

Diuretic Activity of the root of *Homonoia retusa* (GRAH. EX WT.)

MUELL.

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Summary

Traditionally *Homonoia retusa* (GRAH. EX WT.) Muell. (Euphorbiaceae) root part is claimed to possess powerful diuretic activity. The aim of this study is to evaluate the diuretic potential of aqueous extract (AEHR) and 70% of ethanol extract of the root of *Homonoia retusa* (GRAH. EX WT.) Muell. (EEHR) in rats. Different parameters viz. total urine volume, urine concentration of electrolytes such as sodium; potassium and chloride have been evaluated. The rats treated with AEHR and EEHR (250 and 500mg/kg; p.o.) showed higher urine volume when compared to respective control. Excretion of cations (sodium and potassium ions) and anions (chloride ions) in both AEHR and EEHR also increased significantly with respect to the control group. The elevated diuretic potential of AEHR and EEHR was statistically significant ($P < 0.001$) and comparable to that of standard furosemide (10mg/kg; p.o.). The present study shows that the drug has significant diuretic activity.

Keywords: *Homonoia retusa* Muell., Urinary volume, Diuretic activity.

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Introduction

Homonoia retusa (GRAH. EX WT.) Muell is a dioecious shrub, up to 3m tall; branchlets stout, woody; leaves obovate to oblanceolate, 2-4×1-2 cm, coriaceous, margin serrate to dentate, apex obtuse to retuse, acute-cuneate at base, flowers in axillary spikes; capsules 3.5 mm across, globose; seed rounded on the back, smooth, yellow¹.

The shrub is known as Pashanabhesha in Ayurveda, **Pashanabhesha** is a derived word from pashana meaning a **stone** and **bheda** mean to break. This meaning is attributed to the drug since it is claimed to possess the property of disintegrating the calculi or stones in the bladder as well as in Kidney². This is one of the controversial drug in Ayurveda³. Within India, it has been distributed in Coorg, North kanara, Raichur, Shimoga, South kanara, Peninsular india⁴. It is usually inhabiting rocky riverbeds¹.

In traditional medicine, the plant is used in cough, sore throat, diabetes and lithiasis, diuretic⁴. There is paucity of data about the pharmacological activities of *H. retusa*, which prompted us to pursue this pharmacological evaluation of *H. retusa* root to verify the medicinal property. Therefore, the present study was undertaken to evaluate the diuretic activity of AEHR and EEHR in normal rats.

Material and Method

Plant materials

The roots of *Homonoia retusa* were collected from Thenmalai forest, Kerala state. The taxonomic identification of the plant was established in RRI, Bangalore and a voucher specimen has been kept in the laboratory of Gautham College of Pharmacy for future reference.

Preparation of extract

The roots were dried under sunlight, powdered in the hammer mill and passed through sieve no 60 and store in air tight container. The dried, powdered roots were extracted using 70% ethanol in a soxhlet extraction apparatus. The extract cleared of ethanol by distillation under reduced pressure which was kept in the desicator and a weighed amount of the extract was dissolved in normal saline for the experiment. The dried, powdered root was macerated with chloroform: water (1: 1000) for 24 hours, the solvent was evaporated to dryness.

Animals

Swiss albino mice weighing 18-30 g of either sex were used for acute toxicity studies and male albino rats of Wistar strain, in the weight range of 200-250 g were used for diuretic activity studies. The animals was purchased from Sri Venkateswara enterprises, Bangalore and housed in the animal house of Gautham College of Pharmacy, Bangalore at least 2 weeks prior to the study, so that animals could adapt to the new environment. Animal house was well maintained under standard hygienic conditions, at a temperature ($22 \pm 2^{\circ}\text{C}$), room humidity ($60 \pm 10\%$) with 12 h day and night cycle, with food and water *ad libitum*.

Acute toxicity study

Acute toxicity studies were carried out to study acute toxic effects of the drug and to determine minimum lethal dose of the drug extracts. The AEHR and EEHR were administered orally to separate groups of overnight fasted mice at doses of 30, 100, 300, 1000 and 3000 mg/kg. After administration of the extracts, the animals were observed continuously for the first three hours, for any toxic manifestation like increased motor activity, salivation, acute convulsion, coma and death. Thereafter, observations were made at regular intervals for 24 h⁵. Further, the animals were under observation up to a period of one week. On the basis of LD50, two doses were selected for detailed study.

Screening of diuretic activity^{6,7}.

