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Evaluation of Diuretic Activity with Different Concentrations of *Allium Cepa* on Albino Rats

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ABSTRACT

The present study was aimed to explore the diuretic actions of n-butanol extract of *Allium cepa* bulbs in male Westar albino rats. The n-butanol extract of *Allium cepa* was administered at three doses (10, 20 and 30 mg/kg, p.o.) to wistar rats in the present diuretic model. Furosemide, a standard diuretic was used as reference drug. In this study urine volume and urine electrolyte levels were determined. The extract gave positive tests for Alkaloids, Cardiac glycosides and Saponins steroids. The n-butanol extract of *Allium cepa* bulbs increased urine volume and urine electrolytes excretion with a dose of 20 mg/kg, p.o. The study concludes that n-butanol extract of *Allium cepa* bulbs possesses the acute diuretic activity, thus validating the traditional use of this plant as diuretic. However further studies are necessary to isolate and characterized the phytoconstituents responsible for diuretic action and to explore the exact underlying mechanism of *Allium cepa* bulbs.

Key words: *Allium cepa*, diuretic, Furosemide.

INTRODUCTION

A diuretic provides a means of forced diuresis which elevates the rate of urination. Diuretics are the drugs that promote the rate of urine flow and sodium excretion. Diuretics alone or in combination with other drugs are used in a variety of clinical situations like hypertension, heart and renal failure, nephritic syndrome and cirrhosis [1]. There are several categories of diuretics. All diuretics increase the excretion of water from bodies, although each class does so in a distinct way. For high blood pressure, diuretics, commonly known as "water pills," help the body to get rid of unneeded water and salt by excreting them through the urine. Diuretics may be used to treat a number of heart-related conditions, including high blood pressure, heart failure, glaucoma, kidney and liver problems. The two commonly used diuretics, that is, thiazides and furosemide, have been associated with many side effects, like disturbances of electrolytes, acid-base and water balance, changes in uric-acid, carbohydrate and lipid metabolism and drug interactions [2, 3]. To bypass these problems there is a need for safe and effective natural diuretic. Natural medicines are considered to be more safe and economical [4]. As most of the plants contain potassium, along with other nutritional elements like Na^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , etc [5], it can be assumed that they would not lead to potassium depletion.

The onion (*Allium cepa*), which is also known as the bulb onion common onion is the most widely cultivated species of the genus *Allium*. The genus *Allium* also contains a number of other species variously referred to as onions and cultivated for food and other [6].

MATERIAL AND METHODS

Plant, preparation of crude extract and fractions

Allium cepa (AC) bulbs were purchased from local market, identified in the department of Pharmacognosy, Balaji College of Pharmacy, Anantapur, AP, India and voucher specimen (BCP-PCOG- 49) was procured and submitted to the herbarium of the Department of Pharmacognosy, Balaji College of Pharmacy, Anantapur, AP, India.

Sliced *Allium cepa* bulbs were dried under shade and powdered by using grinder mixer. The powdered material (1000g) was taken and was macerated with water and the extract was collected [7]. The obtained extract was transferred in to petri dish and evaporated to dryness at 60° C on water bath, yielding a reddish coloured dry 280 g mass of preparation of n- butanol extract of AC leaves (BAC) (23% w/w). BAC was preserved in desiccator till use. BAC was suspended in 2.0% Tragacanth in distilled water and administered 5ml/kg, per orally (p.o.)

Phytochemical Screening

The powdered extract was subjected to various chemical test to know the presence of absence of sugars, carbohydrates, steroids, glycosides, tannins, alkaloids, flavanoids, proteins and amino acids [8, 9].

