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Evaluation of Diuretic Activity of *Ipomoea aquatica* (Kalmisak) in Mice Model Study

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The diuretic activity of the methanol extract of *Ipomoea aquatica* was investigated in the Swiss albino mice. A dose dependent diuretic activity of 1.93 and 2.44 of the extract was observed at a dose of 250 mg kg^{-1} and 500 mg kg^{-1} body weight, respectively. Good diuretic activity was evident till the fifth hour when mice were orally administrated with the extract at these concentrations. The diuretic activity of the extract was compared with that of the standard drug fursemide at a dose of 500 μg kg^{-1} body weight. The maximum diuresis observed with both the doses at the first hour of study indicated its rapid onset of diuretic action. The diuretic responses with its electrolyte excretion potency of the extract were highly remarkable in comparison with control animals. The extract, at doses of 250 and 500 mg kg^{-1} show a dose dependent increase in volume of urine with moderate increase in Na^+ and Cl^- excretion, accompanied by the excretion of K^+. In all cases the excretion of electrolytes and volume of urine increased was higher than the standard diuretic, fursemide.

Key words: Diuretic activity, Ipomoea aquatica

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Introduction

Plants have fed the world and cured its ills since time immortal. The use of plants in curing and healing is as old as man itself. A vast knowledge of medicinal plants must therefore have accumulated. But most of this knowledge only exists as verbal tradition and only a fraction has got scientific basis till to date.

The use of plant products is increasing in many segment of the population (Eisenberg ***et al.***, 1993). At present thousands of plant metabolites are being successfully used in the treatment of variety of diseases. According to an estimate 80% of the world's population relies upon plants for their medication (Akerele, 1993). Since Bangladesh have a vast resource of medicinal plants, the present study may be a significant way of making the best use of natural resources. The majority of our population, who is impoverished, has to rely upon indigenous system of medication because of their inability to meet the cost of modern medicines. Moreover the standardization of herbal medicines has made then popular in many developed countries (Crake and Simon, 1986).

Previous phytochemical study revealed that the plant ***Ipomoea aquatica*** contains flavonoids and antioxidant activity (Chu ***et al.***, 2000) and various compounds such as alkaloids, myricetin, quercetin, kaempferol, luteolin and pigment (Miean and Mohamed, 2001). The plant is also rich in protein, amino acid, oxalic acid and tetrasaccharide (Reynolds ***et al.***, 1995). The root and leaf of ***Ipomoea aquatica*** contains phenol, which is absent in stem (Singhvi and Sharma, 1984). The young plants, leaves and shoots form a common leaf vegetable with Asians. Owing to its high iron content, it is often fed to patients who are suffering from certain types of anaemia. It is beneficial for nervous and general debility in females. Juice is given in liver complaints and as an emetic, purgative and antidote to optimum and poisoning. Buds are used in the treatment of ringworm and also useful in leprosy, leucoderma and fever (Ghani, 1989).

Therefore, the present research work has been designed to evaluate the diuretic activity of an indigenous medicinal plant ***Ipomoea aquatica***.

Materials and Methods

The experiment was conducted at Pharmaceutical Biotechnology Laboratory, Biotechnology Discipline, Khulna University, Khulna -9208, Bangladesh.

Plant material

The plant ***Ipomoea aquatica*** was collected from Khulna during March 2002 and identified by Bangladesh National Herbarium, Dhaka (Sample # DACB.NO.29, 636). After collection, whole plant was sun-dried for eight days and made into a coarse powder by grinding. 500 g powder of ***Ipomoea aquatica*** was extracted with 800 ml of methanol (40-55°C) in soxhlet apparatus. The extraction was carried out until the process was completed. The methanolic extract was concentrated to one third of its initial volume and a dark greenish colored mass was obtained. The concentrated mass was kept in room temperature to remove methanol by evaporation and finally heated it at 45-50°C until it became methanol free and highly dense.

Table 1: Design of the diuretic activity screening of methanol extract of *Ipomoea aquatica*

Animal group	No. of animals	Mean body weight (gm)	Dose	Route of administration
Group I (control)	5	25	250 mg kg^{-1}	Oral
Group II (Urea)	5	23	500 mg kg^{-1}	Oral
Group III (Fursemide)	5	24	500 µg kg^{-1}	Oral
Group IV (TS-250 mg kg^{-1})	5	25	250 mg kg^{-1}	Oral
Group IV (TS-500 mg kg^{-1})	5	25	500 mg kg^{-1}	Oral

TS: Test sample

Experimental animal

Post weaning Swiss albino mice (20-25 g) of either sex were obtained from the animal house of International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed in standard metal cages and provided with food and water ***ad libitum***. They were deprived of any food and water for the preceding 12 h and were divided into five groups containing 5 animals in each group. They were weighed before the experiment.

