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**A study on the Diuretic Effect of Ethanolic Extract of  
*Crataeva religiosa* in Albino rats**

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**Abstract**

A Study on the diuretic effect of ethanolic extract of *crataeva religiosa* in albino rats was performed to evaluate the diuretic effect of *Crataeva religiosa* (a native plant) in albino rats. 18 adult, male, wistar rats, fasted for 24 hours were divided into 3 groups. Group I received normal feed and water, Group II Hydrochlorothiazide (2.5mg/kg) and Group III - Ethanolic extract of *Crataeva religiosa* (EECR) 200mg/kg orally. Group I served as normal control, group II – standard, Group III as test group. Urinary volume, sodium and potassium concentrations were determined with a Perkino - Elmer model 303 atomic absorption spectrophotometer. Chloride concentrations were measured with a Buchlercotlovc chloridometer. Data were evaluated using student's 't'-test. Probability values less than 0.05 were considered significant. It was observed that the total volume of urine and urinary sodium, potassium, and chloride excretion were significantly increased in experimental groups (GII and GIII) compared to control (GI). The increased urinary volume in the HCT (GII) and EECR (GIII) are due to the diuretic effect of the drug and extract. The natriuretic and kaliuretic effects were observed in the animals belonged to GII and III. Hence it is likely that the natriuretic effect exerted by the plant extract must be similar to that of HCT. Since sodium excretion is always associated with water excretion, natriuretic effect might have contributed for diuretic effect. These observations indicate that the plant extract have got significant diuretic effect.

Key-Words: HCT, EECR, *Crataeva religiosa*

**Introduction**

Diuretics are substances that increase the rate of urine flow and sodium excretion and are used to adjust the volume and /or composition of body fluids<sup>(1)</sup>. They are considered as the ideal pharmacotherapeutic agents for the correction of excess sodium and water load.

Though kidneys serve multiple functions in our body, the most important function that is especially critical is, to control the volume and composition of the body fluids. The kidneys perform their functions by filtering the plasma and removing substances from the filtrate at variable rates, depending on the needs of the body<sup>(2)</sup>. It is important to recognize that the management of edema is to reverse the physiologic aberrations and to promote diuresis. Diuretics are widely used for the treatment of patients with edema. The objective of any diuretic is natriuresis.

Edema formation, represents the accumulation of excessive volume of fluid in the interstitial compartment and is invariably associated with renal Na<sup>+</sup> retention. The most common causes of edema are renal diseases (nephrotic syndrome, acute renal failure, chronic renal failure), congestive heart failure, cirrhosis and obstruction of venous and lymphatic drainage of a limb<sup>(3)</sup>.

The World health organisation feel herbal remedies are cheaper, locally available, time tested and liked by population at large in view of relatively less toxic and safety. Many herbal remedies are being used for urinary complaints<sup>(4)</sup>. Among them the bark of *Crataeva religiosa* was found to have diuretic effect which has been reported in literature. Review of the literature revealed that this rare medicinal plant remained unexplored for many of its claimed pharmacological activities like laxative, carminative, anthelmintic, digestive, expectorant, demulcent, antiemetic, anti-inflammatory and anti arthritic properties. So an attempt has been made to evaluate the diuretic effect of *Crataeva religiosa* in albino rats.

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### Material and Methods

This experimental study was carried out for a period of 6 months, in the central animal house, Institute of Pharmacology, Madurai Medical College (MMC), Madurai, after obtaining ethical clearance from the institutional animal ethical committee, MMC, Madurai.

#### Requirements

##### Animals

The urinary system of rat is similar to that of human being<sup>(5)</sup>. Healthy adult male wistar rats, 6-8 months old, weighing around 200 to 230gms (Mean wt – 214gms) were used. The animals were from inbred colony maintained in the central animal house, MMC. They were fed with commercially available standard pellet diet obtained from AMRUT FEEDS, Pranav agro Industries limited and water *ad libitum*.