Experimental Animal

Male Westar albino rats were obtained from NIN Hyderabad, India. The experiment was conducted as per the permission of Institutional animal ethical committee bearing Ref.NO: BCP/ IAEC/Approval / 12 / 2012. The rats (150-200 g) were grouped randomly and housed in polycyclic cages with paddy husk bedding at a temperature of 22±2° C and relative humidity of 65 ± 5%. They were allowed to free access for standard dry pallet diet (Kris Scientific Shoppe, Bangalore) and water. The experimental protocol was approved by IAEC of Balaji College of Pharmacy, Anantapur, India constituted as per the rules and guidelines provided by the CPCSEA.(1563/PO/a/11/CPCSEA)

Acute Toxicity study

Healthy male Wister Albino rats (150-200 g), 8-12 weeks old were employed. Test substance was suspended in 2% tragacanth in distilled water, freshly prepared; the volume administered was 1 ml/100 g body weight. The animals were fasted overnight with free access to water, weighed before dosing and test substance administered. After drug administration food were withheld for 3-4 h. Animals were observed individually during first 30 min after dosing, periodically during 24 h with special attention given during first 4 h and daily thereafter for total of 14 days. All observations were systemically recorded with individual records being maintained for each animal [10].

Diuretic activity

Male albino rats weighing between 150-200g, deprived of water for 16-18h before the test drugs were administered. The animals were pre-treated with physiological saline (0.9% NaCl) at an oral dose of 5ml/kg body weight, to impose a uniform water and salt load. Four animals were kept in each group [11].

Diuretic Potential study

The rats were randomly divided into 5 groups of 4 animals each

Group I- Vehicle control given 2 % tragacanth suspension in distilled water 1 ml/kg (p.o);

Group II- Standard drug Frusemide (20 mg/kg, p.o); treated with distilled water;

Group III- BAC extract (10 mg/kg, p.o);

Group IV- BAC extract (20 mg/kg, p.o);

Group V- BAC extract (30 mg/kg, p.o)

Parameters viz., Urine volume, Electrolyte excretion (Sodium, potassium and chlorine levels), Diuretic action: Urinary excretion of test group/urinary excretion of control group, Diuretic activity: Diuretic action of extract/Diuretic action of standard, Saluretic index: mMoles/L of test group/mMoles of control group were observed.

Total urine excreted out was collected and the volume was determined. pH of the pooled urine from each animal was determined by using digital pH meter (Global DPH 500, Mumbai) and Na⁺ and K⁺ concentrations was determined on flame photometer (Flame Photometer systronics, Mumbai).

Experimental protocol

Animal were fasted for 18 h prior to the experimentation but with free access to water only. All the rats received priming dose of normal saline 25 ml/kg orally. Immediately after administration vehicle, standard drug and different doses of extracts according to body weight all the rats were placed in metabolic cages (group wise) specially designed to separate urine and faeces at room temperature of 25±0.5°C Urine was collected in a graduated cylinder and its volume was measured for next 5 h. During this period no food and water was made available to animals. Collection time for first drop of urine and total volume of urine collected from both control and treated groups were measured. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 gm body weight. Electrolyte (Na⁺ and K⁺) concentration and pH of collected urine was estimated at the end of the experimental period.

Measurement of Urine output and Analysis of Electrolytes

Na⁺ and K⁺ concentrations were measured using a digital flame photometer. The instrument was calibrated with standard solutions containing different concentration of Na⁺ and K⁺. Cl⁻ concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of potassium chromate solution as an indicator. A pH meter was used to measure the pH of freshly collected urine sample.

Statistical Analysis

The results were expressed as mean values ± SEM (standard error of mean) of 5 rats. Experimental data were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparison test. P value less than 0.05 were considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS AND DISCUSSIONS

Phytochemical Screening

Phytochemical analysis of BAC fraction showed presence of steroids, tri terpenoidal saponins and carbohydrates as major phyto chemical constituents, and alkaloid and cardiac glycoside in slight quantity. Tannins, flavonoids, Anthraquinone and Cyanogenic glycosides were found to be absent. The results were tabulated Table 1 and shown in fig 1.

