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“Pharmacological Screening of Ethanolic Extract of Plant “*Cassia Tora*” Linn. Leaves on Nephrolithiasis Rats”.

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Abstract:

Cassia tora linn has been known traditionally in Indian system of medicine for the treatment of various metabolic disease and disorders. Since, the present study was carried out to know its applicability in urolithiasis condition. In the present study, extracts of *Cassia tora* linn was prepared with Ethanol and used for evaluation of antiurolithiatic activity. In this study we systematically evaluated its property by using Ethylene Glycol induced Albino rats. This study also involves state of the rat animal model to elucidate its probable mechanism of industrial guidelines of FDA, USA for pre-clinical evaluation of anti- Nephrolithiasis drugs.

Keyword: Antiurolithiatic activity, *Cassia tora* linn. Leaves, ethanol, ethylene glycol.

Introduction:

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors^[1].

A kidney stone is a hard, crystalline mineral material formed within the kidney or urinary tract. Kidney stones are a common cause of blood in the urine (hematuria) and often severe pain in the abdomen, flank, or groin. Kidney stones are sometimes called renal calculi. The condition of having kidney stones is termed nephrolithiasis.^[2] Having stones at any location in the urinary tract is referred to as urolithiasis, and the term ureterolithiasis is used to refer to stones located in the ureters. Urolithiasis, referred to the formation of a stone in urinary tract, is one among the diseases that afflicted humans since antiquity.

Despite tremendous advances accomplished in understanding the mechanisms governing the formation of such concretion, the disease remains an enigma since several factors intervene and interfere each other. As many forms of mineralization, urolithogenesis encompasses several physico-chemical steps which occur either sequentially or concurrently that start with supersaturation, then nucleation, growth, and aggregation.^[3] Of course, based on their size, aggregated particles can be attached and retained within urinary tubule epithelium constituting an additional step in calculogenesis. According to clinical and epidemiological studies, calcium oxalate (CaOx) followed by calcium phosphate are the most frequently encountered crystalline components found in analyzed stones. Most stones do not contain one single crystal phase but rather a mixture

of several different crystal phases. This leads to wonder which phase was initiated the crystallization process. The overall probability of forming stones differ in various parts of the world and is estimated at 1-5% in Asia, 5-9% in Europe, 13% in North America and the recurrence rate of renal stones about 75% in 20 years span. It occurs both in men and women but the risk is generally high in men and is becoming more common in young women^[1]. *Cassia tora* linn has been known traditionally in Indian system of medicine for the treatment of various metabolic disease and disorders. Since, the present study was carried out to know its applicability in urolithiasis condition.^[4]

Objectives of The Study:

The present study was carried out with an objective to find out the effect of Ethanolic extract of *Cassia tora* linn on Ethylene Glycol induced Albino rats.

MATERIAL AND METHODS:

Animals: Eight Male Wistar albino rats, weighing between 150 to 280g, were selected and allowed to acclimatize for a minimum ten days prior to the study. The rats were housed in room maintained of $21 \pm 1^{\circ}$ c, relative humidity 50-55% and 12hr light-dark cycle. The rats were caged with one animal in each polypropylene cage and were fed with standard food pellets and water ad libitum. throughout the study. All animal experiments

and maintenance were carried out according to the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the animal ethical committee (IAEC). After ten days the rats were divided into four groups, two rats in each group.

Chemicals and Drugs:

In the present study, the root of *Cassia tora* linn was collected in Warananagar. Soon after authentication, all leaves were dried at room temperature, until they were free from the moisture. Finally the root was subjected to get coarse powder and then passed through sieve no. 44 to get uniform powder. The sieve powder was stored in air tight, high-density polyethylene container before extraction. Ethylene glycol (Merck specialties private ltd, Mumbai.), ethanol (Merck specialties private ltd, Mumbai.).

Plant preparation:

The powdered root (500g) was subjected to hot continuous extraction (soxhlet) with ethanol. After the residue extraction, solvent was distilled off, and excess solvent was completely removed by using a rotary flash evaporator to get reddish-brown semi solid extract (yield: 28.17%). The obtained extracts were then evaluated for anti-urolithiasis activity.

Experimental Design:

The rats were divided into 4 groups of 2 rats in each group. They were grouped as:

The rats from all groups were administering the ethylene glycol except for rats from group I, which served as Normal.

1-Group I: Normal control will receive (saline) vehicle.

