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Antilithiatic effect of Ethanolic extract of *Crataeva religiosa* on Wistar rats

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Abstract

To evaluate the anti-lithiatic effect of *Crataeva religiosa* (a native plant) in wistar rats. 4 adult, male, wistar rats, fasted for 24 hours were divided into3 groups. Group I (Ia+Ib) received normal feed and water, Group II Hydrochlorothiazide (2.5mg/kg) and Group III received Ethanolic extract of *Crataeva religiosa* (EECR) 200mg/kg orally. Antilithiatic activity was studied by standard means, with addition of 0.75% ethylene glycol in drinking water daily for 28days in group Ib, II & III. Group Ia served as normal control, Group Ib served as lithiatic control. 24hours urinary calcium, magnesium, oxalate, phosphate, uric acid and protein were analysed on day 0, 14 and 28. Data were evaluated using student's 't'-test. Probability values less than 0.05 were considered significant. The animals of GII and III had significant reduction in urinary phosphate and urate, compared to animals exposed to ethylene glycol alone (G Ib). It is likely that HCT and EECR might have protected these animals, from the effects of ethylene glycol. Excessive urinary phosphate and urate excretion are the contributory factor for calcium phosphate and / or urate stones, as calcium excretion was also statistically higher in animal receiving ethylene glycol alone. These observations indicate that the plant extract have got significant antilithiatic effect.

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

Introduction

Renal calculi (Nephrolithiasis) is a common disorder estimated to occur in approximately 12% of the populations of the United States[(1)] and 12% in industrialized countries[(2)]. Nephrolithiasis is known for time immemorial. Urinary chemistry is one of the important factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Hence, the study of the urinary chemistry with respect to the stone forming minerals will provide a good indication of the risk of stone formation. Renal stone disease has a significant recurrence rate, with a 50% risk of recurrence at 5 years and a 70% risk at 10 years, when no medical treatment is rendered[(3)].

The goal of urological management is to remove all stone burden with minimally invasive therapy. It is extremely important to avoid residue lest these form nidus for subsequent growth[(4)]. Medical management consists of diet and drugs designed to reduce the risk factors of stone production. Many herbal remedies are being used for urinary complaints such as kidney and bladder stones.

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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.

Among them the bark of Crataeva *religiosa* was found to have lithotriptic effect which has been reported in literature. So an attempt has been made to assess the antilithiatic effect of *Crataeva religiosa* in wistar rats.

Material and Methods

This randomized, experimental, animal study was carried out in the central animal house, Institute of Pharmacology, Madurai Medical College (MMC), Madurai, after obtaining ethical clearance from the instituitional animal ethical committee, MMC, Madurai.

Requirements

Animals*:* Six to eight months old, adult, healthy, male wistar rats, weighing around 200 to 230gms (Mean wt – 214gms), from inbred colonies maintained in the central animal house, MMC, Madurai, were used for this study. 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* 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.

Ethylene glycol (EG)

It was planned to induce lithiasis by creating ionic disturbances by feeding rats with 0.75% ethylene glycolated water for 28 days. Ethylene glycol administration led to oxalate stone formation, as indicated by its high level in urine. Complementary to this anion, the cation calcium level in urine was elevated. These two ions may have contributed to the formation of calcium oxalate stones [5,6,7].

Preliminary Screening

Wistar rats of either sex weighing between 200 – 230 grams were selected and grouped into two each consisting of 6 animals. All the animals in Group I, which served as the control, received 0.5ml/100gms of 2% gum acacia suspension orally. The animals in Group II 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.

Methodology

24 adult, male, wistar rats, fasted for 24hours were divided into3 groups. Group I (Ia+Ib) received normal feed and water, Group II Hydrochlorothiazide (2.5mg/kg) and Group III , EECR 200mg/kg orally , as shown in Table 1.

