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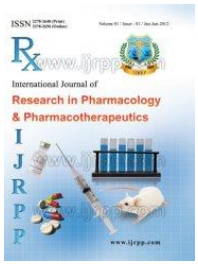

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ANTIUROLITHIATIC ACTIVITY OF ACTIVITY OF *TECOMA STANS* LEAF EXTRACT

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ABSTRACT

Herbal medicines offer vast scope for the successful treatment of urolithiasis. Although most remedies were herbal and proved useful, a systematic scientific evaluation has been reported for only few remedies. *Tecoma stans* a herbal formulation was claimed to be useful in the treatment of urinary stones. Toxicity study confirms that the therapeutic dose of *Tecoma stans* was 430 mg/kg. The antilithiatic effect of *Tecoma stans* was determined on lactose diet + ethylene glycol induced and ammonium chloride + ethylene glycol induced lithiasis in male albino wistar rats. The results obtained after 4 weeks of treatment for the urine estimation of calcium, oxalate, magnesium and protein were significant ($p<0.01$) in reducing the calculus when compared with the standard drug cystone (750 mg/kg) treated animals. Urine calcium oxalate crystals were also reduced in test drug treated and standard treated groups. These observations and results conclude that *Tecoma stans* herbal formulation possesses significant antiurolithiatic activity.

Keywords: *Tecoma stans,* Antiurolithiatic activity**,** herbal medicines.

Introduction

The formation of stone in the urinary tract system, like kidney, ureter, and urinary bladder or in the urethra is called urolithiasis. 'Urolithiasis' = ouron (urine) and lithos (stone). Urolithiasis is one of the major diseases of the urinary tract. [Soundararajan P] Urolithiasis in its different forms is frequently 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 generally stones of two types i.e., non calcium and calcium stones are formed. Calcium, albumin, creatinine, urate and oxalate are some necessary analytical markers in serum and urine for clinical diagnosis of this type of urological disorders [Grases F]. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease [Lopez M] Oxalate, the major stone-forming

constituent, is known to induce lipid per oxidation which causes disruption of the cellular membrane integrity [Coe FL].

Dietary factors that increase the risk of stone formation include low fluid intake and high dietary intake of animal protein, sodium, refined sugars, fructose and high fructose corn syrup [Knight], oxalate [Johri], foreign bodies in urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities like vitamin A deficiencies, The excess vitamin D, and metabolic diseases like hyperthyroidism, cystinuria, gout, intestinal dysfunction etc. [Suman Kumar] Calcium oxalate is considered as main constituent in the renal calculi.

In herbal medicine plant based formulations are used to alleviate the diseases. But the most important challenges faced by these formulations arise because of their lack of complete evaluation[4]. The present study is to investigate antiurolithiatic activity of *Tecoma stans* in animal models.

Materials and Methods

Collection and extraction

The leaves of *Tecoma stans* were collected from thiruchirapaly, Tamil Nadu, India. Leaves were dried in shade, powdered and 100 g of the dried powder was extracted with methanol by hot Soxhlet apparatus. The solvent was removed under reduced pressure and controlled temperature by using rotary flash evaporator to get the sticky mass of extract. The yield obtained was 9.6 % w/w.

Experimental animals

Male albino rats of wistar strain weighing between 150-200gm were used, the animals were fed with commercial rat feed pellets and were given water *ad libitum*. Animals were housed in plastic cages with filter tops under controlled conditions of 12:12 light dark cycle, 50% humidity and 28°C. All animal experiments and maintenance were carried out according to the ethical guidelines.

Toxicity study [5]

Toxicity study was performed to find out the toxic dose and therapeutic dose confirmation. The human therapeutic dose (5gm) was converted into animal therapeutic dose (430mg/kg) and acute toxicity test was performed for that particular dose. Subacute toxicity study was done for the sub minimal therapeutic dose (260mg/kg), therapeutic dose (430mg/kg) and the sub maximal therapeutic dose (460mg/kg).

Pharmacological screening

Lactose (30%) and Ethylene glycol (1%) induced urolithiasis [6]

Adult healthy male albino wistar rats were divided into 4 groups of 6 animals each. Group I served as the control received 1ml/kg of distilled water and rat chow diet *ad libitum* for 4 weeks, Group II received Ethylene glycol intoxicated rats with diet for inducing urolithiasis were fed with a lactose rich Lab diet (which contains 3.68% sucrose, 30% lactose, 23.4% protein, 10% fat, 5.3% crude fiber, 6.9% ash minerals – calcium (0.95%), phosphorus (0.67%), magnesium (0.21%), Vit A 22IU/g, Vit D3 4.5IU/g, Vit E 49 IU/g, with 1% ethylene glycol in drinking water for 4 weeks. Group III received lactose rich lab diet + 1% ethylene glycol + Cystone 750 mg/kg and Group IV received lactose rich lab diet + 1% ethylene glycol + *Tecoma stans* 430 mg/kg for weeks. 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 day of the experiment. The collected urine sample volume were measured followed by centrifugation at 2000 rpm for 20 minutes. After centrifugation of the urine samples were examined under light microscope to ensure the presence of oxalate micro crystals followed by biochemical analysis (urine oxalate, calcium and uric acid). The blood samples were collected from the animals under anesthesia before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium.

