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EFFECT OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS*. LINN. ON URINARY VOLUME & ELECTROLYTE EXCRETION IN ALBINO RATS

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ABSTRACT

Hibiscus rosa-sinensis. Linn grown widely in tropical countries, recently known to have anti-hypertensive effect in the albino rats. This report has shown new dimension to one clinical use & paucity of data available in this regard, the present work is undertaken to study this effect scientifically. Albino rats (100-200gms) deprived of food for 15hrs divided into seven groups of six in each and put in metabolic cages after hydration by normal saline for 24hrs. Aqueous extract of Hibiscus rosa-sinensis was administered in100,200,400 & 600 mg/kg doses per oral. Urinary volume, total Na^+, K^+, Cl^- conc. was estimated at 5^{th} & 24^{th} hr and compared with control group. Furosemide 25mg/kg PO was taken as the standard. Aqueous extract of *Hibiscus rosa-sinensis*(AEHR) was found to increase the urinary volume of the 5^{th} hr and 24^{th} hr sample. Na^+ & Cl^- excretion also significantly increased in 200&400 mg/kg doses. AEHR was found to possesses diuretic activity.

KEYWORDS: **Hibiscus rosa-sinensis, Diuretic effect, Metabolic cages, Albino rats**

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INTRODUCTION

Hypertension currently is a common disorder growing incidence globally.[1]Hypertension (HTN) is one of the major chronic diseases resulting in high morbidity and mortality in the world population.[2] Effective pharmacological control is usually achieved by multidrug regimen, hence frequently associated with various side effects due to increase in number of drug and duration of therapy . Abnormalities in fluid volume and electrolyte composition are usually associated with hypertensive disorder. Therefore drugs that block specific transport system of the renal tubules are valuable tools in the treatment of this condition.[3,4] Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[5]. Herbal products are cost effective, easily available and safe for long term use. *H. rosa-sinensis* belongs to the family, Malvaceae. As a traditional medicine, the fresh juice of the flower is used to treat gonorrhea, the powdered root is used in the treatment of menorrhagia and the infusion of the petals is used as a refrigerant drink in fever. [6] The flowers are considered emollient, and an infusion of the petals is used as a demulcent and refrigerant drink in fevers; [7] it's decotion is given in bronchial catarrh [8] in India. Previous studies show that the plant possesses anti-complimentary, anti-diarrhetic and anti-phologistic [9] activities. The leaves and flowers have been found to be effective in the treatment of heart disorders; [10] used as an anti-spermatogenic and androgenic, [11] anti-tumor, [12] anticonvulsant, [13] anti-diabetic [14] and anti-ulcer [15] activities and also as a hair growth promoter. [16] Recently, its antihypertensive potentiality has been revealed in folklore use of the same which has shown new dimensions to therapeutics.[17] However, paucity of data is available in this regard. With this background the present work was undertaken to investigate the diuretic effect of *H. rosa-sinensis* in a scientific manner.

MATERIALS AND METHODS

Source of plant materials

Fresh flowers of *H. rosa-sinensis* were obtained locally & was authenticated by department of botany, Utkal university, Bhubaneswar.

Preparation of plant extract

Fresh flowers of *H. rosa sinensis* were rinsed and air dried to constant weight at room temperature. It was macerated and extracted with distilled water. The homogenate was filtered using a cloth over a funnel, and the residue discarded. The filtrate was evaporated to dryness over a low temperature water bath. The solid residue (AEHR)left was weighed and stored in a refrigerator at 4^{o}C until required for use.[18]

Treatment of animals

Drugs used

Furosemide was obtained from M/S Hoechst Pharmaceuticals ltd., Mumbai in pure powder form.

Acute toxicity study

Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals (albino rats), animals were kept for observation of behavioral change and death up to 72h. Male wistar albino rats (100-200 gms) were acclimatized to standard laboratory conditions for 15 days. The animals were provided standard rat feed & water ad libitum. Methods of Liptschiz et al 1943 was followed.[19] Food but not water was withdrawn 15 hrs prior to the experiment. At the end of 15 hrs, on the day of experiment, all the rats were hydrated with normal saline 20 ml/kg administered orally through a pediatric nasogastric tube. The rats were arranged into seven groups of six in each & the study protocol was designed as depicted in the table 1.

