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ANTI-UROLITHIATIC ACTIVITY OF METHANOLIC EXTRACT OF ROOTS OF "*CARICA PAPAYA*" LINN IN ETHYLENE GLYCOL INDUCED UROLITHIATIC RATS

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ABSTRACT

The present study was undertaken to evaluate the antiurolithatic effects of the methanolic extract of the root of *Carica Papaya* (CP) on ethylene glycol (EG) induced urolithiatic rats. Rats were given 0.75% v/v EG administration resulted in hyperoxaluria as well as increased renal excretion of calcium, oxalate and phosphate. Extract and cystone treated groups revealed increase the urine output, urinary pH and serum calcium level when compare to EG induced control. Methanolic extract of *C. papaya* root significantly ($p<0.05$) lowered the elevated urinary concentration of calcium, oxalate and phosphorus and also serum level of blood urea nitrogen, creatinine and uric acid. The increased deposition of stone forming constituents in the kidneys of urolithiatic rats was also significantly lowered by preventive treatment using methanolic extract of the roots of *C. papaya*. The results indicate that the root of *C. papaya* is consummate with antiurolithiatic activity like fruit which has been proved by earlier studies.

Key words: Urolithiatic, *Carica papaya*, calcium, oxalate, Ethylene glycol and cystone.

INTRODUCTION

Nephrolithiasis (renal stone formation) 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]. The majority of stones, up to 80%, are composed mainly of calcium oxalate [2]. Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys and bladder. However, the pathophysiologic bases for the formation of kidney and bladder stones are entirely different. Kidney stones form as a result of physicochemical or genetic derangements leading to supersaturation of the urine with stone-forming salts or, less commonly, from recurrent urinary tract infection with urease producing bacteria. Stasis in the upper urinary tract due to local anatomic anomalies may also promote or enhance stone formation in susceptible individuals. In contrast, bladder stones form almost exclusively as a result of urinary stasis and/or recurrent infection due to bladder outlet obstruction or neurogenic bladder. Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches. However these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments. This has stimulated research on traditional remedies showing anti-urolithiatic activity [3].

Carica papaya L. of the family Caricaceae is commonly known as Papaya. Its food and nutritional values are popular throughout the world. The medicinal properties of papaya fruit and other parts of the plants are well known in traditional system of medicine. In Indian Materia Medica describes the traditional uses of *C. papaya* as carminative, diuretic, laxative, stomachic, treatment of urinary calculus, bleeding piles and injuries of urinary tract, abortifacient and antiobase. The dried fruits is known to be helpful in spleenomagaly, hepatomagly, dysentery and chronic diarrhea, ringworm and skin diseases like psoriasis, well known expectorant, sedative and tonic [4-7]. The extracts of fruit, leaves, seeds and roots of *C. papaya* have been extensively studied for many potential uses including, antioxidant [8], diuretic [9], wound healing [10-12], anti-inflammatory [13], antihypertensive [14], antiulcer [15, 16], hypoglycaemic and hypolipidemic [17]. Earlier study on the papaya fruit was proved antiurolithiatic activity [18]. Based on the earlier report the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of methanolic extracts of *C. papaya* root using EG induced urolithiasis model in rats.

MATERIALS AND METHODS

Collections and authentication of plant material

The roots of carica papaya were collected in madanapalle and authenticated by Dr. T Damodharam, M.Sc, M.Phil, Ph.D, Assistnt Professor, S.V. University, Tirupathi.

Preparation of extracts

The collected roots of *carica papaya* were shade dried completely. The dried root was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The 500 g powdered material was loaded in Soxhlet's extractor and defatted with N-hexane. The marc was dried and extracted with methanol in a Soxhlet's apparatus. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators.

Preliminary phytochemical screening

The methanolic extract of the *carica papaya* were subjected to phytochemical test for identification. [19]

Experimental animal

Swiss albino mice and rats of both sex weighing 20±5 g and 150-180 g were used for the present study. The animals were obtained from the venkateaswara agency, Bangalore. They were housed at room temperature of 23±1^{o}C, relative humidity 55±55% under 12 hr light/12 hr dark cycle in the animal house. Mice were fed with commercial pellet diet and water *ad libitum* freely throughout the study. The animals were transferred to the laboratory at least 1 hr before the start of the experiment. All animal procedures were performed after approval from the IAEC (institution of animal ethical committee) and in accordance with the recommendations for the proper care and use of laboratory animals.

