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ORIGINAL ARTICLE

Evaluation of Anti-Urolithiatic activity of Ethanolic Extract of *Erythrina indica* in Ethylene Glycol Induced Urolitiasis on Experimental Animals

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ABSTRACT

The present study was designed to evaluate the antiurolithiatic activity of selected plant ethanolic extract of Erythrina indica. Urolithiasis is the most prevalent condition of the urinary system, characterized by the formation of stones inside the urinary tract. Erythrina indica Linn. belongs to the family Fabaceae is a medium sized quick growing Tree selected for the study due to presence of flavones, glycoside 5, 7, 4'-trihydroxy-3'-methoxy-8-C- prenyl flavone7-O-β-D-glucopyranosyl-(1→3)-α-L-arabino pyranoside. Isoflavonoids such as 5, 4'-di-O-methylal pinum, isoflavone, Cajanin, Indicanine B, and Indicanin eC ,carbohydrates, proteins, glycosides, saponins, alkaloids, flavonoids, ,steroids tannins, and phenolic compounds. HealthyMaleWistarrats(150-200gms) were selected and divided into five groups having six animals in each. Group-I served as normal and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. For Group-III Cystone (750 mg/kg b. wt.) was employed as a standard drug. Group-VI and Group- V received ethanolic extract of Erythrina indica 200 mg/kg b. wt. and 400 mg/kg b. wt. for 28 days. Extracts were given once daily by oral route. Finally based on improvement in urine and serum parameters, antioxidant parameters, it is concluded that the ethanolic extract of leaves of Erythrina indica possess antiurolithiatic activity

***Keywords**: Urolithiasis, Erythrina indica , Ethylene glycol & Ammonium chloride*

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INTRODUCTION

Urolithiasis is a complex urological disorder characterized by the production of calculi in the kidneys, bladder, and urethra. The term Urolithiasis is derived from the Greek 'ouron' means urine, 'oros' means flow, and 'lithos' means stone.[1] urolithiasis is a highly prevalent disease with a rising incidence worldwide. Approximately 7%–13% of the adult population in North America, 5%–9% in Europe, and 1%–5% in Asia are affected by urolithiasis. It is a highly recurrent disease with a relapse rate of 50% within 10 years [2].

Traditionally, urolithiasis was characterized by bladder calculi in children of developing countries. The incidence of upper tract calculi, occurring mainly in industrialized areas, was much lower in children than in adults and approximately 7%of all stones occur in children younger than 16years. Also, in comparison with adult stone formers, children were more likely to demonstrate risk factors outside the metabolic realm such as UTI, anatomic abnormalities and surgical alterations in the urinary tract. Currently, the incidence of upper tract calculi in children without these predisposing factors is on the rise worldwide.[3]

The composition of stones has significantly changed over the past few years, with calcium phosphate and oxalate stones becoming more prevalent. Calcium oxalate produces between 60 and 90 percent of stone formation in adolescents, then comes calcium phosphate (10 %–20 %), struvite (14 %), uric acid (5–10

%), cystine 1 to 5 %, and mixed or other four percent stones, according to a recent epidemiological study from various geographic regions and countries. Additional elements that affect both incidence and intensity of urolithiasis in relation to climate and geography include genetics, gender, and age.[4]

Kidney stones usually go through four stages when they form: crystal nucleation process, crystal growth, crystal reorganization, and crystal continuation. The first step in the formation of kidney stones is urine supersaturation carried on by typical stone-forming substances, which results in crystal growth, retention, agglomeration, and nucleation The transformation of a liquid into a solid in a supersaturated solution is the first stage of crystal formation. Any crystal that is held in the nephron has the potential to become a stone because it serves as a nidus to promote subsequent crystal development and the build-up of ions. If the crystal nuclei do not stay in the kidney, they are either ejected in the urine or develop and combine to create a bigger ureteral calculus (up to 10 mm) [5]. Reduced urine volume raises the concentration of stone-causing compounds, which in turn promotes crystallization and stone formation, making urine volume a critical factor in the pathophysiology of urolithiasis.[6]