Table 1
Protocol of the study (Approved by IAEC)

Groups	Treatment Dose in mg/kg(P.O.)	Mode Of Administation	Nature Of Group
1	NS(20ml/kg)	Through paediatric nasogastric tube	Control
2	FUROSEMIDE(25)	Dissolved in 1 ml NS followed by 20 ml/kg NS	Standard drug
3	AEHR (100)	-do-	Test drug
4	AEHR (200)	-do-	-do-
5	AEHR (400)	-do-	-do-
6	AEHR (600)	-do-	-do-

Metabolic cages with albino rats

Immediately after hydration and treatment the animals were kept in the metabolic cages (3 per one) provided with a wire mesh bottom & funnel with stainless steel sieves to retain faeces and allow the urine to pass. Few drops of toluene were added to each of the collecting cylinder as a preservative to prevent evaporation of urine, since 5th hr & 24th hr urine collection was necessary for the study. The cumulative volume of urine excreted was recorded every hour up to 5hrs & again after 24hr. The electrolyte concentration of cations (Na^+, K^+) was estimated by Ecolyte analyzer & that of anion (Cl^-) was estimated by end point titration. Data were analyzed by one way ANOVA followed by Dunnet's multiple comparison test. [20]

OBSERVATION & RESULTS

The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hr.AEHR (aqueous extract of *H. rosa sinensis*) 5hrs after its administration (vide table 2) shows increase in urinary volume which was significant with 400mg/kg&600mg/kg doses. Excretion Na^+ & Cl^- followed similar pattern with same doses & chloride excretion were highly significant with two higher dosages. K^+ excretion were not significant with all the doses tested. Analysis of Na^+ / K^+ ratio reveals definite natriuretic effect. The diuretic index was found to be more than that of Furosemide treated group in two high doses of AEHR. Cl^- / Na^+ + K^+ ratio suggest no evidence of carbonic anhydrase inhibitory activity.[21] The results are comparable to that of Furosemide. Analysis of 24hr urine sample exhibited similar pattern as that of the 5hr.sample. AEHR exhibited increase in urinary volume, sodium and chloride excretion which were highly significant with 400&600 mg/kg doses. K+ excretion was not significant with all the doses tested. The ionic quotient remained parallel with 5hr values.

DISCUSSION

Diuretic activity of AEHR 5 hr after its administration was manifested in the form of an increase in urinary volume which was highly significant with 400 and 600 mg/kg doses. Excretion of sodium and chloride followed similar pattern with same doses. Potassium excretion was not significant with all the doses tested which indicated potassium conserving property of the herbal extract. Analysis of Na^+/K^+ ratio revealed a definite natriuretic effect.(Na^+/K^+ values greater than 2.0 indicate a favourable natriuretic effect. Ratios greater than 10.0 indicate a potassium sparing effect.) Therefore, though AEHR showed a potassium conservating potentiality, potassium sparing effect was not observed. The diuretic index found to be more than that of Furosemide in the two higher doses. The value of Cl^- / $Na^+ + K^+$ ratio varied between 0.70 to 1.16 with graded doses of AEHR suggesting a negative carbonic anhydrase inhibitory activity. The results were comparable to that of Furosemide.

TABLE 2

Diuretic activity of AEHR in albino rats 5hr after administration

Groups	Treatment (PO) mg/kg	Cumulative urine volume in ml	Total Na^+ (mEq / lt)	Total K^+ (mEq / lt)	Total Cl^- (mEq / lt)	Na^+/K^+ ratio	Diuretic index	$\frac{Cl^-}{Na^+ + K^+}$
1	Control	12 ± 2.33	155±11.9	65.75 ± 11.39	128.17 ± 8.545	2.35	-	0.58
2	Furosemide (25)	15 ± 0.85	**210.5 ± 17.96	87 ± 11.505	*222.17 ± 17.09	2.44	1.42	0.74
3	AEHR (100)	7.8 ± 1.255	142.5±6.29	74.25 ± 2.0965	153.81 ± 9.865	1.91	0.68	0.70
4	AEHR (200)	14 ± 1.255	145±20.205	67.25 ± 18.375	180.99 ± 22.93	2.15	1.31	0.84
5	AEHR (400)	*16.2 ± 0.815	*188.5 ± 11.085	88.5 ± 4.645	***265.07 ± 8.545	2.16	1.45	0.96
6	AEHR (600)	**17 ± 0.575	*198.5 ± 6.29	89.5 ± 3.775	***333.25 ± 35.23	2.23	1.54	1.16