Ammonium chloride (2%) and Ethylene glycol (0.75%) induced Urolithiasis [7]

Male Wistar rats (180-200gm) are acclimatized to laboratory conditions for 1 week and then placed in groups of 4 groups 6 animals each. Ammonium chloride (2%) and (0.75%) ethylene glycol were mixed to the standard chow diet and given to animals for 4 weeks. Group I served as lithiatic control and

received vehicle 1%tween 80, Group II received standard antiurolithiatic drug, cystone (750mg/kg) from 15th day till 28th day [8, 9]. Group III received aqueous extract *Tecoma stans* (430mg/kg) from 15th day till 28th day and served as curative regimen. Group IV received aqueous extract *Tecoma stans* (430mg/kg) from 1st day till 28th day and served as preventive regimen. All drugs were given once daily by oral route using gastric tube. On day 28 animals of all the groups were kept in metabolic cages and urine samples were collected for 24h and analysed for calcium, magnesium, oxalate, inorganic phosphate, protein and creatinine using standard methods [10, 11,13]. The serum creatinine levels and urinary output volumes of all groups were also noted.

Statistical analysis

The results were expressed as Mean±SEM. Statistical analysis was performed by ANOVA test for multiple comparisons followed by Dunnett's test and $P<0.05$ was considered as significant.

Results and Discussion

The acute urolithiasis in both the conventional models was evidenced by the significant elevation in urine and serum biochemical parameters along with the reduced urine output as compared to the normal animals. The *Tecoma stans* (430mg/kg) employing lactose (30%) + ethylene glycol (1%) induced urolithiasis resulted in a significant reduction ($P<0.001$) in urine uric acid (0.86±0.08) and oxalate (2.58±0.69) level as compared to toxic group. Serum calcium (4.24±0.51) and urine calcium (3.42±0.57) level was significantly ($P<0.005$) lowered when compared with the toxic group along significant elevation in urine volume (14.3±2.21, $P<0.05$) output. Hence, the herbal formulation showed a reduction in serum creatinine level, employing both models but the reduction is not significant when compared with urolithiatic control group. The urine analysis of the animals showed that clear and no stone formation in control treated groups, lithiasis control animals showed more number of crystal formation in the urine. The formulation showed reduction in crystal formation when compared with that of the standard drug cystone treated animals. Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Administration of ammonium chloride (2%) and ethylene glycol (0.75%V/V) to the animals for 14 days forms renal calculi composed mainly of calcium oxalate. The urine output was markedly decreased in lithiatic control animals on 28th day, however in herbal formulation and standard drug treated animals the urinary volume was increased when compared to the lithiatic group. This suggested that formulation have mild diuretic effect. Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, curative and preventive groups these levels were significantly decreased ($P<0.01$). The formulation treated curative and preventive regimen groups, the urinary outputs increased significantly ($P<0.01$). The chronic administration of 0.75%V/V ethylene glycol to animals resulted in hyperoxaluria. And oxalate, calcium, phosphate and protein excretion were significantly ($P<0.01$) lowered, in curative and preventive regimen groups. In lithiatic control group the magnesium excretion was gradually following ethylene following ethylene glycol treatment. Administration of the formulation enhances the magnesium excretion significantly ($P<0.01$) in both curative and preventive regimens. The creatinine clearance of lithiatic control arts were decreased but it was improved significantly ($P<0.01$) in standard and test treated groups. The data on serum analysis showed significant increase ($P<0.01$) in creatinine levels in lithiatic control rats when compared to normal rats.

All these results indicates the inhibitory and curative potential of herbal formulation has both prophylactics as well as treatment property in urolithiasis of rats. The phytoconstituents of the active ingredients like alkaloids, phytosterols, mucilage and fixed oils in the plant may be responsible for antiurolithiatic action. Further more studies were required for the exact mechanism of antiurolithiatic action of the herbal formulation selected for the present study.

Table – 1 Urine and Serum biochemistry of 30% Lactose and 1% Ethylene glycol induced Urolithiasis 0n 28th day of experiment

Treatment	Urine volume (ml)	Urine calcium (mg/dl)	Urine oxalate (mg/dl)	Uric acid	Serum creatinine (mg/dl)	Serum calcium (mg/dl)
Group I Control	19.2±0.55	3.11±0.67	0.39±0.06	0.78±0.03	8.62±0.42	5.22±0.35
Group II Lithiasis control	10.65±0.38 ***	4.15±0.38*	4.9±0.87***	1.9±0.10	9.24±0.54	3.58±0.11***
Group III Cystone	15.8±0.47***	2.55±0.61*	1.98±0.57***	0.83±0.08	8.75±0.84	4.89±0.75**
Group IV S.S	14.3±2.21*	3.42±0.57*	2.58±0.69***	0.86±0.08***	8.57±0.94	4.24±0.51*

