Original Article

ANTINEPHROLITHIATIC EFFECT OF CRATAEVA MAGNA LOUR. DC ROOT ON ETHYLENE GLYCOL INDUCED LITHIASIS

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Abstract

The extract of Crataeva magna was screened for antinephrolithiatic activity in male Wistar rats were summarized based on the ionic changes in both Urine & Serum. The present study was carried out to evaluate Antinephrolithiatic activity in rats using 0.75% ethylene glycol in drinking water was given orally for 28 days. The plant Crataeva magna is used in analgesic, anti protozoal, hypoglycemic, anti inflammatory, hypotensive, anti spasmodic purposes. The aqueous extract of leaves of Crataeva magna results maximum yield value than that of petroleum ether extract, chloroform extract and alcohol extract through successive maceration process. The aqueous extract of leaves of Crataeva magna showed maximum control of lithiasis in Wistar rats. Urinary risk factors of urolithiasis were monitored at the end of 7th, 14th, 21st, and 28th days. Urinary volume was increased in nephrolithiatic as well as drug-treated rats. Increased urinary excretion of Calcium, Oxalate, Uric acid, Phosphorus and Protein in nephrolithiatic rats was brought down significantly by the administration of Crataeva Magna. Decreased magnesium excretion in hyperoxaluric rats was normalized with respect to calcium oxalate and other crystallizing salts such as uric acid, which may induce epitaxial deposition of calcium oxalate. Simultaneous treatment with the extract reduced Calcium and Oxalate ion concentration in Urine confirming the stone inhibitory effect. In Ethylene glycol induced nephrolithiasis, the nephrolithiasis was significantly reduced and the stone formation was normalized by administration of 200 mg/kg and 400 mg/kg dose orally and the property was comparable to the standard drug. This study has established the antinephrolithiatic activity of Crataeva magna and thus, justifies the uses of this plant for lithiasis.

Key words: Nephrolithiasis, Ethylene glycol, Crataeva magna, rats.

Introduction

In modern medicine no satisfactory effective therapy is still available to dissolve or to prevent recurrence of urinary stones. Nephrolithiasis is worldwide in distribution and a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-80% on males and 47-60% females[1]. Nephrolithiasis in its different forms is frequently encountered during urological complications. Some common causes are inadequate urinary drainage, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities, viz. Vitamin A deficiencies, Vitamin D excess, metabolic diseases like hyperparathyroidism, cystinuria, gout and intestinal dysfunction[2]. Present day medical management of nephrolithiasis mainly involves the surgical removal of stones. Techniques such as ESWL PCNL do not assure the prevention of recurrence of the stone. They cause side effects such as hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney[3].

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The present study was undertaken to evaluate the pharmacological evaluation of the effect of the plant Crataeva magna on the healing of experimentally induced nephrolithiasis in rats.

Materials and Methods

The Root part of plant Crataeva magna was collected from young matured plant from Alagar Kovil, Madurai during the month of Nov-Dec and identified by the botanist of Department of Botany, American College, Madurai by comparing with the voucher specimen present in the herbarium. After authentification fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for further studies.

About 200gm of coarse dried powder of plant of the Crataeva magna was taken in the soxhlet apparatus and extracted successively using different solvents according to their increasing order of polarity, for the present investigation. (i.e. Pet. ether, Chloroform, Ethanol, Aqueous). The extraction for each solvent was carried out for 18 to 24 hours. The extract was collected by evaporating the solvents by slow heat treatment. Total 2 kg of pulverized whole plant was subjected under solvent extraction to produce the

required amount of test extract. Calculated amount of dried aqueous extract was suspended in 0.5% w/v of sodium-CMC in normal saline solution to get the test doses (200mg/kg per ml for both extract)[4].

Wistar male rats aged 16-20 weeks weighing 160-200g were obtained from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai University. The experimental protocol is approved by the Institutional Animal Ethics Committee, Annamalai University and animals were maintained under standard conditions for an acclimatization period of 15 days before performing the experiment. All rats were housed individually in metabolic cages and temperature maintained at 22±2°C.

Animals were divided into five groups. Each group consists of 6 animals. Group I animals served as a control and were maintained using commercial pelleted feed. Group II animals received 0.75% ethylene glycol in drinking water ad libitum for 28 days. Group III (Standard drug treated rats) were fed with 0.75% Ethylene glycol + Cystone (5 ml/kg) (Himalaya Lab., India) for 28 days. Group IV animals received 0.75% ethylene glycol in drinking water ad libitum along with aqueous extract of Crataeva magna 200mg/kg. Group V animals received 0.75% ethylene glycol in drinking water ad libitum along with alcoholic extract of Crataeva Magna 200mg/kg.

The crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. The urine samples of the test animals in different groups were collected in their respective end day of the experiment. The collected urine sample volume were measured followed by centrifugation at 3000 rpm for 10 minutes. After centrifugation the urine samples were examined under light microscope (LAICA, DME Germany 400X) to ensure the presence of oxalate microcrystal followed by biochemical analysis (urine oxalate, calcium and uric acid). The blood samples were collected from the animals under anaesthesia (ether) before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium.

Results were indicated in terms of Mean ± SEM. Statistical significance of data were determined by One way - ANOVA followed by comparison between different groups using 'Tukey Kramer' multiple comparison test. Differences between the data were considered significant at $P<0.05$.

Results and Discussion

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans[5] and also earlier studies have shown that the amount of stone deposition in female rats was significantly less[6]. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in nephrolithiasis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (0.75%) administration, young male albino rats form renal calculi composed mainly of calcium oxalate[7]. Urinary volume is markedly increased in experimental groups. The increased urine volume in all the drug-treated groups might be due to the diuretic effect of the drug. Similar results are also observed when lupeol is used as an antiurolithic agent[8,9].

The results of urine and serum biochemistry showed significant reduction in urine calcium, uric acid and oxalate level, serum calcium with significant elevation in urine volume output, the markers previously reported which affirmed potent antiurolithiatic activity[10]. We suggest that magnesium, which was excreted in large amounts in the urine of treated rats, binds oxalate in the gut, reducing its intestinal absorption[11]. The analysis of crystalluria showed that untreated rats excreted more calcium oxalate of both types, and as larger crystals, than excreted by treated rats. This may by important as large particles have a greater chance of being trapped within the renal tubules, whereas small particles can be flushed easily from the kidney. Crystalluria could occur similarly in both healthy and stone forming subjects[12], but the latter might tend to excrete large and aggregated particles[13]. Thus, the earlier results in vivo are in accord with those obtained in vitro[14]. Why treated rats excrete smaller particles might be related to the involvement of magnesium, as it was excreted more by the treated rats. Indeed, magnesium is considered a potent inhibitor of Calcium oxalate crystallization in vitro[15,16] and binds to oxalate to form a soluble complex, consequently reducing the concentration available for calcium oxalate precipitation[17]. Experiments in animals models showed that magnesium provided some protection against calcium oxalate deposition in kidneys[18], but clinical studies showed that magnesium had no beneficial effects in impeding the formation of calcium oxalate kidney stones[19,20]. Similar results were obtained in experimentally induced calcium oxalate nephrolithiasis in rats, confirming its minimal effect in lithogenesis[21]. The results of serum and urine biochemistry is indicated in Table-01 comprises, the urine and serum biochemistry data of Ethylene glycol (0.75%) induced nephrolithiasis model data. There was a significant increase in the urine output of Ethlene glycol treated rats ($P < 0.001$) in the 7th day of Ethylene glycol treatment. Treatment with Aqueous & Alcoholic extracts reduced the oxalate excretion significantly. A maximum oxalate excretion was observed with EG- treated rats on the 28th day. Calcium excretion was increased in Ethylene glycol treated rats on day 28. Administration of Crataeva magna decreased the calcium excretion significantly.

Conclusion

Crataeva magna root has a potent therapeutic effect on calcium oxlate stone formation, confirming the folklore about its antinephrolithiatic activity. Kidney stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. According to our results, root of Crataeva magna was

effective for prevention and treatment of Calcium Oxalate kidney stone in rats. A dose of 200mg/kg of the plant significantly decreased the number and size of Calcium oxalate deposits in different segments of the renal tubules. A higher dose of the plant leaves had also preventive and therapeutic effects on calcium oxalate kidney stone. Further studies to determine the same effects on human beings are recommended.

Table-01
Urine and serum parameters in control and experimental animals

Parameter	Group I control	Group II lithiasis induced	Group III Cystone	Group IV AqE	Group V AlcE
Body weight					
Initial	167 ± 6.46	175 ± 4.26	177 ± 4.47	181 ± 2.71	162 ± 2.47
Final	186 ± 7.02	183 ± 4.28	190 ± 4.23	190 ± 2.85	172 ± 2.30
Urine (mg/dl)					
Oxalate	0.35 ± 0.04	3.03 ± 0.24***	1.31± 0.21***	2.55 ± 0.11	2.34 ± 0.41
Calcium	1.15 ± 0.22	3.27 ± 0.31*	2.31± 0.31*	3.12 ± 0.14	2.43 ± 0.31
Uric acid	0.73 ± 0.06	1.2 ± 0.12	0.71± 0.04	1.88 ± 0.19	1.78 ± 0.19**
Serum (mg/dl)					
Creatinine	7.40 ± 0.42	8.22 ± 0.40	7.12 ± 0.21	7.92 ± 0.61	7.58 ± 0.31
Calcium	4.11 ± 0.31	2.31 ± 0.21***	2.11 ± 0.24**	1.81 ± 0.11	2.01± 0.15

Values are given in Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. For, n=6

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