The method of Lipschitz et al.⁸ was employed for the assessment of diuretic activity, male albino rats weighing 200 - 250 g were selected, and the tail base was pressed to empty the bladder of remaining urine. The animals were divided into 6 groups of 6 each. Group I was maintained as control and administered 5ml/kg of normal saline p.o. Group II was maintained as Standard and administered 10 mg/kg of Furosemide p.o. Group III and IV were administered AEHR 250 and 500 mg/kg p.o. respectively. Group V and Group VI were administered EEHR 250 and 500 mg/kg body weight p.o. respectively. All animals were hydrated with 5 ml/kg of distilled water prior to drug administration. Animals were deprived of food and water 18 h before the experiment. They were hydrated with 5 ml/kg of water prior to drug/ extract administration. Immediately after dosing, animals were placed in metabolic cages (2 in one cage), specially designed to separate urine and feaces. The urine was collected in measuring cylinder up to 5 h after dosing. During this period, animals were deprived of food and water. The parameters measured were

total urine volume, urine concentration of Na^+ , K^+ and Cl^- . Concentration of Na^+ and K^+ were determined using Flame photometer⁹. While Cl^- concentration was estimated titrimetrically using 0.02N AgNO_3 with 5% potassium chromate as indicator^{10,11} appearance of brick red precipitate was taken as the end point.

The ratio, urinary excretion in test group:

Urinary excretion in control group has been used for the measure of diuretic action for the treated groups.

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test group}}{\text{Urinary excretion in control group}}$$

The relative diuretic potency can be determined¹². To obtain the diuretic activity,

$$\text{Diuretic activity} = \frac{\text{Diuretic action of extract}}{\text{Diuretic action of standard}}$$

The sum of Na^+ and Cl^- excretion was estimated for saliuretic activity. The ratio Na^+/K^+ was estimated as a natriuretic activity. The ratio $\text{Cl}^- / (\text{Na}^+ + \text{K}^+)$ (ion quotient) was derived to estimate carbonic anhydrase inhibition¹³.

Statistical analysis

The data were expressed as Mean \pm S.E.M. and statistically analyzed using one way ANOVA followed by Tukey-Kramer's Multiple comparison test, $P<0.05$ was considered significant.

Results

The hot extraction of coarse powder (50g) of *H. retusa* was carried out with 70% ethanol yielded 5.20% w/w and extraction of coarse powder with chloroform water yielded (1.49% w/w). The phytochemical analysis of the both extract shown the presence of phytosterols, phenolic compounds and tannins, carbohydrates and flavonoid glycosides, gums and mucilage.

Acute toxicity study

No toxic symptoms or death was observed in any of the animals with either extracts up to the dose of 3000 mg/kg, till end of the study.

Effect on urine volume

Table I-showed that, the cumulative urine volume was measured at 5th h of control (1.2 ± 0.025), furosemide (2.7 ± 0.57), aqueous 250 mg/kg (1.5665 ± 0.04), aqueous 500 mg/kg(1.8 ± 0.076), alcohol 250 mg/kg (1.715 ± 0.06), alcohol 500 mg/kg (1.815 ± 0.1165). The results were significant at $p < 0.001$ with Furosemide and alcohol extract 500 mg/ kg; $p < 0.01$ for aqueous extract 500 mg/ kg and alcohol extract 250 mg/kg, $p < 0.05$ for aqueous extract 250 mg/ kg, when analyzed by Tukey - Kramer multiple comparison test. The urine volume of furosemide, aqueous extract and alcohol extract treated groups increased 2.16, 1.253, 1.44, 1.372 and 1.452 fold respectively when compared with the control group.

Effects on electrolyte excretion

Table II- shows the diuretic responses with its electrolyte excretion potency of the AEHR and EEHR are highly significant in comparison with control animals. There was a significant increase ($p < 0.001$) in urine concentration of Na^+ , K^+ , Cl^- in the treated groups.

Effect on natriuretic, saliuretic and carbonic anhydrase inhibition

From the electrolyte excretion of Na^+ , K^+ and Cl^- of AEHR and EEHR at both dose levels (250 mg and 500 mg/kg), the natriuretic (Na^+/K^+), saliuretic activity(Na^+ and Cl^-) and carbonic anhydrase inhibition ($\text{Cl}^-/\text{Na}^+ + \text{K}^+$) were estimated and compared with control. No carbonic anhydrase inhibition was detected. The natriuretic ratio of AEHR and EEHR 250 and 500 mg/kg were found as 0.61, 0.6365, 0.624 and 0.6405 compared to control 0.5875 and is significant natriuretic. The significant saliuretic activity is also been showed by both AEHR and EEHR.