2-Group II: Animal served as control.

3-Group III: Animal was orally administered a standard (Spironolactone 2 mg/kg).

4-Group IV: Animal was maintained on normal diet, along with oral administered of ethanol extract at 100 mg/kg, respectively.

Evaluation of Antiurolithiatic Activity:

The extract treatment was started the days of the administration of ethylene glycol and continued for 30 days, but the body weights of all animals were recorded at the beginning and at weekly intervals throughout the experiment. Twenty-four hour urine samples were collected using separator by placing each group in a metabolic cage during the last week. Urine samples were acidified with 2ml of 1M HCL and centrifuged at 100g for 10 min at 4°C to remove contaminating Sediments aliquots were stored at 20°C until they were assayed. After 30 days treatment with ethanolic extract of *cassia tora* linn, experimental blood samples from all the Groups were withdrawn by retro arbitral, blood samples were allowed to clot at room temperature and the serum was separated by centrifugation at 1050g for 20 min. Serum samples were stored at 70°C until analysis. The left and right kidneys were dissected out.

The left and right kidneys were used for the measurement of dry and wet weight. The left and right kidneys were immediately fixed in 10% neutral buffered formalin for histopathological examination.

BIOCHEMICAL ESTIMATION:

1. Serum analysis: blood urea nitrogen, uric acid.

The test was carried out using diagnostic reagent kit (Span diagnostic Ltd. Surat, India) for the *In vitro* determination of Blood urea nitrogen, uric acid analyzed by using an auto analyzer.

2. Serum analysis: creatinine.

This method is based on the deproteinisation of samples with picric acid and the determination of creatinine in the supernatant by the addition of NaOH solution. The assay was performed exactly described in Arzneibuch (D. L.) DDR-'83. To 250 µl of sample, 1.5ml of picric acid were added, mixed and centrifuged for 10 min at 3000g. The supernatant was mixed with 50 µl of NaOH solution (final concentrations: 41 mmol/l picric acid, 69 mmol/l NaOH) and the absorbance was measured between the 20th and 30th min at 530 nm.

This method is based on the deproteinisation of samples with picric acid and the determination of creatinine in the supernatant by the addition of NaOH solution. The assay

was performed exactly described in Arzneibuch (D. L.) DDR-'83. To 250 μ l of sample, 1.5ml of picric acid were added, mixed and centrifuged for 10 min at 3000g. The supernatant was mixed with 50 μ l of NaOH solution (final concentrations: 41 mmol/l picric acid, 69 mmol/l NaOH) and the absorbance was measured between the 20th and 30th min at 530 nm.^[5,6]

3. Urine analysis: calcium oxalate crystals.

The test was carried out using diagnostic reagent kit (Span diagnostic Ltd. Surat, India) for the *In vitro* determination of calcium oxalate. The calcium present in the serum was precipitated with naphthyl hydroxamic acid (calcium reagent). The precipitated was then dissolved in EDTA reagent and calcium from this solution was complexed with color reagent to give a colored complex that was measured colorimetrically, and analyzed using an autoanalyzer.

4. Renal tissue samples analysis:

At the end of the experiment, on day 30, the rats were killed by cervical dislocation and kidneys excised, washed with normal saline and weighed. The kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was broiled in 10 ml of 1 N hydrochloric acid for 30 min.^[7-11]

Results:

Effect of *Cassia tora* linn extract on Nephrolithiasis rats body weight changes:

Table 1, show significant changes in body weight in Ethylene Glycol induced rats comparable to Normal group rats. It was also noticed that significant changes in body weight in animals treated with *Cassia tora* linnethanolic (100 mg/kg p.o) compared to Standard drug in nephrolithiasis rats.

1. Effect of Ethanolic Extract *Cassia tora* linnon body weight in Nephrolithiasis rats (0.2 ml/kg)

Table 1.

Groups	body weight (g)
Group-I	261.57
Group-II	250.00
Group-III	257.00
Group-IV	253.00

Effect of *Cassia tora* linnextract on body weight in nephrolithiasis rats was calculated from first day of study and every week regularly. The body weight of each animal was recorded one hour prior to administration of drug extract on recording day.

