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Research Article

EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF *TRAPA NATANS L.* FRUITS

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ABSTRACT

The objective was to investigate the Antiurolithiatic activity of ethanolic extract of *Trapa natans* L. fruits on 0.75% ethylene glycol-induced urolithiasis in Wistar albino male rats. Urolithiasis was produced in wistar albino male rats by adding 0.75% ethylene glycol to drinking water for 28 days. The ethanolic extract of *Trapa natans* L. fruits was assessed for its preventive action in urolithiasis it's given from 1 day to 28 day. Various renal functional and injury markers evaluated using urine volume, calcium, Phosphate, uric acid, Creatinine, magnesium, oxalate, Protein serum, kidney histopathology.The *Trapa natans* L fruits extract treatment increased the urine output significantly compared to the control. The *Trapa natans* L fruits extract treatment significantly reduced the urine excretion of calcium, Protein, creatinine, oxalate, uric acid, and phosphate and increase magnesium level and also found lower amount of stones in kidney histopathology. These results suggest the usefulness of ethanolic extract of *Trapa natans L.* fruits as an Antiurolithiatic agent.

Keywords: Ethylene glycol, *Trapa natans L.*, Urolithiasis, calcium.

INTRODUCTION

Urolithiasis, one of the common and painful ailments of the urinary tract disorder, has been occurring in humans from centuries[1]. Kidney stones are hard, solid particles that form in the urinary tract. In many cases, the stone are very small and can pass out of the body without any problem. However if a stone (even a small one) blocks the flow of urine, excruciating pain may result, and prompt medical treatment may be needed[2].

Renal lithiasis is one of the oldest disease known to human being and has been documented in ancient Greek. Urinary stones have been found in remains of Egyptian mummies dating back as far as 7000 years[3]. Urinary stone are the third prevalent disorder of the urinary system. It has been reported that 91% of the urinary calculi contain calcium in some form, while 8% and 1% are composed of uric acid and cystine, respectively[4]. It is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70-80% in men and 47-60% in women[5].

Stone formation was identified and concluded as an imbalance between promoter (calcium, oxalate, uric acid, inorganic phosphate etc.) and inhibitors (citrate, magnesium, potassium, pyrophosphate and urinary glycoprotein etc.) further more free radical generate due to oxidative stress, damage epithelium of kidney or bladder thereby producing a favourable environment for crystal attachment to surface[6]. The present day medical management of lithiasis includes lithotripsy and surgical procedures. Unfortunately, these techniques do not correct the underlying risk factor. As per reported activity of *Trapa natans* L useful as analgesic, anti-inflammatory, antioxidant, antimicrobial and as per Ayurveda, the fruits of Trapa natans L useful as Diuretics so those all activity help in treatment of Urolithiasis. In the present study, an effort has been made to establish the scientific validity of the antiurolithiatic property of the *Trapa natans* Linn fruits using 0.75% ethylene glycol induced Urolithiasis using male Wistar albino rats.

MATERIALS AND METHODS

Plant collection and Authentication

The fruits of *Trapa natans* Linn were received from local market of Jodhpur, Rajasthan and

were identified by Dr. S. L. Meena, Scientist C & in charge Botanical survey of India, Jodhpur. A voucher specimen (voucher no. BSI/AZRC/I.12012/2014-15/Tech. (Pl. Id)/132) dated 20:05:2014 were deposited botanical survey of India, Jodhpur and G. D. Memorial College of Pharmacy, Jodhpur, (Raj)

Preparation of plant extract

The fruits of *Trapa natans* L. were washed and dried under shade for 15 days.The dried fruits of *Trapa natans* Linn (500 g) were powered and first extracted using Soxhlat apparatus with petroleum ether as solvent for 24 hours then after dry secondary extract with ethanol for 48 hours. The ethanolic extract was evaporated under reduced pressure to give solid residue, which was stored at 0-4C for subsequent experiment. The yield of ethanolic extract was 8.40% w/w.

Experimental animals

Adult male albino rats of wistar strain (150-200 g) were procured from animal house G. D. Memorial College of Pharmacy, Jodhpur (Raj.). The animals were acclimatized to standard laboratory conditions (temperature: 23 $\pm$ 2 C) and maintained on 12-hour light/dark cycle. They were provided with regular free access to drinking water ad libitum for the period of 28 days. Institutional Animal Ethics Committee (IAEC) approval (Protocol no.: 1491/PO/a/11/CPCSEA dated 07:06:2014) was obtained and care of the animals was taken as per the guidelines of CPCSEA, Ministry of social Justice and Empowerment, Government of India.