Table I. AEHR and EEHR on urine volume and diuretic at 5th h in rat.

Extract/ Drug	Dose	Urine volume(ml)	Diuretic action	Diuretic activity
Control	(5ml of 0.9% Nacl/ kg)	1.25±0.0285		
Furosemide	10 mg/kg	2.7±0.0575***	2.16	
Aqueous	250 mg/kg	1.566±0.04*	1.253	0.231
Aqueous	500 mg/kg	1.8±0.076 **	1.44	0.266
Alcohol	250mg/kg	1.715±0.06**	1.372	0.254
Alcohol	500 mg/kg	1.815±0.116***	1.452	0.268

Table II. AEHR and EEHR on electrolyte excretion, saliuretic and natriuretic activity at 5th h in rat.

Extract / Drug	Dose	Na ⁺ (mEq/l)	K ⁺ (mEq/l)	Cl ⁻ (mEq/l)	Na ⁺ /K ⁺ ratio	Na ⁺ +Cl ⁻	Cl ⁻ / Na ⁺ +K ⁺
Control	(5ml of 0.9% Nacl/kg)	7.083±0.464	6.025±0.3815	22.65±0.665	1.175± 1.216	29.73±1.12 5	1.72±0.78 6
Furosemide	10 mg/kg	36.41±0.316** *	26.58±0.893** *	94.65±1.33** *	1.369± 0.353	131.06±1.6 45	1.502±1.1 10
Aqueous	250 mg/kg	19.92±0.958* **	16.24±0.287** *	35.33±0.665* **	1.22± 3.33	55.25±1.62 3	0.977±0.5 3
Aqueous	500 mg/kg	28.58±0.965** *	22.44±0.118** *	44.66±1.76** *	1.273± 8.177	73±2.725	0.875±1.6 5
Alcohol	250mg/kg	31.15±1.307** *	24.925±2.374* **	44.66±0.665* **	1.249± 0.550	75.82±1.97	0.796±0.1 8
Alcohol	500 mg/kg	34.13±1.679** *	26.625±0.325* **	64±2.084***	1.281± 5.167	98.3±3.76	1.05±1.03

Values expressed as Mean ± S.E.M

sOne way ANOVA: p value found to be 0.0001 considered extremely significant (for parameters).Tukey-Kramer's multiple comparison test *p<0.05, **p<0.01, ***p<0.001; when compared with the control group.

Discussion

Acute toxicity studies help to evaluate the drug's acute toxic effect and to determine minimum lethal dose. It was found that both aqueous and alcohol extract of the *H. retusa* were safe up to a dose of 3000 mg/kg in Swiss albino mice.

Both AEHR and EEHR produced significant dose dependent increase in urine volume as well as urine concentration of Na^+ , K^+ and Cl^- . The increase in the ratio of excreted sodium to potassium ion for the extracts in treated groups, compared to control, indicates that the extract increases sodium ion excretion to a greater extent than potassium. This is a very essential quality for a good diuretic. K^+ excretion in aqueous extract 250 mg/ kg and 500 mg/ kg and alcohol extract 250 mg/ kg body weight treated groups was less when compared with that of standard. These findings suggest that extracts of *H. retusa* posses a potassium sparing property. The drug also posses significant saliuretic activity.

The presence of polyphenolic compounds, carbohydrates, proteins in *Portulaca oleracea*¹⁴ and steroids, tannin and carbohydrates in *Jussiaea suffruticosa* Linn.¹⁵ are reported to possess diuretic activity and natriuretic activity. Preliminary phytochemical analysis of the AEHR and EEHR revealed the presence of phenolic compounds and tannins, phytosterols, carbohydrates and glycosides, fixed oil and fats, gums and mucilage. These constituents in AEHR and EEHR may be responsible for the observed diuretic activity.

On the basis of results obtained, it is evident that both AEHR and EEHR possess effective hypernatraemic, hyperchloremic, and hyperkalaemic diuretic property, which provides the pharmacological evidence to support the folk claim for the root as an effective diuretic agent.

Conclusion

In conclusion, the present study the results demonstrate that AEHR sand EEHR possess significant effect on urinary excretion of water and electrolytes. It has shown that the active constituents in AEHR and EEHR had similar diuretic spectrum to that of furosemide. The results and this study substantiate the traditional use of this drug as one of the botanical sources of the drug *Pashanabhesha*.

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