Table 1: Phytochemical Screening of BAC Extract

Test for phyto-constituents	Observation
Alkaloids	+
Tannins	-
Flavanoids	-
Cardiac glycosides	+
Anthraquinone glycosides	-
Cyanogenic glycosides	-
Saponins	- +
steroids	++

- = Compound not detected; + = Compound detected

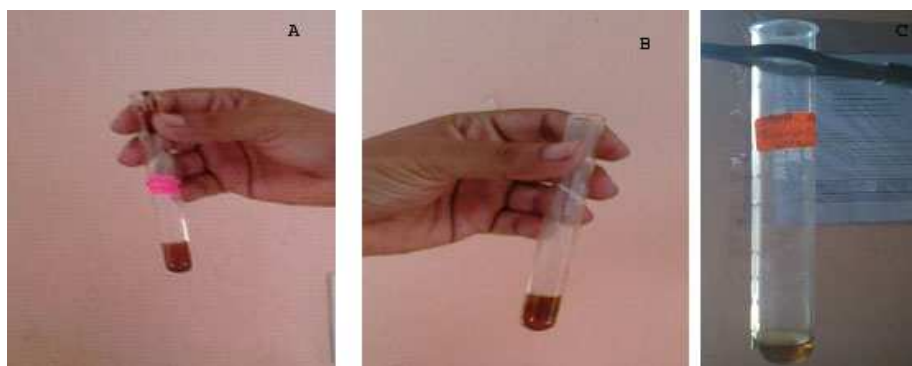


Fig.1. Test for A) alkaloids B) Saponins C) Cardiac glycosides

Diuretic Activity:

The BAC extract induced a significant ($P < 0.05$) increase in urinary Na^+ loss at 20 mg/kg dose with a Na^+/K^+ ratio of 1.659. Frusemide caused the expected increase in the renal excretion of Na^+ , Cl^- and K^+ as a result urine Na^+/K^+ ratio was decreased to 1.474 as compared to control. The effect of *A. cepa* on sodium excretion was comparatively more on sodium than that of potassium. Extract at 20 mg/kg dose produced 26.84% increase in Na^+ excretion against 132.17% increase by Furosemide when compared to control. BAC extract had significant natriuretic and aquatic responses in experimental animals. The Effect of BAC Fraction on Urine Output in Rats. The values were represented in table 2 and 3 and shown in figures 2, 3, 4, 5 and 6.

Table 2: Effect of BAC Fraction on Urine Output in Rats

Treatment (mg/kg, p.o)	Collection time for 1st drop of urine in min. (M±SEM)	Total volume of urine on the 5 th h. (M±SEM)
Vehicle control	35.8±1.08	6.8±0.11
Frusemide	18.2±0.91***	1.8±0.24**
BAC extract	25.8±1.51*	8.9±0.81
BAC extract	19.5±0.97	9.9±0.22
BAC extract	18.3±1.23	4.8±0.15

n = 6. * $p < 0.01$, *** $p < 0.001$ and ns = not significant when compared to control group