Preparation of sample solution

Urea (62.5 mg) was accurately weighed and taken into a graduated test tube. Then it was dissolved slowly in saline by gentle shaking. Finally the volume was adjusted up to 2.5 ml with saline solution. To prepare suspension of the methanol extract of ***Ipomoea aquatica*** 31.5 mg of the extract was measured accurately and suspended in saline solution using 0.1% tween-80 as the suspending agent. The final volume was adjusted to 2.5 ml so that each animal of group IV received 0.5 ml, which was equivalent to a dose of 250 mg kg^{-1} body weight. Similarly 63 mg of the extract was measured accurately and suspended in saline solution using 0.1% tween-80 as the suspending agent. The final volume was adjusted to 2.5 ml so that each animal of group V received 0.5 ml, which was equivalent to a dose of 500 mg kg^{-1} body weight.

Screening of diuretic activity

The test animal was divided into five groups, containing five mice in each group. Group-I was provided only with saline solution and 0.1% tween-80 i.e. control. Group-II was given urea at a dose of 500 mg kg^{-1} body weight and was considered as positive control group. Group-III was provided with standard diuretic drug Fursemide at a dose of 500 mg kg^{-1} body weight. Group-IV and Group-V received the test materials, obtained by methanolic extract, at a dose of 250 and 500 mg kg^{-1} of body weight, respectively. These preparations were given by oral route (Table 1). Twenty-four hours prior to the experiment, the test animals were placed into metabolic cages with total withdrawal of food and water. After oral administration of test samples, the urinary output of each group was recorded at different time intervals from the graduated urine chamber of metabolic cage. Urine samples, which are collected from metabolic case, were analyzed for Na^+ and K^+ concentration by flame photometric method (Mukherjee ***et al.***, 1997) while Cl concentration was determined by the Argentometric titration method (Ramesh and Anbu, 1996).

The volume of the urine excreted in 5 h of study by each group was expressed as percent of the liquid administered giving rise to a measure of "Urinary Excretion"(U. E.)- independent of group weight.

$$\text{Urinary Excretion} = \frac{\text{Total urinary out put}}{\text{Total liquid administered}} \times 100$$

The ratio of urinary excretion (U.E) in test group and control group was denoted Diuretic Action which was used as the measure of degree of diuresis.

$$\text{Diuretic Action} = \frac{\text{U.E. in test group}}{\text{U.E. in control group}}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic action of drug}}{\text{Diuretic action of urea}}$$

Results and Discussion

The hot extraction of coarse powder (500 gm) of ***Ipomoea aquatica*** was carried out with distilled methyl alcohol which yielded 3.8 gm of methanolic extract after evaporation of solvent The research was launched on the basis of its folkloric use as a diuretic.

Since the plant ***Ipomoea aquatica*** has traditional use as a diuretic, the effect of methanol extract of the plant on urination was investigated in Swiss albino mice. The urinary output at different hours of study has been presented in Table 2. The result of the experiment (Table 3) revealed that the diuretic activity of methanol extract at a dose of 250 g kg^{-1} body weight was comparable to that of the standard drug fursemide at a dose of 500 mg kg^{-1} body weight. However the diuretic activity of the extract at a dose of 500 mg kg^{-1} body weight was much higher than that observed with the standard drug fursemide. The methanol extract of ***Ipomoea aquatica*** at an oral dose of 250 g kg^{-1} and 500 mg kg^{-1} body weight showed maximum diuretic activity at 2.89 and 3.38, respectively at the first hour of the study. This indicated the rapid onset of diuretic action, which is dose dependent, of the test material. The diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is with in 1.00-1.50, little if it is between 0.72-1.00 and nil if it is less then 0.72. In this respect after fifth hour of drug administration, the methanol extract of Ipomoea ***aquatica*** showed good diuretic activity of 1.93 and 2.44 at doses of 250 mg kg^{-1} and 500 mg kg^{-1} body weight respectively till the fifth hour of study.

The diuretic responses with its electrolyte excretion potency of the extract were highly moderate in comparison with control animals. The extract, at doses of 250 mg kg^{-1} and 500 mg kg^{-1} show a dose dependent increase in volume of urine with remarkable increase in Na^+ and Cl^- excretion, accompanied by the excretion of K^+. In all cases the excretion of electrolytes and volume of urine increased was higher than the standard diuretic fursemide (Table 4).