##### Drugs and Chemicals

##### Preparation of extract of *Crataeva religiosa*

The barks of *Crataeva religiosa* (Fig.1) were collected and dried in shade for 10 days. It was then coarsely powdered. 200 gm of the powdered bark was soaked in sufficient quantity of 90% ethanol overnight. The contents were transferred to a soxhlet apparatus and extracted for about two hours using hot water bath. The process was repeated several times with fresh bark powder to get sufficient quantity of extract. The semisolid extract obtained was weighed accurately and utilized for experimental studies. The extract was suspended in 2% gum acacia in distilled water to yield the required concentration (50mg/0.5ml).

##### Gum acacia

This is the dried gummy exudate obtained from the stem and branches of *Acacia senegal* or other African species of acacia and is used here as a suspending agent for the oral administration of the standard drugs and test compound in 2% strength.

##### Hydrochlorothiazide (HCT)

Tablet Hydrochlorothiazide 12.5mg (cardicare (micro pharmaceuticals ltd) was powdered and mixed with 25ml of 2% gum acacia suspension. The suspension prepared, provided 0.5mg/ml and it was administered orally in the dose of 2.5mg/kg body weight<sup>(6)</sup>.

##### Appliances / Equipments

##### Metabolic cage

A metabolic cage is provided with a wire mesh bottom (Fig.II) and a funnel to collect the urine. Stainless-steel sieves are placed in the funnel to retain feces and allow urine to pass.

##### Oral feeding tube<sup>(7)</sup>

A 16 gauge hypodermic needle of 3 or 4 inch length serves as a useful stomach tube for the rat. The needle is blunted and a small ball of solder applied around the

distal end. A gentle 20 – 30° bend is made about 2 cm proximal to the solder. The tube thus constructed is attached to an 2 ml syringe.

##### Preliminary Screening

Albino rats of either sex weighing between 200 – 230 grams were selected and grouped into two each consisting of 6 animals. Group I, which served as the control, received 0.5ml/100gms of 2% gum acacia suspension orally. Group II, animals were given ethanolic extract of *Crataeva religiosa* in the dose of 200mg/kg body weight. There was no appreciable change noted in the behavioral profile, and autonomous profile.

##### Acute toxicity studies

Pairs of albino rats weighing between 200 – 230 gms were selected. Six such animal pairs were subjected to the toxicity studies. 2% gum acacia suspension containing *Crataeva religiosa* at various strengths of 50mg, 100mg, 200mg, 400mg, 600mg, 800mg per ml were prepared and 0.5ml/100 grams were given to all the rat pairs. The animals were observed for about 24 hours. Observations were made with special reference to the behavioral profile (awareness, mood, motor activity), neurological profile (central excitation, motor incoordination, muscle tone, reflexes) and autonomic profile. Then the animals were observed for mortality at the end of 24 hours.

##### Methodology

18 adult male wistar rats fasted for 24 hours were divided into Group I which received normal feed and water, Group II - Hydrochlorothiazide, (2.5mg/kg) and Group III EECR 200mg/kg orally (Table I). Diuretic activity was assessed after 24 hours. Urinary sodium and potassium concentrations were determined with a Perkin - Elmer model 303 atomic absorption spectrophotometer. Chloride concentrations were measured with a Buchlercotlovic chloridometer.

##### Results and Discussion

No significant change in urinary specific gravity and albumin were noted in GI, GII and GIII after 24 hours. The urine volume (Fig. III), sodium, potassium, chloride and pH were given in (Table II). The results were expressed as mean ± SD. Data were evaluated using student's 't'-test. Probability values less than 0.05 were considered significant.

##### Conclusion

Diuretics are considered as the ideal pharmacotherapeutic agents for the correction of excess sodium and water load. The observations emanated in the present study indicated that the total volume of urine and urinary sodium, potassium, and chloride excretion were significantly increased in experimental groups (GII and GIII) compared to

