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Pharmacological Evaluation of Antiurolithiatic Activity of Methanolic Extract of Cassia Fistula Leaves

Ankita[1]*, Divya Singh
1. Research Scholar, Jaipur College of Pharmacy, Jaipur, Rajasthan
2. Professor, Jaipur College of Pharmacy, Jaipur, Rajasthan

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ABSTRACT:
Objective: The present study aims to evaluate the anti-urolithiatic activity of the methanolic extract of Cassia fistula leaves using in vitro and in vivo models.
Methods: The in vitro anti-urolithiatic potential was assessed using calcium oxalate crystallization and nucleation assays. For in vivo evaluation, ethylene glycol-induced urolithiasis in Wistar rats was employed. Rats were divided into control, disease, standard, and treatment groups, receiving the methanolic extract at different doses. Biochemical parameters such as urinary calcium, oxalate, and creatinine levels were analyzed, along with histopathological examination of kidney tissues.
Results: The extract significantly inhibited calcium oxalate crystallization in vitro. In vivo studies demonstrated a marked reduction in urinary stone-forming constituents and renal damage, indicating a protective effect. Histopathological analysis revealed a decrease in crystal deposition and improved renal architecture in treated groups.
Conclusion: The methanolic extract of Cassia fistula leaves exhibits significant antiurolithiatic activity, likely due to its diuretic, antioxidant, and crystallization-inhibitory properties. These findings support its potential as a natural therapeutic agent for urolithiasis. Further studies are required to elucidate the active constituents and mechanisms involved.
Keywords: Cassia fistula, urolithiasis, calcium oxalate, nephroprotection, phytotherapy, kidney stones.

I. INTRODUCTION:

Urolithiasis, commonly known as kidney stone disease, is a prevalent urological disorder characterized by the formation of urinary calculi in the kidney, ureter, or bladder. The condition affects a significant portion of the global population, with an increasing incidence attributed to dietary habits, metabolic disorders, dehydration, and genetic predisposition. Kidney stones primarily consist of calcium oxalate, calcium phosphate, uric acid, struvite, and cystine. Among these, calcium oxalate stones are the most common and are often associated with hyperoxaluria and hypercalciuria. The pathophysiology of urolithiasis involves supersaturation of urinary constituents, nucleation, crystal aggregation, and retention in the renal system, leading to stone formation and associated complications such as renal colic, hematuria, urinary obstruction, and renal impairment.

Current treatment options for urolithiasis include pharmacological interventions, extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and surgical removal. However, these approaches have limitations, such as high recurrence rates, side effects, and complications. Pharmacological management typically involves the use of diuretics, citrate therapy, and alkali supplements to prevent stone formation and facilitate stone dissolution. Despite these advancements, the recurrence of kidney stones remains a significant challenge, necessitating the exploration of alternative and complementary therapies, particularly from natural sources.

Medicinal plants have been extensively investigated for their potential role in preventing and treating urolithiasis. Various phytoconstituents, including flavonoids, saponins, tannins, alkaloids, and polyphenols, have demonstrated diuretic, antioxidant, and anti-inflammatory properties that can mitigate stone formation. Cassia fistula, commonly known as the golden shower tree, is a well-known medicinal plant belonging to the Fabaceae family. It is widely distributed across tropical and subtropical regions and has been traditionally used in Ayurvedic and folk medicine for treating various ailments, including skin diseases, gastrointestinal disorders, diabetes, and urinary tract infections.

The therapeutic potential of Cassia fistula is attributed to its rich phytochemical composition, which includes flavonoids, phenolic compounds,

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tannins, glycosides, and alkaloids. Previous studies have reported that different extracts of Cassia fistula exhibit antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and nephroprotective activities. The presence of bioactive compounds such as anthraquinones and flavonoids suggests its possible role in inhibiting stone formation and providing renal protection.

The methanolic extract of Cassia fistula leaves has been selected for this study due to its high content of phytochemicals with potential antiurolithiatic properties. Methanol is known to extract a broad spectrum of bioactive compounds, thereby enhancing the therapeutic efficacy of the plant extract. The antiurolithiatic activity of Cassia fistula may be attributed to its ability to inhibit calcium oxalate crystallization, reduce oxidative stress, and enhance diuresis, thereby preventing the formation and growth of kidney stones.

