracts at AE200 and AE400 as compare to a negative control.

Effect on Urinary Electrolyte Excretion

The urine samples collected over the five hours were analyzed for the electrolyte content (Na^+, K^+, and Cl^-) and presented in **Table 2**. A significantly increased sodium loss was observed in rats treated with the aqueous extract at all doses. The increased sodium excretion produced by *A. esculentus* (100), *A. esculentus* (200) and *A. esculentus* (400) was 45%, 94%, and 118%, respectively, ($p<0.05$), compared to the negative control. Furosemide (10) produced the maximum sodium excretion (124%) which was significantly ($p<0.05$) greater than the effect produced by aqueous extracts. Enhanced potassium loss was observed at the doses of 200 and 400 mg/kg, (55% and 96%) respectively, compared to the negative control. These values were, however, significantly lower than produced by Furosemide (10) 117%, ($p<0.05$). In the case of chloride excretion, *A. esculentus* (200) 35% and *A. esculentus* (400) 48% produced a significant ($p<0.05$) loss than the negative control. In this regard, no apparent differences were observed between the standard and the above mentioned two doses. **Table 2** also shows the saluretic indices of the aqueous extract at three different dose levels. *A. esculentus* (400) for Na^+ (2.12), K^+ (1.91) and Cl^- (1.48) were nearly similar to the indices of Furosemide (10): 2.24, 2.21,

and 1.44 for the three ions, respectively. The Na^+/K^+ ratios of *A. esculentus* (100) (1.43), *A. esculentus* (100) 1.90 and *A. esculentus* (200) 1.57 were higher than the ratio for the standard drug (1.41). The carbonic anhydrase inhibitory activity of *A. esculentus* (200) and *A. esculentus* (400) was 0.63 and 0.61, respectively.

Hypoglycemic Activity of Aqueous Extracts

The development of diabetic conditions was evaluated on the third day after alloxan injection. All diabetic rats groups developed diabetes mellitus, as is shown by their higher fasting blood glucose levels compared to the non-diabetic rats. The rats in the normal control had an average normal blood glucose level of 96 mg/dl. **Table 3** shows that all diabetic rats groups were given Glibenclamide or aqueous extracts of A. esculentus at doses of 200 and 400 mg/kg BW for 10 days. On day 10, the fasting blood glucose levels in groups receiving A. esculentus at doses 200 and 400 mg/kg BW were significantly ($p<0.05$) different compared to that of the diabetic control group. On the other hand, Glibenclamide showed a significant ($P<0.05$) decrease in blood glucose level at a dose of 2.5 mg/kg (25.27% decreases) as compared to diabetic control group.

Qualitative Photochemical Screening

The aqueous extract of *Abelmoschus esculentus* was tested for the composition of medicinally active compounds and it was found to be positive for alkaloids, flavonoids, polyphenols, and saponins **(Table 4)**.

Table 1: Effect of aqueous extracts of *Abelmoschus esculentus* fruits on Urine Volume in Rats

Experimental Group	Dose	Liquid administered(ml)	Volume of Urine (mL)					% Urinary excretion	Diuretic action	Diuretic activity
			1 h	2 h	3 h	4 h	5 h			
Control	Normal Saline	1.2	1.20 ±.05	1.8 ±.05	2.05 ±.06	2.28 ±.05	2.45 ±.06	21	1	
Furosemide	10 mg/kg	1.24	2.43±.1.0 ***	3.8± 0.07***	4.15± 0.06***	4.91± 0.07***	5.53± 0.11***	105	2.26	1
Aqueous extract of *A. esculentus*	100 mg/kg	1.2	1.25±.04	1.93±. 08	2.48± 0.06	2.90±0 .08	3.43± 0.09*	65	1.4	0.63
Aqueous extract of *A. esculentus*	200 mg/kg	1.26	1.93± 0.04***	2.73± 0.04***	3.18± 0.06***	3.83± 0.6***	4.99± 0.10***	95	2.03	0.92
Aqueous extract of *A. esculentus*	400 mg/kg	1.3	2.25± 0.05***	3.18± 0.07***	3.62± 0.07***	4.180± 0.06***	5.25± 0.09***	100	2.14	0.97

Table 2: Effect of Aqueous Extracts of *Abelmoschus esculentus* Fruits on Urinary Electrolyte Excretion in Rats