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

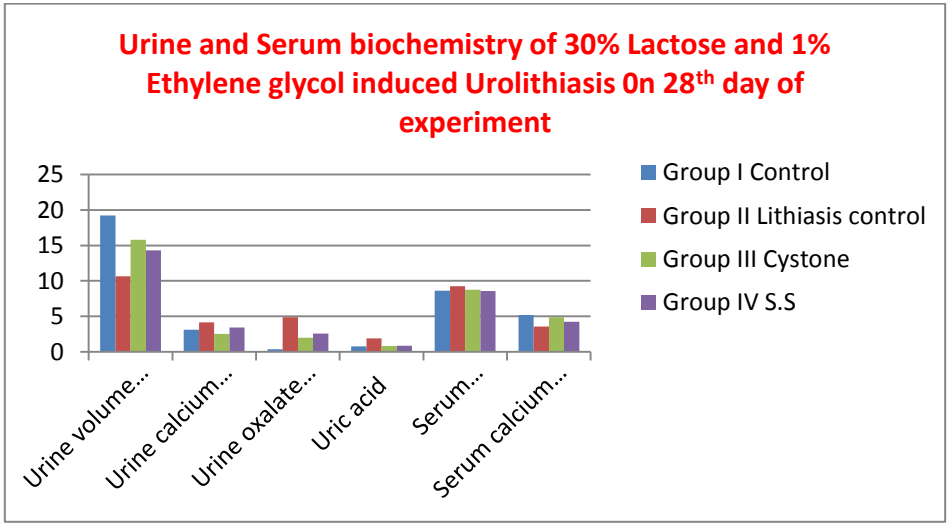
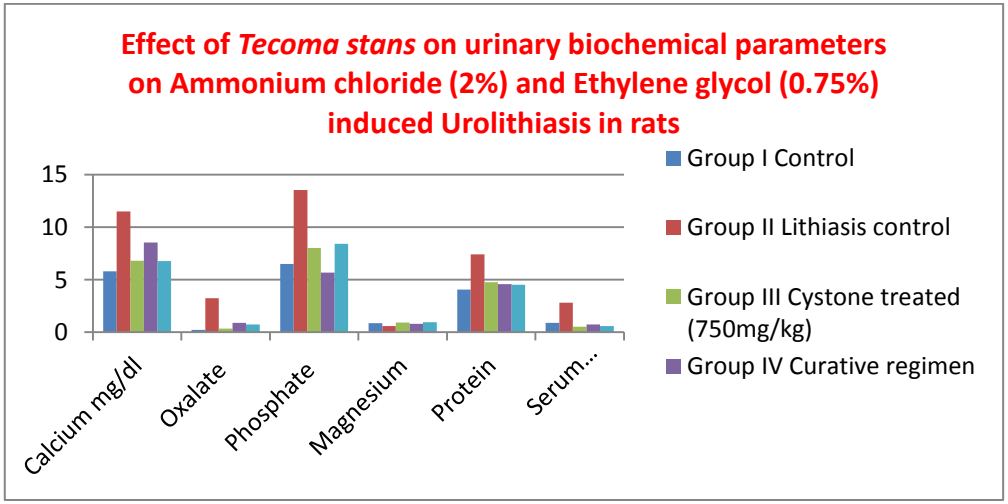


Table – 2 Effect of *Tecoma stans* on urinary biochemical parameters on Ammonium chloride (2%) and Ethylene glycol (0.75%) induced Urolithiasis in rats

Treatment	Calcium mg/dl	Oxalate	Phosphate	Magnesium	Protein	Serum creatinine mg/dl
Group I Control	5.8±0.64	0.22±0.08	6.5±0.47	0.84±0.10	4.06±0.25	0.88±0.09
Group II Lithiasis control	11.50±0.45*	3.22±0.45*	13.53±0.35*	0.57±0.08*	7.40±0.33*	2.80±0.3*
Group III Cystone treated (750mg/kg)	6.8±0.57**	0.34±0.09**	8.03±0.77**	0.9±0.08**	4.75±0.32**	0.53±0.28**
Group IV Curative regimen	8.54±0.27**	0.87±0.05*	5.67±0.47**	0.78±0.06**	4.57±0.07**	0.72±0.14**
Group V Preventive regimen	6.77±0.27**	0.74±0.03**	8.4±0.45**	0.94±0.06**	4.50±0.44**	0.57±0.03**

n=6, Values are expressed as mean±SD for in each group. One way ANOVA followed by Dunnett's test. *P<0.001, **P<0.01



Conclusion

All these results concluded to the inhibitory and curative potential of herbal formulation has both prophylactics as well as treatment property in urolithiasis of rats. The phytoconstituents of the active ingredients like alkaloids, phytosterols, mucilage and fixed oils in the plant may be responsible for antiurolithiatic action.

Further more studies were required for the exact mechanism of antiurolithiatic action of the herbal formulation selected for the present study. These observations and results conclude that *Tecoma stans* herbal formulation possesses significant antiurolithiatic activity.

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