2. Effect of Ethanolic Extract *Cassia tora* linnon Urine sample in Nephrolithiasis rats (0.2 ml/kg).

Calcium and oxalate excretion were significantly increases in 24h urine of ethylene glycol induced nephrolithiasis rats when compared with normal rats. It was also decreases significantly when treated with ethanolic extract of *Cassia tora* linncompared with Group II and shows similar significance result with standard. As per the report of urine

analysis by girnar laboratory, Jaysingpur stated that as follows:

Table 2.

Groups	Crystals in urine	Significant
Group-I	Calcium oxalate crystals are not seen	--
Group-II	Calcium oxalate crystals are seen	**
Group-III	Calcium oxalate crystals are seen	*
Group-IV	Calcium oxalate crystals are seen	*

Effect of Cassia tora linn extract on nephrolithiasis rats was studied by urine biochemical analysis. Biochemical parameters were estimated by using Span diagnostic kits purchased from local market. Results obtained from serum biochemical analysis are expressed by presence of calcium oxalate crystals in test. Urine biochemical results were collected from each group and evaluated and considered as significant result.

3. Effect of Ethanolic Extract *Cassia tora* linnon Serum sample in Nephrolithiasis rats (0.2 ml/kg).

As per the report of serum sample analysis by girnar Laboratory, Jaysingpur stated that as follows:

Table 3.

S.No	Group -I	Group-II	Group-III	Group-IV
Blood	8.13±0	13.00±	10±	9.12±0.
Urea	.38	0.48	0.14	11
Nitrogen				
Uric acid	1.58±0 .06	4.30±0. 11	2.78±0 .02	1.98±01 3**
creatinine	0.70±0 .01	0.90±0. 03	0.82±0 .05	0.75±0. 03**

*Effect of Cassia tora linn extract on nephrolithiasis rats was studied by serum biochemical analysis. Biochemical parameters were estimated by using Span diagnostic kits purchased from local market. Result obtained from serum biochemical analysis are expressed as Mean ± SE and evaluated by student's' test. Serum biochemical results were collected from each group and statistically evaluated by Chi-square test for coming to conclusion. P value less than 0.05 was considered as significant. **p<0.01*

4. Effect of Ethanolic Extract of *Cassia tora* linn on Dry and wet Kidney weight in Nephrolithiasis rats (0.2 ml/kg).

Table 4

Animal Groups	Dry Kidney weight	wet Kidney weight
Group-I	0.094±0.0016	0.421±0.0054
Group-II	0.112±0.0020	0.925±0.0019*
Group-III	0.096±0.0022	0.902±0.0012
Group-IV	0.087±0.0018	0.735±0.009*

Values are mean ± SE evaluated by student's' test. P value less than 0.05 was considered as significant. *p<0.01

STATISTICAL ANALYSIS:

All the data collected in the present study are expressed as Mean ± SE and were analyzed by student t' test for coming to conclusion.

Images of kidney

Normal Control Test Standard



DISCUSSION:

The ethylene glycol-induced calcium oxalate urolithiasis model in rats has been characterized and extensively used in the study of the mechanism, therapy and prevention of stone formation. In the past, since increased calcium intake was considered to be an important risk factor for stone formation, calcium restriction was

recommended as an obvious intervention to prevent kidney stones in calcium stone-forming patients suffering from hypercalciuria. However, this practice has been questioned due to a large epidemiologic study which reported an increased risk for stone formation in subjects consuming a low calcium diet due to secondary hyperoxaluria, although these data had been largely indirect. Furthermore, several investigators have observed that calcium restriction has a deleterious effect on bone in hypercalciuric patients.

In healthy subjects, observed that an increased calcium intake can totally abolish the hyperoxaluria induced by an oxalate load. However, in an experimental model of genetic hypercalciuric rats, observed that, although urinary calcium proportionally increased with increasing dietary calcium content, a parallel decrease in urinary oxalate did not occur. By knowing the biochemical studies Primary stone formation and recurrence of stone formation is one of the biggest challenges faced by urologists today and remain a major source of morbidity in humans. Despite intensive studies in the last decade many aspects of nephrolithiasis / urolithiasis, the complete pathogenesis and thus prevention, still remains to be clarified. Studies have concentrated on the metabolic defects in urine and mechanisms of supersaturation, reasons for cytotoxic damage of renal tubular cells, and interference with some natural inhibitors.

It is believed that, like urinary metabolic defects, biochemical variations in blood are less likely to occur in first time stone formers than in patients with recurrent disease. Increased excretion of oxalate and deposition of calcium oxalate crystals in the renal tubules is associated with renal epithelial injury and that products of cellular damage can act as heterogeneous nucleators of both calcium oxalate and calcium phosphate crystals in animal model.