Antilithiatic activity was studied by standard means in the same group with addition of 0.75% ethylene glycol in drinking water daily for 28days in group Ib, II & III. Group Ia served as normal control, Group Ib served as lithiatic control. 24hours urinary calcium, magnesium, oxalate, phosphate, uric acid and protein were analysed on day 0, 14 and 28. These animals were followed up meticulously during the study period and up to a period of 4 weeks after the project work.

Calcium and inorganic phosphate were estimated in an autoanalyzer. Oxalate was determined by the method of Hodgkinson and Williams. Proteins were estimated colorimetrically at 650nm. Magnesium was analysed by using ERBA magnesium Arsenazo Method, End point – TRANSASIA and uric acid were measured by using "COBAS MIRA" plus autoanalyser.

Statistical analysis

The results were expressed as mean ± SD. Data were evaluated using student's 't'-test. Probability values less than 0.05 were considered significant.

Results and Discussion

Every day the animals were assessed after the administration of drugs. On close follow up of the animals which received HCT (or) EECR (or) placebo, none of them showed any behavioural abnormalities (or) weight loss. Similarly animals belonging to G II and III did not show any evidence of bowel disturbances or change in eating habits / drinking water. These indicated that the extract did not have any systemic toxicity. None of the rats considered for the present study expired, either during study period or during post study follow up of another 28 days. The guidelines provided by ethical committee for the animals were adhered strictly. The results are given in Table 2. From the results of this study, it is obvious that the plant *Crataeva religiosa* has got either inhibiting or dissolving effect of calcium oxalate crystal formation. The elevation of urinary calcium and oxalate in GIb indicate that these two ions contributed significantly to ethylene glycol induced stone formation. It has been reported that 75-80% of the renal stones analyzed contain calcium oxalate and phosphate[8]. It is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria. An increased urinary calcium concentration is a factor favoring nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth. The extract of *Crataeva religiosa* reduced urinary calcium and oxalate in GIII, suggesting the antilithiatic activity comparable to that of HCT.

The oxalate excretion was relatively more (statistically significant $P<0.01$) in GIII when compared with GII, thereby indicating that the plant extract enhances oxaluria better than HCT. Whether enhanced oxaluria in GIII is related to oxalate content of the plant extract or true ability of the nephrons for the oxalate excretion. This needs further study.

Thiazides have been used already for absorptive hypercalciuric oxaluria to prevent recurrent stone formation[9]. As the effect of plant extract theoretically was similar to thiazide with reference to oxalate and calcium excretion, it is believed that the antilithiatic action of plant extract on renal tubules will be almost similar to HCT.

Normal urine contains many inorganic and organic inhibitor of crystallization, magnesium is one such well-known inhibitor. The fall in urinary magnesium in G Ib suggests that stone formation was induced in that group. EECR restored magnesium ion level, suggesting that it inhibited stone formation comparable to that of HCT.

A gradual increase in urinary phosphate and uric acid excretion is observed in G Ib animals. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition. As the animals of GII and III had significant reduction in urinary phosphate and urate compared to animals exposed to ethylene glycol alone (G Ib), it is likely that HCT and EECR might have protected them from the effects of ethylene glycol. Excessive urinary phosphate and urate excretion are the contributary factor for calcium phosphate and / or urate stones, as calcium excretion was also statistically higher in animal receiving ethylene glycol alone. These observations indicate that the plant extract might have acted via it's antilithiatic effect.

The predominance of uricacid crystals in calcium oxalate stones and the observation that uricacid binding proteins are capable of binding to calcium oxalate and modulates its crystallization also suggests its primary role in stone formation. EECR (G III) treatment restores phosphate and uric acid levels to normal thus reducing the risk of stone formation, comparable to that of HCT.

Increased excretion of proteins in G Ib has been observed. Super saturation of urinary colloids results in precipitation as a crystal initiation particle, which when trapped, acts as a nidus leading to subsequent crystal growth. Administration of EECR (G III) has profound effects on reducing the protein excretion and thus preventing the nidus formation for crystal nucleation, comparable to that of HCT. The extract of *Crataeva religiosa* decreased the excretion of calcium oxalate, uric acid, phosphate and protein which was comparable to that of HCT.