Several in vitro and in vivo models have been employed to evaluate the anti-urolithiatic activity of medicinal plants. In vitro studies often include nucleation, aggregation, and crystallization assays to assess the inhibitory effects of plant extracts on calcium oxalate stone formation. In vivo models, such as ethylene glycol- and ammonium chloride-induced urolithiasis in rats, mimic the metabolic and physiological conditions associated with kidney stone formation in humans. These models allow for the evaluation of biochemical markers, urinary parameters, and histopathological changes in renal tissues following treatment with plant extracts.

The present study aims to evaluate the anti-urolithiatic activity of the methanolic extract of Cassia fistula leaves using in vitro and in vivo models. The objectives include assessing the inhibitory effects on calcium oxalate crystallization, analyzing biochemical markers of urolithiasis, and examining histopathological changes in renal tissues. This study seeks to provide scientific validation for the traditional use of Cassia fistula in the management of kidney stone disease and to explore its potential as a natural alternative to conventional urolithiasis treatments.

II. MATERIAL AND METHODS

2.1. Selection of plant part and Authentication:

Locally, fresh Cassia fistula leaves will be gathered from the Jaipur, Rajasthan, area market. The plant will be verified at Rajasthan University's Botany Department in Jaipur.

2.2. Extraction of Leaves:

The coarsely powdered leaves material will extract with Methanol by cold maceration technique.

2.3. Phytochemical investigation:

The extract will be analysed for a variety of chemical constituents, including alkaloids, glycosides, carbohydrates, phenols, saponins, flavonoids, gums and mucilages, proteins, sterols, and steroids.

2.4. Pharmacologic Activity

2.4.1 In vitro anti-urolithiatic activity by preparation of synthetic urine

Nucleation assay

This method was selected for the investigation of oxalate crystallization due to its simplicity and adequate reproducibility.The model examines crystallization both in the absence and presence of an inhibitor to evaluate the inhibitory efficacy of any employed chemical species.

Procedure: Solution of calcium chloride and sodium oxalate will prepare at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 mL of calcium chloride solution mix with 100 mL of herb extracts at different concentrations (25 mg/ml to 125 mg/ml). Crystallization will start by adding 950 mL of sodium oxalate solution. The temperature was maintained at 37 ºC. The OD of the solution was monitored at 620 nm. The rate of nucleation will estimate by comparing the induction time in the presence of the extract with that of control. Data represented in percentage inhibition (18, 19).

The growth of crystals is expected due to the following reaction:

$$CaCl_2 + Na_2C_2O_4 \longrightarrow CaC_2O_4 + 2NaCl$$

% Inhibition = [(Control- Sample) / Control] X 100

2.4.2 Sub-acute oral toxicity study and dose selection

In accordance with OECD norms, the extract will undergo acute toxicity testing. The six animal groups received varying amounts of the extract: 2000 mg/kg, 300 mg/kg, and 50 mg/kg. The animals will be monitored for a change in body weight and mortality as well as for the usual three days following the administration of the dose. If it is discovered that the extract is not significantly harmful up to a dosage of 2000 mg/kg. Up to 2000

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mg/kg, the extract will then be extremely acceptable.

Dose selection The 1/5th and ¼th of the maximum tolerable dose 2000mg/kg will be selected for the studies.

2.4.3 Ethylene Glycol induced urolithiasis

Ethylene glycol induced hyperoxaluria model (20) will be used to assess the antilithiatic activity in albino rats. Ethylene glycol (0.75%) in drinking water will be fed to different groups for induction of renal calculi till 28th day (20).

2.4.4. Ethylene glycol + ammonium chloride induced urolithiasis

Wistar rats (weighing 180–220 g) will be divided with matched bodyweights into various groups of 6 animals each, which will be then randomly selected to receive various treatments.

After being split into groups of six animals each with matched bodyweights, wistar rats (weighing 180–220 g) will be chosen at random to receive different treatments.