Table 2: Urinary output of mice at different time intervals after oral administration of methanol extract of *Ipomoea aquatica*

	Volume of Urine (ml)					Urinary Excretion (V_o/V_1) X 100				
Group	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Group I (Control)	0.7	2.1	3.1	4.0	4.2	28	84	124	160	168
Group II (Urea)	0.8	4.0	4.5	4.6	4.6	32	160	180	184	184
Group III (Fursemide)	2.0	4.2	5.0	6.2	7.0	80	168	200	248	280
Group IV (TS-250 mg kg^{-1})	2.3	4.9	6.3	7.5	8.8	92	196	252	300	352
Group V (TS-500 mg kg^{-1})	2.7	5.4	8.4	10.0	11.2	108	216	336	400	448

V_o= Total Urinary output; V_1 = Total Fluid input (2.5 ml); TS= Test sample

Table 3: Diuretic action and diuretic activity at different time intervals after oral administration of methanol extract of *Ipomoea aquatica*

	Diuretic action (UE_t / UE_c)					Diuretic Activity (DA_t / DA_u)				
Group	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Group I (Control)	---	---	---	---	---	---	---	---	---	---
Group II (Urea)	1.14	1.90	1.45	1.15	1.09	---	---	---	---	---
Group III (Fursemide)	2.50	2.00	1.61	1.55	1.67	2.19	1.05	1.11	1.35	1.53
Group IV (TS-250 mg kg^{-1})	3.29	2.33	2.03	1.88	2.10	2.89	1.23	1.40	1.63	1.93
Group V (TS-500 mg kg^{-1})	3.86	2.57	2.71	2.50	2.66	3.38	1.35	1.87	2.17	2.44

TS = Test sample

UE_t = Urinary excretion in test group; UE_c = Urinary excretion in control group

DA_t = Diuretic action of the test sample; DAu = Diuretic action of the urea

- Diuretic activity > 1.50 indicates good diuretic activity;
- Diuretic activity between 1.00 to 1.50 indicates moderate diuretic activity;
- Diuretic activity between 0.72 to 1.00 indicates little diuretic activity;
- Diuretic activity < 0.72 indicates no diuretic activity

Table 4: Dose response study of diuretic effects of *Ipomoea aquatica* extract through electrolyte excretion in urine in mice

					Electrolyte excretion (mM)			
Treatment	Dose	No. of animal	Total urine volume (ml)	Diuretic activity of extract	Na^+	K^+	Cl^-	Na^+/K^+
Group I (Control)	250 mg kg^{-1}	5	4.2	---	0.11	0.037	0.194	2.97
Group III (Fursemide)	500 µg kg^{-1}	5	7.0	---	0.14	0.041	0.231	3.41
Group IV (TS-250 mg kg^{-1})	250 mg kg^{-1}	5	8.8	1.23 to 2.89	0.143	0.044	0.253	3.25
Group V (TS-500 mg kg^{-1})	500 mg kg^{-1}	5	11.2	1.35 to 3.38	0.143	0.043	0.264	3.33

TS= Test sample

From the above observation it can be suggested that the methanolic extract of ***Ipomoea aquatica*** is an effective hypernatraemic, hyperchloremic and hyperkalaemic diuretic; which supports the claim about the ***Ipomoea aquatica*** being used as a diuretic.

A large number of indigenous drugs have been claimed to have a diuretic effect in the Ayurvedic system of medicine. Gujral ***et al.*** (1955) found that the flowers of ***Viola odorata***, ***Nymphaea alba***, ***Nelumbium speciosum***; roots of ***Asparagus recemosus***, ***Arundo karka***, ***Portulaca***

oleracea posses diuretic effect in different animal models. Diuretic activity has also been observed in extract of the rhizomes of ***Nelumbo nucifera*** Gaertn (Mukherjee ***et al.***, 1997). Malalavidhane ***et al.*** (2000 and 2001) found that an aqueous extract of ***Ipomoea aquatica*** is as effective as the oral hypoglycemic drug tolbutamide in reducing the blood sugar levels of Wistar rats.

Though the extract appeared to cause good diuresis at both dose levels, the actual mode of action, which may be due to its effect either on loop permeability or reduction of antidiuretic hormone (ADH) secretion or inhibition of carbonic anhydrase enzyme, is not clear from the test result. The exact mechanism of diuretic activity exhibited by the extract can only be established after more investigation of the extract and screening of diuretic activity of isolated pure constituents.

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