Conclusion

To conclude, the antilithiatic effect was considered based on reduced excretion of oxalate, calcium, as well as uric acid and phosphate in a significant manner.The antilithiatic effect of EECR might have been carried out by inhibitory effect of, as reflected in the form of decreased magnesium excretion. Proteinuria was significantly more in GIb animals, a contributary factor for stone formation, which was inhibited by HCT and EECR.

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Table 1: Feed administered to animals in different groups

Group	No. of animals	Category	Materials administered (28 Days)
I a	6	Control 2% Gum acacia	Normal feed & water
I b	6	Lithiatic Control 2% Gum acacia	Normal feed & water with 0.75% ethylene glycol
II	6	Standard	Normal feed & water with 0.75% ethylene glycol and HCT (2.5mg/kg)
III	6	Test	Normal feed & water, with 0.75% ethylene glycol and EECR(200mg/kg).

Table I1: Effect of *Crataeva religiosa* on oxalate, calcium, magnesium, phosphate, uricacid and protein excretion in [experimental hyperoxaluria] 0.75% ethylene glycol induced albino rats

Oxalate

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	0.36 ± 0.03	0.36 ± 0.03	0.37 ± 0.04	0.36 ± 0.05
14	0.36 ± 0.03	3.31 ± 0.15***	2.22 ± 0.19***	2.52 ± 0.19**
28	0.33 ± 0.14	4.23 ± 0.01***	1.61 ± 0.09***	1.78 ± 0.07***

CALCIUM

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	0.71 ± 0.01	0.72 ± 0.02	0.75 ± 0.03	0.72 ± 0.02
14	0.73 ± 0.03	1.63 ± 0.02***	1.58 ± 0.13^{NS}	1.61 ± 0.05 ^{NS}
28	0.76 ± 0.03	2.02 ± 0.05***	1.31 ± 0.18***	1.39 ± 0.08***

MAGNESIUM

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	0.91 ± 0.03	0.92 ± 0.01	0.91 ± 0.03	0.92 ± 0.02
14	0.88 ± 0.01	0.69 ± 0.03**	0.85 ± 0.02***	0.84 ± 0.02***
28	0.88 ± 0.02	0.62 ± 0.03***	0.80 ± 0.02***	0.82 ± 0.03***

PHOSPHATE

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	6.67 ± 0.06	6.58 ± 0.07	6.58 ± 0.07	6.60 ± 0.08
14	6.68 ± 0.05	9.13 ± 0.05***	7.05 ± 0.05***	6.97 ± 0.06***
28	6.65 ± 0.06	12.5 ± 0.17***	7.14 ± 0.13***	7.02 ± 0.06***

URICACID

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	1.12 ± 0.06	1.10 ± 0.04	1.07 ± 0.03	1.11 ± 0.03
14	1.13 ± 0.03	2.01 ± 0.09***	1.75 ± 0.05**	1.89 ± 0.02*

28	1.14 ± 0.04	3.08 ± 0.18***	1.48 ± 0.06***	1.51 ± 0.03***

PROTEIN

DAYS	GROUP I a	GROUP I b	GROUP II	GROUP III
0	3.04 ± 0.14	2.89 ± 0.15	3.10 ± 0.14	3.13 ± 0.16
14	3.05 ± 0.15	12.41 ± 1.40***	3.90 ± 0.14***	3.27 ±0.13***
28	3.03 ± 0.15	20.19 ± 1.38***	3.49 ± 0.19***	3.36 ± 0.13***

GIa-normal control, GIb-lithiatic control, GII-HCT, GIII-EECR
Values are expressed as mg/24 hours Urine Sample. 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. Groups compared Ib Vs Ia, Ib Vs II, Ib Vs III.

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