Group I rats, which served as the vehicle treated control, were given the test material once every 24 hours, while group II rats, which were left untreated, received a stone-inducing treatment consisting of 0.75% (w/v) ethylene glycol and 1% (w/v) ammonium chloride for 21 days. After five days, the water supply was changed to 0.75% EG alone in water, in addition to vehicle treatment. In addition to receiving a stone-inducing therapy akin to the untreated group, the other treatment group will receive a test sample or a conventional antiurolthic medication dissolved or suspended in a vehicle once per 24 hours.

2.4.5. Serum analysis

After 28th day of experimental period, blood will collect through cardiac puncture/ retro orbital puncture from animals under anesthetic condition. Serum will separate by centrifugation at 10,000 rpm for 10 min. and analyse creatinine, BUN, urea, uric acid, ALP, SGPT, SGOT and bilirubin.

III. RESULT & DISCUSSION:

Table No:1 Solubility determination of extract

S. No.	Solvent	Solubility of methanolic extract
1.	Water	Soluble
2.	Acetone	Insoluble
3.	Chloroform	Partial soluble
4.	Methanol	Soluble
5.	Petroleum ether	Partial soluble
6.	Ethylacetate	Partial soluble
7.	DMSO	Soluble

3.1. Phytochemical testing

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test
1.	**Alkaloids**	
1.1	Mayer's reagent test	Absent
1.2	Wagner's reagent test	Absent
1.3	Hager's reagent test	Absent
2.	**Carbohydrates**	
2.1	Molish's test	Present
2.2	Fehling's test	Absent
2.3	Benedict's test	Absent
2.4	Barfoed's test	Absent
3	**Proteins and Amino Acids**	
3.1	Biuret test	Absent
4.	**Flavonoids**	
4.1	Alkaline reagent test	Present

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4.2	Lead Acetate test	Present
5.	**Glycoside**	
5.1	Borntrager test	Absent
5.2	Legal's test	Absent
5.3	Killer-Killiani test	Absent
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present
6.2	Lead Acetate test	Present
6.3	Gelatin test	Absent
7.	**Saponin**	
7.1	Foam test	Absent
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Present
8.2	Libbermann-Burchard's test	Present

3.2. In vitroAntiurolithiatic activity

Table 3: Inhibition of COM crystals after addition of 1 ml of extract of Cassia fistula leaves

S. No.	Concentration (mg/mL)	% Inhibition	
		After 5 minutes	After 40 minutes
1	25	8	45.76
2	50	10.47	51.34
3	75	13.54	57.76
4	100	19.88	59.87
5	125	21.25	65.05

3.3. Acute oral toxicity

The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were ussed for toxicity studies, i.e 5mg/Kg, 50 mg/Kg, 300 mg/Kg, 2000 mg/Kg. animals were observed individually for next 4 hours after dosing for the presence of mortality during this period and 72 hours after sample administration.

Table 4: Acute oral toxicity of extract

S. No.	Dose	Lethality	Mortality
1.	5 mg/Kg	0/3	Not observed
2.	5 mg/Kg	0/3	Not observed
3.	50 mg/Kg	0/3	Not observed
4.	50 mg/Kg	0/3	Not observed
5.	300 mg/Kg	0/3	Not observed
6.	300 mg/Kg	0/3	Not observed
7.	2000 mg/Kg	0/3	Not observed
8.	2000 mg/Kg	0/3	Not observed

*0/3- zero animal dead out of three animals

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Ethylene glycol induced urolithiasis

2.5. Size and Weight of Kidney

Table 5: Size and Weight of both kidneys

S. No.	Group	Dose (mg/Kg)	Right Kidney		Left Kidney	
			Size (mm)	Weight (gm)	Size (mm)	Weight (gm)
1	Control (Group I)	Normal Saline (10 ml/Kg)	13.50±1.252	0.56±0.118	13.94±1.410	0.57±0.152
2	Standard (Group II)	750	12.81±1.186^{NS}	0.46±0.056^{NS}	12.98±1.014^{NS}	0.48±0.061^{NS}
3	Extract treated (Group III)	250	13.08±1.045^{NS}	0.51±0.061^{NS}	13.32±0.792^{NS}	0.55±0.112^{NS}
4	Extract treated (Group IV)	500	12.85±0.984^{NS}	0.49±0.091^{NS}	13.21±0.563^{NS}	0.50±0.081^{NS}

Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

2.6. Blood parameters

KFT

Table 6: Changes in serum parameters in EG induced urolithic group

S. No.	Group	Dose (mg/Kg)	Creatinine (mg/dl)	BUN (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	1.18±0.402	49.80±5.317	3.36±1.465	3.95±0.572
2	Standard (Group II)	750	1.07±0.539^{NS}	37.53±0.4762.528*	1.92±0.619*	1.63±0.677*
3	Extract treated (Group III)	250	1.10±0.569^{NS}	46.77±1.616*	3.12±0.190*	2.43±0.378*
4	Extract treated (Group IV)	500	0.98±0.337^{NS}	42.07±2.771*	2.50±0.20*	2.07±0.516*

Table -7 LFT- Blood parameters

S. No.	Group	Dose (mg/Kg)	SGPT (IU/L)	SGOT (IU/L)	ALP (mg/dl)	Bilirubin (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	98.17±7.57	112.66±7.469	208.34±7.962	1.24±0.144
2	Standard (Group II)	750	56.18±7.768*	60.23±8.003*	112.99±8.84*	0.57±0.083*
3	Extract	250	81.00±3.788^{NS}	88.43±5.291*	169.96±7.5	1.10±0.063*

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	treated (Group III)				15*	
4	Extract treated (Group IV)	500	60.56±5.028*	67.91±3.999*	130.28±2.785*	0.67±0.04*

Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

2.7. Kidney homogenate analysis

Table 8: Changes in kidney retention of stone formation constituents in EG induced urolithic group and experimental animals

S. No.	Group	Dose (mg/Kg)	Phosphorus (mg/dl)	Calcium (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	3.98±0.412	5.00±0.454
2	Standard (Group II)	750	1.57±0.35*	2.38±0.429*
3	Extract treated (Group III)	250	3.5±0.485*	3.94±0.332*
4	Extract treated (Group IV)	500	2.31±0.231*	3.41±0.358*

Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

From the result of ethylene glycol induced urolithiasis method it was found that administration of 0.75 % (v/v) ethylene glycol aqueous solution to rats causes hypercalciurea and calcium, phosphate excretion were grossly increased in Group I animals. Methanolic extract of leaves of Cassia fistula significantly (P<0.05) reduced the elevated levels of calcium and phosphate in kidney in Group III and IV compared to Group II. 500 mg/Kg dose of extract was more significantly decreased calcium and phosphate level compared to 250 mg/Kg. However, standard drug Cystone lowered the elevated levels of calcium and phosphate more significantly.

2.8. Blood parameters

KFT

Table 9: Changes in serum parameters in EG induced urolithic group

S. No.	Group	Dose (mg/Kg)	Creatinine (mg/dl)	BUN (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	1.20±0.275	53.23±3.318	4.70±0.857	3.87±0.405
2	Standard (Group II)	750	0.86±0.079*	40.56±5.311*	2.43±0.443*	1.88±0.405*
3	Extract treated (Group III)	250	0.99±0.253*	44.26±2.57*	3.97±0.201*	2.05±0.252*
4	Extract treated (Group IV)	500	0.83±0.055*	41.93±2.391*	3.12±0.327*	1.85±0.09*

Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

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From the result of ethylene glycol and ammonium chloride induced urolithiasis method it was found that administration of 0.75 % (v/v) ethylene glycol and 1% (w/v) ammonium chloride aqueous solution to rats remarkably increased creatinine, urea, uric acid and blood urea nitrogen in urine in calculi induced Group I. 250 mg/Kg and 500 mg/Kg dose of Cassia fistula extract significantly (P<0.05) reduced the elevated levels of creatinine, urea, uric acid and blood urea nitrogen in Group III and IV respectively. 500 mg/Kg dose of extract was more significantly decreased elevated levels of creatinine, urea, uric acid and blood urea nitrogen compared to 250 mg/Kg dose. However, standard drug Cystone lowered the elevated levels of creatinine, urea, uric acid and blood urea nitrogen in blood serum more significantly.