Fig.2. Urinary excretion by different concentrations of *Allium cepa*

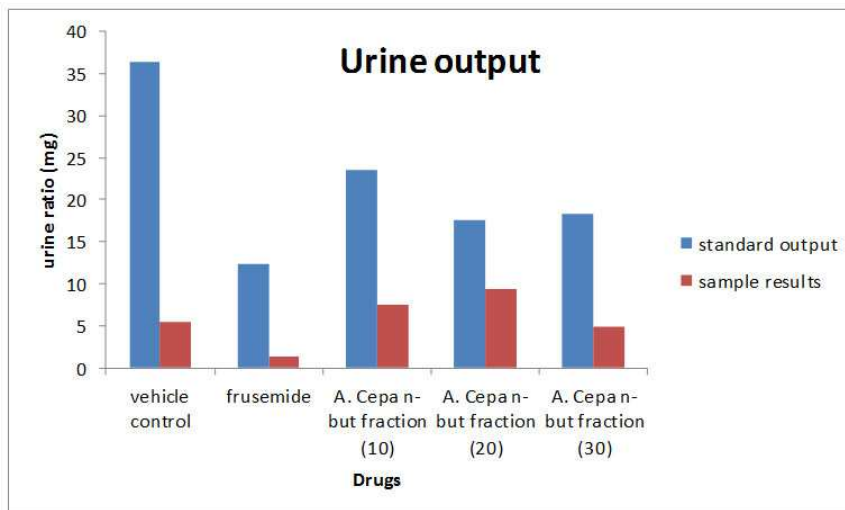


Table 3: Effect of BAC extracts on Urinary Electrolyte Excretion in Rats

Treatment (mg/kg, p.o)	Electrolyte excretion				
	Na ⁺ in mEq/L (M ± SEM)	K ⁺ in mEq/L (M ± SEM)	Cl ⁻ in mEq/L (M ± SEM)	Na ⁺ /K ⁺ ratio	% increase in Na ⁺ excretion
Vehicle control	159.11±9.25	95.85±5.67	75.84±5.08	1.659	-
Furosemide	371.01±10.58 ***	251.67±9.99***	228.12±9.12***	1.474	133.17
BAC extract	174.65±8.87	126.50±7.41	76.12±5.89	1.381	9.77
BAC extract	203.81±5.95*	125.84±9.14	97.86±5.28	1.619	26.84
BAC extract	187.44±5.89*	124.57±9.8	85.84±4.84	1.504	17.81

N = 6. *p<0.01, ***p<0.001 and ns = not significant when compared to control group

Fig.3. Sodium excretion in the urine by different extraction and formulation of *Allium cepa*

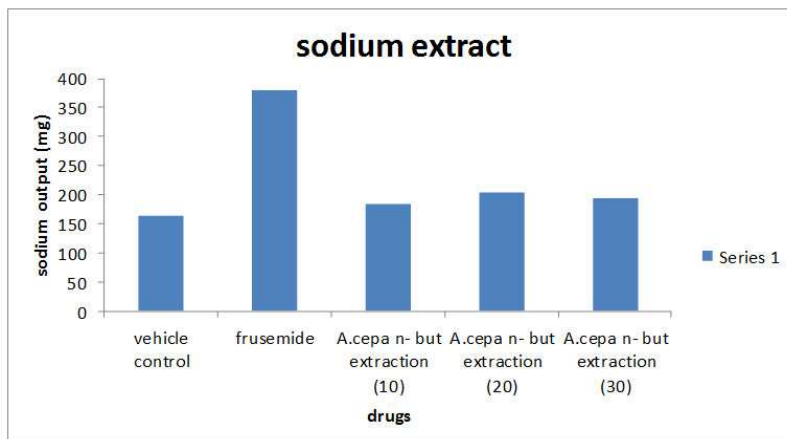


Fig.4. Potassium excretion in the urine by different concentrations of *Allium cepa*

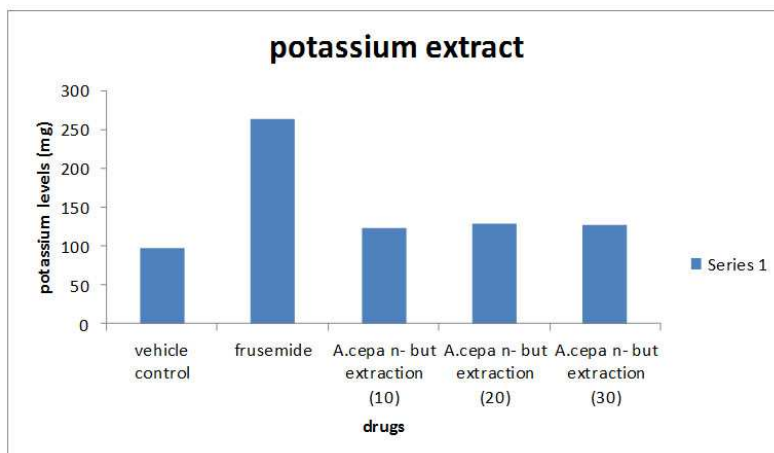


Fig.5. Chlorine excretion in the urine by different concentrations of *Allium cepa*