Anti-inflammatory drugs (NSAIDs) used orally and intravenously are suggested as first-line pain management strategies. Opioids, used for persistent pain. Additionally investigated as a viable pain management alternative is intravenous lidocaine.[7]

Various herbs possess diuretic, antilithic, alkalizing, antispasmodic, and anti-inflammatory properties that can aid in preventing stone formation, promoting stone dissolution, alleviating symptoms, and supporting kidney health. For instance, diuretic herbs like dandelion leaf and parsley increase urine flow, helping flush out stone-forming substances, while antilithic herbs such as gravel root interfere with crystallization. Alkalizing herbs like marshmallow root raise urine pH, reducing the risk of uric acid stones. Medical herbs and their products are more advantageous since they naturally help in the healing process.[8]

Erythrina indica Linn. Belongs to the family Fabaceae is a medium sized quick growing Tree attaining 18 m in height armed with dark colored, conical prickles. Mature dried stem Bark about 0.5-2.0 cm thick, smooth, exfoliating in narrow strips; outer surface yellowish to yellowish grey, lenticels found at short intervals longitudinal lines on the outer surface, yellowish to cream colored.

E. indica showed the presence of flavone glycoside 5, 7, 4'-trihydroxy-3'-methoxy-8-C- prenyl flavone7-O-β-D-glucopyranosyl-(1→3)-α-L-arabino pyranoside. Isoflavonoids such as 5, 4'-di-O-methylal pinumisoflavone, Cajanin, Indicanine B, and Indicanine C , carbohydrates, proteins, glycosides, saponins, alkaloids, flavonoids, ,steroids tannins, and phenolic compounds.[9]

Erythrina indica, a plant used in cameroonian folk medicine for the treatment of trachoma, elephantiasis, and microbial infections [10]. *E. indica* leaves are reported to exhibit sedative but no analgesic activity.[11] It is also reported to exhibit antioxidant activity.[12]

MATERIAL AND METHODS

Chemicals and reagents:

Ethylene glycol was received from Finar chemicals Ltd, Ahmadabad, Ammonium chloride was obtained from Reachem Labs Pvt. Ltd., Chennai, Cystone were received from Himalaya's drug company, Bangalore , and Phosphate kit were obtained from MS Diagnostics Pvt. Ltd., Hyderabad. All reagents used in the present study were of analytical grade.

PREPARATIONOF EXTRACT:

Leaves of *Erythrina indica* was taken, powdered in a grinder-mixer to obtain a coarse powder and then passed through 40mesh sieves. About 200gms of powder was extracted by using ethanol by soxhlet apparatus process up to 24hrs. The solution was filtered through Whatman filter paper and the resultant filtrate was distilled under reduced pressure for recovery of solvent. The dried extract thus obtained was kept in desiccators and used for further experiments.[13]

EXPERIMENTALANIMALIS:

Wistar albino rats of male sex (150-230 gm) procured from Raghavendra enterprises (Bangalore), were used in the present study. The animals were housed in the clean propylene cages and maintained under standard conditions(25±2ºC,relativehumidity 44–56%and12-hours light and dark cycles respectively) and fed with standard rat diet (M Mysore feeds, Bangalore) and purified drinking water *ad* libitum for 1 week before and during the experiments. Animals were handled with human care. Institutional Animal Ethical Committee (IAEC) of P. Rami Reddy Memorial College of Pharmacy (1423/PO/Re/S/11/CPCSEA) approved the present study

EXPERIMENTALDESIGN:

The experimental design used to carry out the anti-urolithiatic activity of ethanolic extract of *Erythrina indica* in ethylene glycol (EG) induced urolithiasis in rats.