Experimental group	Dose	Urinary Electrolyte Concentration (mMol/L)			Saluretic Index			Na^+/K^+	$Cl^-/Na^+ + K^+$
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Control	Normal Saline	63.21 ±1.80	44.88±1.38	90.78 ±1.82				1.4	0.84
Furosemide	10 mg/kg	140.03±1.20***	99.56±2.40***	130.48±2.92***	2.24	2.21	1.44	1.41	0.55
Aqueous extract of *A. esculentus*	100 mg/kg	84.23 ±2.03*	59.30±2.08	102.22±2.46	1.33	1.32	1.23	1.43	0.71
Aqueous extract of *A. esculentus*	200 mg/kg	129.36±3.04**	68.13±2.35*	124.29±2.27**	2.05	1.52	1.37	1.9	0.63
Aqueous extract of *A. esculentus*	400 mg/kg	134.17±1.83***	85.60±2.32**	134.10±2.47***	2.12	1.91	1.48	1.57	0.61

Table 3: Effect of Aqueous Extracts of *Abelmoschus esculentus* Fruits on Blood Glucose Level of Rats

Experimental group	Dose	Fasting blood glucose (mg/dl) (mean ± SEM)			
		Day 0	Day 3	Day 6	Day 10
Control	Normal Saline	100.75 ± 1.35	101.09 ± 1.41	101.09 ± 1.30	99.34± 1.35
Diabetic Control		134.99 ± 2.59	135.87 ± 2.51	138.48 ± 2.19	140.09 ± 2.43
Glibenclamide	2.5 mg/kg	136.08 ± 1.12	127.42 ±1.02	104.56 ± 0.70**	101.69 ± 0.53***
Aqueous extract of *A. esculentus*	200 mg/kg	138.03 ± 2.66	129.64 ± 1.68	113.05 ± 1.02**	108.10 ± 0.60**
Aqueous extract of *A. esculentus*	400 mg/kg	137.50 ± 2.04	127.96 ± 1.38	111.93 ± 1.30**	106.18 ± 1.05**

Table 4: Phytochemical Screening of Aqueous Extracts of *Abelmoschus esculentus* Fruits

Phytochemical	Test Method	Observation	Result
Alkaloids	Dragendorff	Red-orange precipitate was formed	Presence of alkaloids confirmed
	Wagner	A red-brown precipitate was formed	Presence of alkaloids confirmed
Flavonoids	Alkaline reagent test	Yellow fluorescence was present	Presence of flavonoids
	Shinoda test	Red-pink color was formed	Presence of flavonoids
Polyphenols	Lead acetate test	A bulky white precipitate was formed	Presence of polyphenols
Saponins	Frothing test	A stable persistent froth was formed	Presence of saponins
Tannins	Ferric chloride test	Blue or purple or green precipitate was formed	Absence of tannins

DISCUSSION

In metabolic disorder, targeting glycemic control, hypertension, and lipid management is important for reducing morbidity and mortality, and improving long-term quality of life for patients diagnosed with type 2 diabetes mellitus **[1]**. Diuretic therapy is convenient in reducing the syndrome of volume overload that work on kidneys to excrete increased amounts of salt and water from the body, which, in turn, reducing stroke volume, decreases cardiac output, oxygen demand and as a result, decrease blood pressure **[14]**. Plants with anti-diabetic (hypoglycemic) properties have been said to stimulate the β-cells of the pancreas by activating the regeneration of the pancreatic cells.[15] This study was undertaken to evaluate

the diuretic and anti-diabetic activity of *Abelmoschus esculentus* in rats. In the present study, an attempt was made to elucidate the role of aqueous extracts of Okra fruits to find safer and more effective molecule.

In view of urine output, the aqueous extract of the plant showed a marked increase in diuresis that appeared to be a function of time and dose. *A. esculentus* (100) did not produce a visible effect throughout the experiment, but *A. esculentus* (200) and *A. esculentus* (400) were able to produce a significant diuresis throughout the observation time **(Table 1)**. Besides, the diuretic actions of *A. esculentus* (200) and *A. esculentus* (400) are 2.03 and 2.14, respectively, which are nearly similar to the diuretic action of Furosemide (10) 2.28. The diuretic activity of the aqueous extract in the two effective doses was a mild type since their values were 0.92 and 0.97 for *A. esculentus* (200) and *A. esculentus* (400), respectively **(Table 1)**. The activity of diuretic is better if it is more than 1.50, moderate if it is between 1.00–1.50, mild if it is between 0.72–0.99, and nil if it less than 0.72 **[16, 17]**.