2.9. LFT

Table 10: LFT- Blood parameters

S. No.	Group	Dose (mg/Kg)	SGPT (IU/L)	SGOT (IU/L)	ALP (mg/dl)	Bilirubin (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	110.88±11.40	125.20±9.438	221.60±11.137	1.53±0.172
2	Standard (Group II)	750	58.58±9.284*	64.58±9.329*	120.03±10.965*	0.61±0.15*
3	Extract treated (Group III)	250	85.41±5.811*	96.03±8.489*	175.55±10.303*	1.15±0.07*
4	Extract treated (Group IV)	500	67.27±10.096*	72.96±10.30*	137.51±15.193*	0.65±0.107 *

Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

From the result of ethylene glycol and ammonium chloride induced urolithiasis method it was found that administration of 0.75 % (v/v) ethylene glycol and 1% (w/v) ammonium chloride aqueous solution to rats remarkably increased ALP, SGPT, SGOT, Bilirubin in urine in calculi induced Group I. 250 mg/Kg and 500 mg/Kg dose of Cassia fistula extract significantly (P<0.05) reduced the elevated levels of ALP, SGPT, SGOT, Bilirubin in Group III and IV respectively. 500 mg/Kg dose of extract was more significantly decreased elevated levels of ALP, SGPT, SGOT, Bilirubin compared to 250 mg/Kg dose. However, standard drug Cystone lowered the elevated levels of ALP, SGPT, SGOT, Bilirubin in blood serum more significantly.

2.10. Kidney homogenate analysis

Table 11: Changes in kidney retention of stone formation constituents in EG + NH_4Cl induced urolithic group and experimental animals

S. No.	Group	Dose (mg/Kg)	Phosphorus (mg/dl)	Calcium (mg/dl)
1	Control (Group I)	Normal Saline (10 ml/Kg)	4.92±0.341	8.95±0.419
2	Standard (Group II)	750	2.21±0.259*	4.62±0.393*
3	Extract treated (Group III)	250	4.15±0.424*	7.31±0.450*
4	Extract treated (Group IV)	500	2.95±0.506*	6.39±0.507*

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Values are expressed as Mean ± SD at n=6, one way ANOVA followed by Bonferroni test, *P< 0.05 as significant compared to the control group.

IV. CONCLUSION:

In present investigation anti-urolithiatic activity of methanolic extract of Cassia fistula leaves was confirmed on various animal models. Extract was prepared using maceration technique. Extract was characterized on the basis of phytochemical testing. Phytochemical testing revealed that extract was rich in flavonoids, carbohydrate, tannins and phenolic compounds, saponin, sterols and terpenoid type of compounds. Safety of extract was confirmed as OECD 423 guidelines which revealed that extract was safe upto 2000 mg/Kg. Hence, its $1/8^{th}$ i.e. 250 mg/Kg and $1/4^{th}$ i.e. 500 mg/Kg were selected as dose for further in vivo investigation.

Assessment of protective potential was done on the basis of level of calcium, phosphate in kidney and BUN, creatinine, urea, uric acid, ALP, SGPT, SGOT, Bilirubin in serum and level of enzymes involved in oxidative stress: LPO, SOD, GSH. It was observed that level of BUN, creatinine, urea, uric acid, ALP, SGPT, SGOT, Bilirubin was significantly less (P<0.05) in animals provided extract at 250 and 500 mg/Kg

In extract treated animals at both dose of 250 and 500 mg/Kg level of LPO, SOD, GSH was found to be increased significantly (P<0.05) as compared to control group. Thus from present investigation it can be concluded that methanolic extract of Cassia fistula provides significant protection against ethylene glycol, ethylene glycol and ammonium chloride induced calculi. Extract possess antiurolithiatic and protective activity against toxicity at both doses 250 and 500 mg/Kg. This effect can be attributed to different mechanism of action and one of them found in present study was effect on enzyme regulating oxidative stress. Further study is required to ascertain mechanism of action for said activity.

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