control (GI). The increased urinary volume in the HCT (GII) and EECR (GIII) are due to the diuretic effect of the drug and extract. There was no alteration in the urinary specific gravity and albumin content in GII and GIII. Statistical analysis also revealed that GIII had a significant diuretic effect almost comparable to that of GII. Physiologically, albuminuria indicate glomerular pathology or injuries. Urine specific gravity depends upon tubular system of the nephron during physiological situations. Since the animals received EECR had significant diuresis, similar to those received HCT, it is likely that the extract might have had diuretic effect. As albuminuria and alteration in specific gravity were not noticed in animals belonged to group II and III, it is likely that the plant extract is safe, similar to HCT. However further evaluation is required to understand the molecular mechanism, and site of action of this diuretic effect. The natriuretic and kaliuretic effects were observed in the animals belonged to GII and III. Hence it is likely that the natriuretic effect exerted by the plant extract must be similar to that of HCT. Since sodium excretion is always associated with water excretion, natriuretic effect might have contributed for diuretic effect. The possible explanation for significant excretion of urinary chloride among GII and III could be attributable to sodium and potassium excretion. The excretion of  $\text{Cl}^-$  molecules usually gets adjusted with entry of  $\text{HCO}_3^-$ . Interestingly, the urinary pH had also increased from  $7.1 \pm 0.4$  to  $8.4 \pm 0.6$  in GII animals and from  $7.2 \pm 0.3$  to  $8.2 \pm 0.5$  in GIII animals respectively, when analysis were made 24hours after oral administration of drugs and plant extract to the respective group. As the difference in the two samples were significant one has to consider the following explanation, that is as the bicarbonate excretion was relatively more in GII than GIII, is it due to bicarbonate reclamation in the renal tubular cells or reabsorption of bicarbonate from urine at tubular level.

The gist of the finding derived from the investigations under this project is furnished in the following concluding lines.

- Ethanolic extract of *Crataeva religiosa* has diuretic effect similar to HCT.
- It has natriuretic and kaliuretic effects.
- It also enhanced chloride excretion.

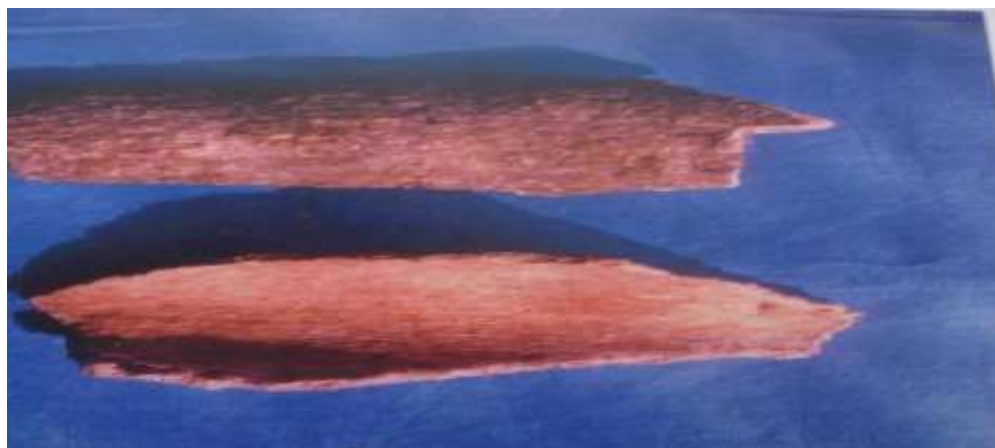
Further molecular studies are required to confirm or refute this observation.