The diuresis increases caused by the aqueous extracts reflected correspondingly in the elimination of electrolytes. It significantly increased the excretion of urinary electrolytes (Na^+, K^+, and Cl^-) in a dose-dependent manner. Although the aqueous extract increased the excretion of K^+ as compared to the negative control, it was significantly lesser than that induced by the reference drug. Besides, the aqueous extract increased the saluretic index and had a dose and time dependent diuresis. This evidence suggests that the aqueous extract might act via the mechanism of loop diuretics. Loop diuretics raise the urinary flow rate and urinary excretion of Na^+, K^+, and Cl^- by inhibiting Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the loop of henle, stimulating renal prostaglandins production, and inhibiting carbonic anhydrase enzyme in the proximal convoluted tubule. The aqueous extracts of the pods of *Abelmoschus esculentus* used in the present study produced a similar Na^+ and Cl^- excretion profile to that of the standard. However, there is a difference when K^+ excretion is considered. This could suggest that the aqueous extract may act via several mechanisms. The aqueous extract has a K^+ saving effect in comparison to the excretion of Na^+ and Cl^-. So, the advantage of the aqueous extract as compare to the conventional agents. Since one of the main adverse effects of loop and thiazide diuretics is hypokalemia which may require oral

administration of potassium supplements or potassium-sparing diuretics that reduce urinary K^+ excretion.

Alloxan induction caused significant ($p<0.05$) hyperglycemia, which may be due to destruction of β cells of Islets of Langerhans. In this study, Alloxan-induced diabetic rats treated with Glibenclamide, *A. esculentus* 200 and 400 mg/kg BW for 10 days. The result shown a significant reduction of glucose levels in animals treated with Aqueous extracts of the *A. esculentus* 200 and 400 mg/kg, almost the same as the Glibenclamide-treated compare to positive-control diabetic rats ($P>0.05$). This suggests that aqueous extracts can increase insulin secretion by pancreatic β cells to a similar extent to Glibenclamide.

Previous studies revealed that there are numerous compounds which could be accountable for a plant's diuretic and hypoglycemic activities via several mechanisms **[18-20]**. The phytochemical screening of the aqueous extract of *Abelmoschus esculentus* showed that the presence of active phytochemical groups such as, alkaloids, polyphenols, flavonoids and saponins. It is reasonable to suggest that these secondary metabolites may act individually or synergistically to produce the observed diuretic, saluretic, natriuretic and hypoglycemic activities of *Abelmoschus esculentus*.

The flavonoids are the natural antagonist ligands for A1 adenosine receptors, while antagonistic activity to the receptor is known to associate with diuretic activity **[21, 22]**. The adenosine A1 receptors are responsible for the reabsorption of 60–70% of filtered sodium and water in the proximal tubules. Interestingly, flavonoids have both diuretic and potassium-sparing activities. The Potassium loss that occurs with many diuretics may leads to hypokalemia. For this cause, potassium-sparing diuretics are recommended **[23]**. The extract of plant has less effect on the elimination of potassium **(Table 2)**. Hence its potassium sparing capacity has to be examined. Plants which contain active moieties like glycosides, alkaloids, terpenoids, and flavonoids have antioxidant property and are claimed to possess antidiabetic activity **[20, 24]**. The antioxidant property of the flavonoids may have played a role in the slight reduction in blood glucose. Collectively, shreds of evidence suggested that the plant *Abelmoschus esculentus* has diuretic and hypoglycemic activity via several mechanisms due to the phyto-constituents.

CONCLUSION

The findings of this study indicated that possibly mechanisms of diuresis and hypoglycemic activity correspondingly potassium-sparing diuretics and antioxidant properties because of its flavonoids and alkaloids content in *Abelmoschus esculentus.* Hence, taking into account that the pods/fruits of the aqueous extracts of *Abelmoschus esculentus* possess significant diuretic and hypoglycaemic effects and can be used in the development of Okra-based nutraceutical for the management of metabolic syndrome.

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