The body weights of rats at the beginning of the study were not significantly different between the four groups. However, at the end of the study, the rats treated with 0.75% Ethylene Glycol weighed less than those on the normal diet. The Group-III and Group IV treatment with Standard and Extract of *Cassia tora* linn significantly increases the level of body weight in urolithic rat. The present study were urinary chemistry with respect to stone forming minerals will provide a good indication of risk of stone formation. In the present study, observed hypercalciuria in ethylene glycol induced urolithic rat might be a factor favoring the nucleation and precipitation of calcium oxalate of apatite (calcium phosphate) from urine and subsequent crystal growth. The *Cassia tora* linn significantly reduced the level of calcium oxalate in urolithic rat. Uric acid is known to promote calcium oxalate crystal growth. Predominance of the uric acid crystals in calcium oxalate stones and the observation

that uric acid binding protein are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. In the present study, higher concentration of urinary uric acid was observed in ethylene glycol induced urolithiasis rats. *Cassia tora* linn treatment restored the uric acid level to normal thus reducing the risk of stone formation.

The serum uric acid and BUN were remarkably increased in ethylene Glycol induced animal while serum creatinine was only slightly elevated in group-II indicating marked renal damage. However ethanolic extract of *Cassia tora* linn treatment in curative and prophylactic regimen significantly lowers the elevated serum level of creatinine, uric acid and BUN in Group IV. There was a significant increase in the kidney weight of animals receiving 0.75% ethylene glycol which was almost normalized in the *Cassia tora* linn treated animals (Table 4). ethylene glycol feeding for 30 days resulted in renal tissue deposition of calcium and oxalate. The increased deposition of calcium and oxalate in the renal tissue is known to lead to papillary calcification and eventual calculi formation. *Cassia tora* linn administration significantly reduced both calcium and oxalate levels in kidneys, which is known to prove beneficial in preventing calculi formation due to supersaturation of these lithogenic substances. *Cassia tora* linn used traditionally in ayurveda, for various kind of

the disease and disorders and an anti-tumor effect. Aphrodisiac effect, anthelmintic, diarrhea, anti-fertility effect, on the impact of blood vessels. The earlier photochemical analysis is demonstrated the presence of the alkaloids, Quinones, Glycosides, Saponin, Fat and oils, triterpenes and Steroids. It has also been evaluated antioxidant, anti-inflammatory, antimicrobial, anti-fungal, antistress, anti-diarrheal, and antiulcer properties. Recently, studies have implicated the generation of oxygen derived free radicals and lipid peroxidation as one of the most important mechanisms involved in the pathogenesis of Nephrolithiasis. Antioxidants are known to inhibit lipid peroxidation and scavenge free radicals. By knowing the above pathophysiology of nephrolithiasis and the plant profile of *Cassia tora* linn is having a essential chemical constituents that reduce the formation of calculi in kidney as well as ureter and urinary bladder. The use of ethanolic extract of *Cassia tora* linn is also appeared to be safe for long-term usage, as there were no short term and long term toxicity reports. Therefore, it can be considered that use of *Cassia tora* linn extract will be beneficial, safe and effective in management of nephrolithiasis.

CONCLUSION:

On detail study, the alcohol extracts of *cassia tora* linn , was found to be effective then Spironolactone in reducing stone forming

constituents both in urine and renal tissues and also reduced, the calcium and oxalate out of this the alcohol extract of *cassia tora* linn , were found to be more effective and highly significant in the reduction of calculi, which can be used as anti urolithiatic agent. The result exhibited by ethanolic extracts of *Cassia tora* linn shown significant anti urolithiasis activity. Further studies will extensive investigation, isolation, purification of active phytoconstituentns with potent antiurolithiatic activity. The presented data indicate that administration of ethanolic extract of *Cassia tora* linn to rats with ethylene glycol induced lithiasis reduced and prevented the growth of urinary stones, supporting folk information about the anti-urolithiatic activity of plant. Based on these results, we conclude that the *Cassia tora* linn ethanolic extract is more potent in protecting the animals from nephrolithiasis. All of their effects observed in this study are similar to Spirinolactone treatment. Hence, this *Cassia tora* linn is considered as an anti-nephrolithiasis drug in treatment of nephrolithiasis.

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