(P < 0.05), ** P < 0.01, *** (P < 0.001)

Analysis of 24 hour post dosing urine sample revealed similar results with regards to urinary volume, sodium, chloride and potassium excretion as observed in 5th hour sample that indicates a continuation of the diuretic effect of AEHR up to 24 hours. The ionic quotient remained parallel with the 5th hour values.

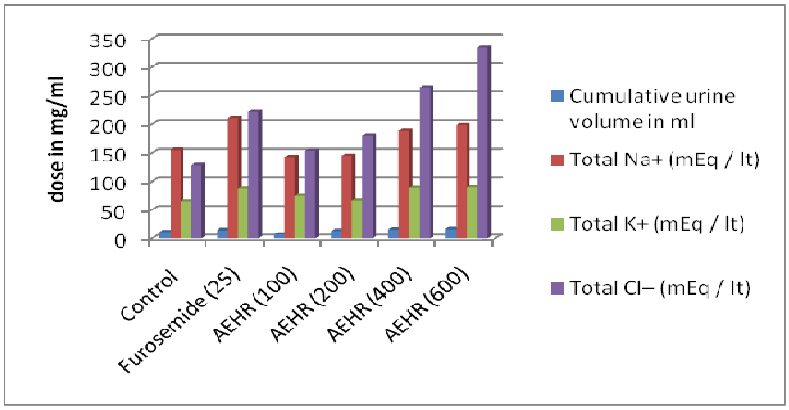
Table 3

Diuretic activity of AEHR in albino rats 24hr after its administration

Groups	Treatment (PO) mg/kg	Cumulative urine volume in ml	Total Na^+ (mEq / lt)	Total K^+ (mEq / lt)	Total Cl^- (mEq / lt)	Na^+/K^+ ratio	Diuretic index	$\frac{Cl^-}{Na^+ + K^+}$
1	Control	41±2.33	170±10	72.5±4.785	145.26±4.925	2.34	-	0.59
2	Furosemide (25)	****66.25±6.885	**195±8.66	89.5±2.10	***273.44±24.17	2.17	1.61	0.96
3	AEHR (100)	41.5±2.87	142.5±2.5	72±10.83	187.96±9.865	1.97	1.01	0.87
4	AEHR (200)	****54.75±3.805	162.5±24.62	76.75±16.75	209.35±20.375	2.11	1.33	0.87
5	AEHR (400)	****73.5±11.84	*180±8.165	78.75±9.455	***290.53±9.865	2.28	1.79	1.12
6	AEHR (600)	****89±10.15	*187.5±7.5	83.75±2.84	***367.43±25.55	2.23	2.17	1.35

p<0.05, p<0.01, p<0.001, All the values are mean ± SE

Graph 1
Diuretic activity of AEHR 5 hrs after administration



From literature survey it is quite evident that diverse herbal product posses significant diuretic potentiality of clinical usefulness reported by various workers in different set up of studies. Herbal products usually contains many active components viz. flavonoides, steroid, glycosides, saponins, organic acids etc. which either alone or in combination is responsible for the diuretic activity . *H. rosa sinensis* popularly known as 'China rose' contain flavonoids which may be responsible for its diuretic activity.

CONCLUSION

The results of the present investigation clearly indicates potential diuretic activity of *H. rosa sinensis* Linn in rats. Hence phytochemical analysis, separation of active ingredients and further investigation in this line is essential to establish its therapeutic benefits. [22] As we all know that, hypertension is an emerging disease worldwide, based upon the results of the present study in animal model, indigenous drugs may be tried as an adjunct therapy in the treatment of hypertension of various etiology as well as various types of edema, heart failure cases.

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