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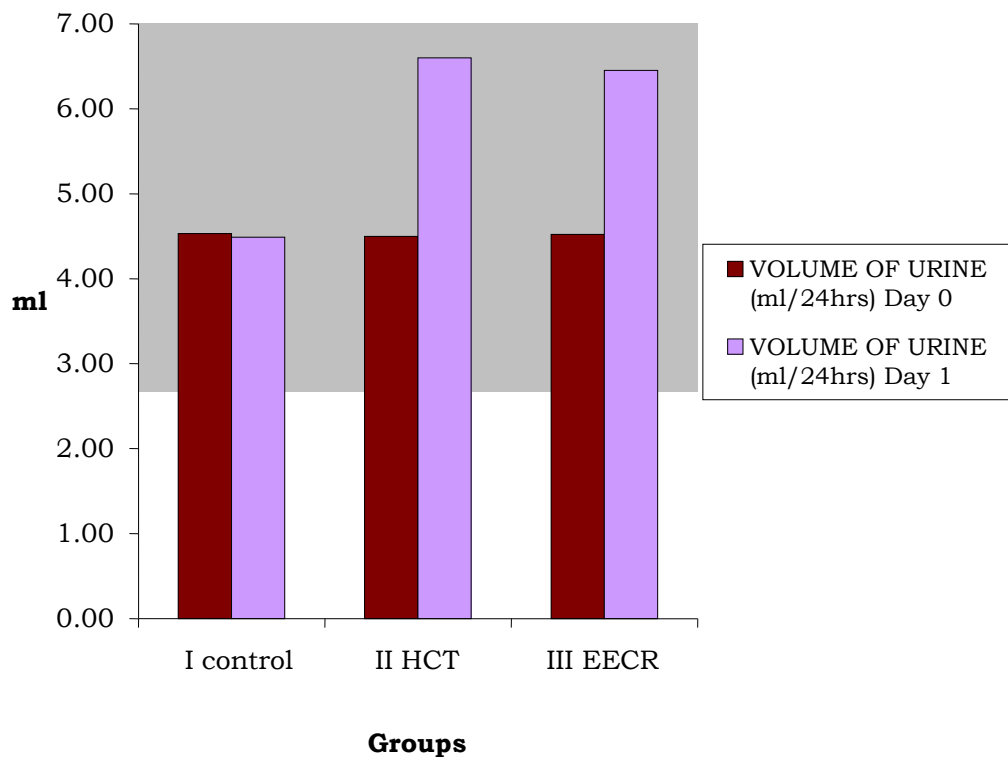


*Fig. : I - Crataeva religiosa – Stem Bark*



**Fig. II - Wistar rats kept in metabolic cage**

**Figure III**  
**VOLUME OF URINE (ml/24hrs)**



**Table I: Feed Administered to animals in different groups**

Group	Category	Diuretic effect
		Materials administration (24 Hours)
I a (No.6)	Control 2% Gum acacia	Normal feed and Water
II (No.6)	Standard	Normal feed, water and HCT (2.5mg/kg)
III (No.6)	Test	Normal feed, water and EECR (200mg/kg).



Table II: Diuretic Activity

GROUP	DAYS	VOLUME OF URINE (ml/24hrs)	pH	SPECIFIC GRAVITY	ALBUMIN mg/dl	SODIUM meq/l	POTASSIUM M meq/l	CHLORIDE meq/l
I (Control)	0	4.53 ± 0.24	7.1 ± 0.4	1.010 ± 0.05	20 ± 0.2	70.6 ± 0.82	58.5 ± 1.67	91 ± 0.89
	1	4.49 ± 0.27	7.3 ± 0.2	1.010 ± 0.02	20 ± 0.1	71.8 ± 0.54	56.5 ± 1.68	91.5 ± 0.78
II HCT 2.5mg/kg	0	4.50 ± 0.36	7.1 ± 0.4	1.010 ± 0.05	20 ± 0.1	69.6 ± 1.58	56.5 ± 1.84	91.2 ± 0.76
	1	6.60 ± 0.24 ***	8.4 ± 0.6 *	1.012 ± 0.03	21 ± 0.3 <sup>NS</sup>	104.5 ± 1.64 ***	78.8 ± 2.57 ***	106.7 ± 2.42* **
III EECR 200mg/kg	0	4.52 ± 0.33	7.2 ± 0.3	1.010 ± 0.03	20 ± 0.2	69.7 ± 2.06	58.2 ± 2.10	91.2 ± 0.76
	1	6.45 ± 0.26 ***	8.2 ± 0.5 *	1.010 ± 0.05	20 ± 0.03 <sup>NS</sup>	93.7 ± 2.46 ***	69.7 ± 1.97 ***	96.7 ± 1.02 ***

Values are expressed as Mean ± SD for six animals in each group.

Values are significantly different compared to control when \*P < 0.05, \*\* P < 0.01 \*\*\* P < 0.001

NS – Not significant

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