Healthy Male Wistar rats(150-200gms) were selected and divided into five groups having six animals in each. Group-I served as normal and received regular rat food and drinking water adlibitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. Group-III received standard antiurolithiatic drug Cystone (750 mg/kg b. wt.). Group-VI and Group- V received ethanolic extract of EEEI 200 mg/kg b. wt. and 400 mg/kg b. wt. for 28 days. Extracts were given once daily by oral route.[14]

Collection of blood samples

The blood samples were collected from the retrorbital venous plexus of rats without any coagulant for the separation of serum, at the regular intervals of the treatment. After collecting the blood in ependroff tubes they were kept for 1 h at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters. [15]

RESULTS

Effect of ethanolic extract of *Erythrina indica* on urine biochemical parameters:

Effect of EEEI on urinary output:

Table1: Effect of EEEI on urinary output in ethylene glycol induced urolithiasis

Groups	Treatment	Urinary output (ml/day)
I.	Normal(Received1%water)	25.03 ± 0.64
II.	Control(Received EG in distilled water)	10.16 ± 0.34[a]
III.	Standard(Received EG in distilled water+ cystone)	22.33 ± 0.45[c]
IV.	Test-1(Received EG in distilled water+ Low dose of drug)	17.26 ± 0.86[b]
V.	Test-2(Received EG in distilled water+ High dose of drug)	20.42 ± 0.28[c]

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c]P<0.001 with respect to Disease control on 28[th] day by One way ANOVA followed by Dunnett: Compare all columns vs. Disease control

There was a significant decrease in the urine levels on 28th day in rats treated with ethylene glycol (EG)(G-II)when compared to the normal group(G-I) .The group(GIII)rats treated with standard drug cystone showed a significant increase in urine levels on 28[th] day when compared to control (G-II).Groups IV and V receiving EEEI (200 mg/kg and 400 mg/kg) showed significant increase in urine levels on 28th day when compared to control group suggesting to treat kidney stones by our extract. The results of urine levels were shown in aboveTable.1signifies that the high dose of EEEI was effective when compared to low dose of EEEI.

Table3: Effect of EEEI on Serum calcium levels, creatinine, uric acid, urea levels in ethylene glycol induced urolithiasis on28th day

Groups	Treatment	Calcium (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
I.	Normal (Received 1 % water)	8.426 ± 0.32	0.82 ±0.62	3.91 ±0.56	9.06 ± 0.31
III.	Standard (Received EG in distilled water + cystone)	10.126 ± 0.36c	1.96±0.23c	11.43 ± 0.23c	10.32 ± 0.13c
IV.	Test-1 (Received EG in distilled water + low dose of drug))	13.521 ± 0.32b	2.53±0.45b	13.52 ± 0.36b	12.642 ± 0.23b
V.	Test-2 (Received EG in distilled water + high dose of drug)	10.254 ± 0.131c	2.15±0.34c	12.41± 0.61c	11.23± 0.36c

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c]P<0.001 with respect to Disease control on 28[th] day by One way ANOVA followed by Dunnett's: Compare all columns vs. Disease control

Administration of ethylene glycol induced a significant increase in calcium, creatine levels, urea& uric acid on 28thday in control group (G-II) when compared to normal group (G-I). On treatment with cystone induced a significant reduction in calcium levels on 28th day in standard group (G-III) when compared to control group(G-II).Groups IV and V receiving EEEI of (200 mg/kg and 400 mg/kg) showed significant decrease in calcium, creatine levels, urea & uric acid levels, on 28[th]day when compared to control group.

The results of calcium, creatine levels, urea & uric acid levels were shown in above Table.3 signifies that the high Dose of EEEI was effective when compared to low dose of EEEI.

Table 4: Effect of EEEI on SOD and CAT levels

Groups	Treatment	SOD (U/mg protein)	CAT(µM H2O2 consumed/mg protein)
I.	Normal(Received1%water)	18.76 ± 0.61	51.54 ±0.24
II.	Control(Received EG in distilled water)	6.24 ± 0.15[c]	17.18±0.72[c]
III.	Standard (Received EG in distilled water + cystone)	16.46 ± 0.38[c]	48.32±0.54[c]
IV.	Test-1 (Received EG in distilled water +lowdose of drug)	12.53 ± 0.28[b]	41.54±0.63[b]
V.	Test-2 (Received EG in distilled water +high dose of drug)	14.82 ± 0.51[c]	46.±0.51[c]

Data represents the Mean ± SEM values (n=6). Statistical significance<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnett's: Compare all columns vs. Disease control.