DOSE DETERMINATION

Acute oral toxicity studies

Acute oral toxicity studies was performed as per OECD-423 guidelines with methanolic extract of roots of *carica papaya* using albino mice of either sex, selected by random sampling for acute toxicity study. Animals are fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night with the mice). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hr in mice. Three animals are used

for each step. The dose level to be used as the starting dose is selected from one of four levels, 5, 50, 500 and 2000 mg/kg body weight.. Animals are observed individually after dosing at least once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr, except where they need to be removed from the study and humanely killed for animal welfare reasons are found dead. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in animal, then the same dose was repeated again to confirm the toxic dose, if mortality was not observed, the procedure was repeated for higher doses. [20]

Table 1: Study period and observation parameters of acute toxicity studies

Initial once observation	First 30 min and periodically 24 hr
Special attention	First 1-4 hr after drug administration
Long term observation	Up to 14 days
Direct observation parameters	Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

ANTIUROLITHIATIC ACTIVITY

Experimental protocol:

Rats were divided randomly into five groups (n = 6) and were treated as follows. Animals of group I was untreated and served as normal control. Rats of group II were received 0.75% ethylene glycol in purified drinking water *ad libitum* for 15 days and purified drinking water for the next 15 days. Rats of group III, IV and V were received 0.75% ethylene glycol in purified drinking water *ad libitum* for 15 days and fed orally with Cystone 750mg/kg, methanolic extract of 200 & 400 mg/kg for next 15 days respectively.

Estimation of urine variables

On 30th day, animals were placed in metabolic cages and urine sample was collected upto 12

hours measured the volume of urine and urine pH by digital pH meter. The urine samples subjected to analysis of calcium, phosphate and oxalate were evaluated using Automated Clinical biochemistry Analysis System (analytical nova).

Estimation of Serum variables

On 30th day after 2 hours of last dose, animals were anaesthetized with ketamine 25mg/kg. Blood was collected from orbital venous plexus in non-heparinized tubes and centrifuged at 2000 rpm for 20 min to obtain serum. Serum levels of calcium, Blood Urea Nitrogen (BUN), uricacid, creatinine, were evaluated using Automated Clinical biochemistry Analysis System, analytical nova. [21]

Statistical analysis

Experimental data are expressed as mean±standard error of mean (SEM). Statistical analysis was performed by one-way ANOVA followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. Data were considered significant at $p < 0.05$.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of roots of *carica papaya* shows presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

Acute oral toxicity

The methanolic extract of CP had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000mg/kg. Hence the LD50 of methanolic extract of CP were considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 200mg/kg and double the dose of ED50 (400mg/kg).

Antiurolithiatic activity

Administration of MECP 400 mg/kg shows significant alteration in urine pH when compare to control and cystone treated group also shows huge variation in urinary pH compare to ethylene glycol induced urolithiasis group. The MECP 200mg/kg revealed significant changes in urinary pH comparable to induced control but not like Group III and V almost equal to normal control. (Table 1)

Table 1: Effect of *carica papaya* on urine volume and urine pH in ethylene glycol induced urolithiasis on 30th days

GROUPS	TREATMENT	URINARY VOLUME IN ML	URINARY pH
I	Normal control	23.54±0.92*	7.89±1.27*
II	Ethylene glycol	12.16±0.43	5.24±1.89
III	Cystone 750mg/kg	22.34±0.21*	7.85±1.28*
IV	MECP 200mg/kg	18.72±0.58*	7.15±0.95*
V	MECP 400mg/kg	21.10±0.61*	7.82±1.38*

Significant difference at *P<0.05 when compared to ethylene glycol control. Values are Mean ± SEM from 6 animals in each group.

Estimation of urine variables

Table 2 shows that on 30th day treatment, there was significant (P<0.05) decrease in calcium, oxalate and phosphate in Group III, IV & V compared to Group II. The both cystone (G-III) and MECP 400mg/kg (G-V) effect were comparable to normal control (G-I).

Table 2: Effect of *carica papaya* on urine variables in ethylene glycol induced urolithiasis on 30th day

GROUPS	TREATMENT	Calcium mg/dl	Oxalate mg/dl	Phosphate mg/dl
I	Normal control	3.09±0.98*	1.36±0.45*	6.39±0.25*
II	Ethylene glycol	9.52±1.21	4.35±1.10	8.25±0.96
III	Cystone 750mg/kg	3.48±1.25*	1.56±0.64*	6.67±0.47*
IV	MECP 200mg/kg	5.89±0.74*	1.61±0.96*	7.12±0.41*
V	MECP 400mg/kg	4.24±0.52*	1.59±0.58*	6.85±0.56*

Significant difference at *P<0.05 when compared to ethylene glycol control. Values are Mean ± SEM from 6 animals in each group.

Estimation of Serum variables

The table 3 shows that there is significant increase in serum BUN, Creatinine and uricacid

level from ethylene glycol induced urolithiasis group when compare with normal control. Administration of MECP 200 and 400 mg/kg revealed that there is significant ($p<0.05$) decrease in all serum parameters except calcium when compare to urolithiasis induced group. The calcium level in serum decreased with group II and increased with all other group the values non significant with normal control. But the effect of MECP 400mg/kg almost equal to the standard drug cystone (Group III). This shows the extract act dose dependent manner.