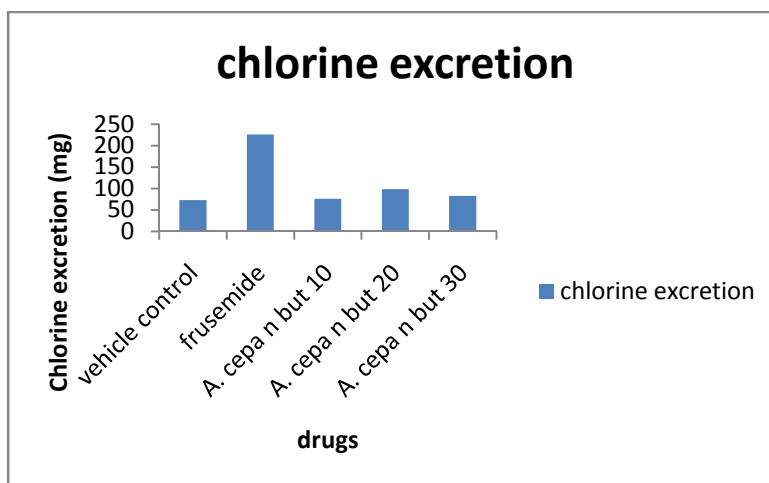
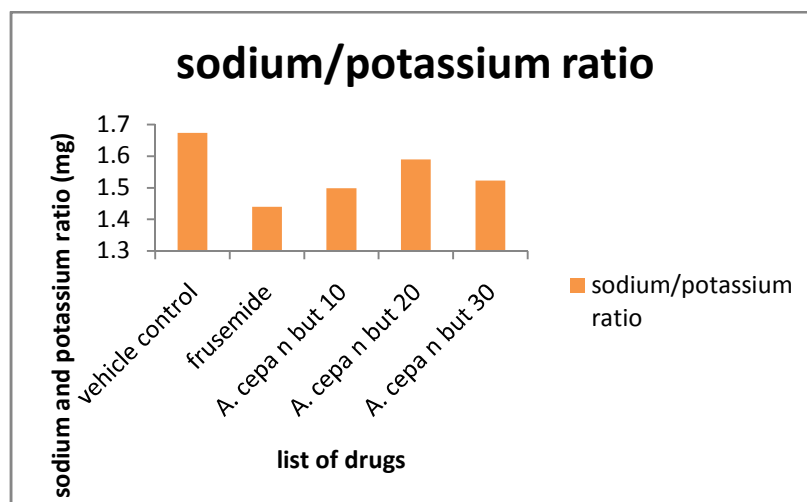


Fig.6. Sodium/ Potassium ratio



Acute Toxicity study

The LD₅₀ is greater than the test dose 500 mg/kg. The *in vivo* diuretic study was conducted at a dose level of 10 and 20 mg/kg, as higher doses are likely to provoke hypotension, Bradycardia and T-wave inversion.

The present study clearly demonstrated the diuretic effect of the onion bulb BAC extract. The study confirmed the ethnopharmacological and Ayurvedic use of *A. cepa* as a diuretic agent, but further studies are necessary to evaluate the mechanisms involved in its biological activity and safety following repeated exposure.

SUMMARY

The diuretic activity of dried onion bulb extracts targeting the steroidal content. The rats were randomly divided into 5 groups of 4 animals each as vehicle control (2 % tragacanth suspension), standard drug Frusemide (20 mg/kg, p.o) and BAC extract extract (10 mg/kg and 20 mg/kg, 30 mg/kg, p.o) treated. Urine was collected in a graduated cylinder and its volume was measured for next 5 h. Na⁺, K⁺ and Cl⁻ concentrations were measured. At 20 mg/kg dose onset of diuresis and total volume of urine formed was significantly (P<0.01-0.05) higher. 5th hour urine volume at 20 mg/kg dose was 9.9±0.22ml as compared to control. Extract at 20 mg/kg dose produced 26.84% increase in Na⁺ excretion against 133.17% increase by furosemide when compared to control signifying natriuretic response. The study confirmed the ethnopharmacological and Ayurvedic use of *A. cepa* as a diuretic agent.

CONCLUSION

These results provide rational for its medicinal use as a diuretic agent. We can conclude n-butanol extracts of *A. cepa* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic Furosemide. The present study provides a quantitative basis for explaining the use of *A. Cepa* as a diuretic agent. All the fractionations were found less efficacious than the parent crude extract suggesting the existence of additive and/or synergistic effect in the crude extract.

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