A significant decrease in the SOD&CAT levels on 28th day was observed in rats treated with ethylene glycol (G-II) when compared to the normal group(G-I).The group(G-III) rats treated with standard drug cystone showed a significant increase in SOD&CAT levels on 28th day when compared to control(G-II).Groups (IV and V) receiving EEEI (200 mg/kg and 400 mg/kg) showed significant increase in SOD&CAT levels on 28th day when compared to control group suggesting to treat the kidney stones by extract .The results of SOD&CAT were shown in b Table. 4 signify that the high dose of EEEI was effective when compared to low dose of EEEI.

Table 5: Effect of EEEI on urine calcium levels, phosphate levels, and oxalate levels in ethylene glycol induced urolithiasis on 28th day

Groups	Treatment	Calcium (mg/dl)	Phosphate (mg/dl)		Oxalate (mg/dl)	
I	Normal(Received1%water)	3.23 ±0.14	5.42	± 0.21	4.34 ±0.16	
II	Control(Received EG in Distilled water)	11.35 ± 0.18[c]	9.22	± 1.42[c]	10.34	±0.34[c]
III	Standard(Received EG in distilled water +cystone)	5.21 ±0.27[c]	4.13	± 0.32[c]	3.21	±0.21[c]
IV	Test-1 (Received EG in distilled water + low dose of drug)	7.32 ±0.34[b]	6.42	± 0.23[b]	5.62	±0.42[b]
V	Test-2 (Received EG in distilled water + high dose of drug)	4.53 ±0.38[c]	5.21	± 0.61[c]	4.32	±0.23[c]

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c]P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnett's: Compare all columns vs. Disease control.

There was a significant increase in the calcium, phosphate & oxalate levels, on 28th day in rats treated with ethylene glycol (G-II) when compared to the normal group(G-I). The group(G-III) rats treated with standard drug cystone showed a significant decrease in calcium levels on 28th day when compared to control (G-II).Groups IV and V receiving EEEI (200 mg/kg and 400 mg/kg) showed significant decrease in Calcium levels on 28th day when compared to control group suggesting to treat the kidney stones by our extracts. The results of Ca were shown in above Table. 5 signify that the high dose of EEEI was effective when compared to low dose of EEEI.

DISCUSSION

Urolithiasis is defined as Formation of stone in the urinary system, i.e. in the kidney, ureter, and urinary bladder or in the urethra. The conventional medications used to treat urolithiasis are frequently insufficient. As a result, it's important to look for alternatives to the currently prescribed medications for urolithiasis treatment because they may not be as effective or safe. A number of medicinal plants are evaluated mainly against calcium oxalate and magnesium ammonium phosphate types of kidney stones, employing various experimental models of urolithiasis In the present study the ethanolic extract of leaves

of *Erythrina indica* was evaluated for antiurolithiatic activity against 0.75% ethylene glycol and 1% ammonium chloride for induction of renal calculi by using cystone (750 mg/kg) as a standard lithiatic drug for 28 days. There is a significant restoration of urine and serum parameters exhibiting antiurolithiatic activity of these plants.

CONCLUSION

Urolithiasis is a condition that occurs when these stones exit the renal pelvis and move in to the remainder of the urinary collecting system, which includes the ureters, bladder and urethra. India has a reputation as one of the oldest civilizations with a vast storehouse of medicinal herbs. India's forests are the main source of a huge variety of medicinal plants, which are mostly gathered as raw materials for medicine production. The ethanolic extract of leaves of *Erythrina indica* showed protection against 0.75%ethyleneglycol and 1%ammoniumchloride for induction of renal calculi by using cystone (750 mg/kg) as a standard lithiatic drug for 28 days. There is a significant restoration of urine and serum parameters, antioxidant parameters exhibiting antiurolithiatic activity of ethanolic extract of *Erythrina indica* . Finally based on improvement in urine and serum parameters, antioxidant parameters, it is concluded that the ethanolic extract of leaves of *Erythrina indica* possess anti urolithiatic activity.

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