Table 3: Effect of *carica papaya* on serum variables in ethylene glycol induced urolithiasis on 30th day

GROUPS	TREATMENT	BUN mg/dl	Creatinine mg/dl	Uric acid mg/dl	Calcium mg/dl
I	Normal control	3.09±0.98*	1.36±0.45*	6.39±0.25*	4.51±0.25*
II	Ethylene glycol	9.52±1.21	4.35±1.10	8.25±0.96	1.21±0.59
III	Cystone 750mg/kg	3.98±0.58*	1.52±1.05*	6.74±0.45*	3.98±0.12*
IV	MECP 200mg/kg	5.89±0.74*	1.61±0.96*	7.12±0.41*	3.29±0.28*
V	MECP 400mg/kg	4.24±0.52*	1.59±0.58*	6.85±0.56*	3.57±0.23*

Significant difference at *$P<0.05$ when compared to ethylene glycol control. Values are Mean ± SEM from 6 animals in each group.

DISCUSSION

This study showed that the methanolic extract of *carica papaya* root had a preventive effect on CaOx calculus formation in the rat kidney. Administration of ethylene glycol caused statistically increases in the level of calcium, oxalate, phosphate in urine and serum calcium level decreased. But increase the nitrogenous waste product in serum. The decrease of serum calcium concentration indicates an increase of urinary calcium and calcium oxalate stone formation. This suggestion is in agreement with several studies [22] who reported that the level of serum calcium was decreased and urinary calcium increased in rats treated with ethylene glycol. Moreover, Soundararajan *et al*. (2006) [23] showed that calcium oxalate excretion was significantly increased in urine of ethylene glycol induced urolithic rats. Additionally, they stated that ethylene glycol disturbs oxalate metabolism by way of increase the substrate availability that increase the activity of oxalate synthesizing enzymes in rats. Moreover, several investigations demonstrated that ethylene glycol treatment increased urinary calcium excretion significantly in lithiatic rats [24, 25]. The calcium level maintained

with MECP treated group in serum and decreased in urine calcium indicates that reduce the chance of stone formation. The increase in uric acid excretion was observed in urolithiatic induced group. Increased excretion of uric acid excretion has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility it contains protein that binds to calcium oxalate and modulates the crystal nature this is a important in role in formation in stone formation [26]. The treatment with MECP rats decreases the uric acid excretion and reduces the stone formation.

In urolithiasis GFR is decreased due to obstruction of urine out flow by formation of stone. Due to this, waste product, particularly nitrogenous substances like creatinine, uric acid and BUN accumulation in blood. The result of carica papaya treated groups decreases the above parameters in serum confirmed the antiurolitiatic activity. The concentration rather than the amount of the crystallising solutes is what ultimately establishes stone formation, reduced urinary volume will amplify the saturation of all solutes and raise the risk of all stone formation and the strong evidence that urine volume increases with CP once again support the beneficial effect on decrease the incidence of stone formation in kidney.[27]

The basis for calcium stone formation is supersaturation of urine with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities can change the composition or saturation of the urine so as to enhance stone-forming propensity. Among the metabolic conditions are hypercalciuria, hypocitraturia and hyperoxaluria.[28] However, the role of other factors like inhibitors, infection, matrix formation as well as urinary obstruction should not be ignored.[29]

There is evidence that in response to ethylene glycol administration, young male Albino rats form renal calculi composed mainly of calcium oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased excretion of oxalate and its urinary concentration. [30] Therefore, this model was used to evaluate the effect of *carica papaya* root extract on calcium oxalate urolithiasis. Consistent with some previous reports, stone induction by ethylene glycol caused an increase in oxalate excretion [31] and cotreatment with *carica papaya* root extract reduced the rate of increase in the oxalate excretion.

The exact mechanisms involved in the effect of *carica papya* on CaOx calculi are not clear; however, the following mechanisms are possible. Firstly, hyperoxaluria is a major risk factor

in calcium oxalate stone formation; the methanolic extract of *carica papaya* was able to reduce the urine oxalate in treatment groups on day 30. Thus, it seems that the preventive effect of *carica papaya* extract on CaOx formation can be in part attributed to alteration of urine oxalate concentration. *Carica papaya* could possibly control the levels of oxalate by inhibiting the synthesis of oxalate.

CONCLUSION

Overall, the results indicate that administration of the methanolic extract of *carica papaya* root, at dose of 200 and 400 mg/ kg, to rats with ethylene glycol-induced lithiasis, reduced and prevented the formation of urinary stones. The root *carica papaya* is good and valuable herbal medicine to prevent kidney stone formation. The mechanism and constituents underlying this effect is unknown, so further studies need to identify and isolate the active constituent of the root.

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