Acute Toxicity Study[7]

Acute toxicity studies was performed as per the OECD guidelines (no. 2000) using female wistar albino rats to evaluation of Antiurolithiatic activity. A single dose (2g/kg) of the extract will orally administrated to overnight fasted (food but not water withheld), Furthermore the observation include change in skin, fur, eyes, locomotors activity and behaviour pattern was recorded continuously for 12 hours, and daily for the next 2 weeks for any mortality.

Antiurolithiatic activity

Experimental Design[8]

0.75% ethylene glycol induced urolithiasis model was used to assess the antiurolithiatic activity in wistar albino rats. Animals were divided in four group containing 6 animals in each groups. Group 1 served as normal control and received regular rat food and drinking water ad libitum. Group 2 served as urolithiatic control and received regular rat food and drinking water ad libitum (0.75% ethylene glycol v/v) in drinking water for 28 days. Group 3 served as test received rats food and drinking water ad libitum (0.75% ethylene glycol v/v) in drinking water for 28 days and also given ethanolic extract of *Trapa natans* Linn (300 mg/kg) orally for 28 days. Group 4 served as control received rats food regular and water ad libitum (0.75% ethylene glycol v/v) in drinking water for 28 days and also given standard antiurolithiatic drug Cystone (750 mg/kg) orally for 28 days. All extract and standard were given once daily by oral route.

Analysis of urine and serum[8]

Collection and analysis of urine

Animals were kept in separate metabolic cages and urine sample of 24 hrs. Urine was collected on day 14, and day 28. A drop of concentrated hydrochloric acid was add to urine before being store at 4 C. Urine was analysis for calcium, phosphate, oxalate, protein, creatinine, magnesium and uric acid content.

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anaesthetic conditions and the animals will be sacrifices by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min. and analysed for calcium, magnesium, phosphate, oxalate, creatinine and uric acid content.

Histopathological Studies

Kidney sample were weight and fixed rapidly with 10% neutralized formalin (ph. 7.4). Section of kidney fixed in paraffin was prepared and stained with haematoxylin and eosin and observed for Histopathological changes.

RESULT

Acute toxicity study

From the acute toxicity study and also reported that the LD_{50} cut off dose was found to be 3g/kg body weight for the extract. Hence the therapeutic dose was taken 300 mg/kg for ethanolic extract.

Urine analysis

Table 1 depicts the urinary biochemical data were obtained at 14^{th} and 28^{th} days respectively in each group. In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution of male wistar rats results in hyperoxaluria. There was

an increase in urinary calcium, phosphate, uric acid, creatinine. oxalate, protein and decrease level of magnesium in calculi induced animal [table 1.1 group-2] however, Supplementation with *Trapa natans* Linn (300mg/kg) significantly this changes and restore to parameter near normal value [table 1.1 group-3]. The results were consistent with Cystone treated animals [table 1.1 group 4].

Serum analysis

Renal stone induction caused impairment of renal functions of untreated rats as evident from the markers of glomerular and tubular damage, i.e., elevated serum creatinine, uric acid. These markers were significantly reduced in the animals which were treated with *Trapa natans* Linn. The serum calcium, phosphate, was significantly increased in calculi induced animals compare to group 1 indicating marker tubular damage. However treatment with *Trapa natans* L. fruits extract significantly lowers the elevated serum calcium, phosphate level. [Table 1.2 group 3] the result was constant with Cystone treated animals [Table 1.2 group 4].

Histopathological study

Section of Kidney from animals treated with ethylene glycol showed that deposition of microcrystals [figure 2]. There was mark dilation in tubules, tubular damage and infiltration of inflammatory cells into the interstitial space. However kidney section on animals treated with *Trapa natans* L. fruits extract (GC) showed improvement of above symptoms and reduced crystals deposition as shown in [figure 3] but significantly improved with Cystone treated in GD as shown in [figure 4].

DISCUSSION

In the present study 0.75% ethylene glycol induced urolithiasis in male rats is used because their urinary system resembles that of humans. It's observed that chances of kidney stone formation are more in men as compare to women. So chances of stone formation is female rats are less than male rats. So, we had selected man wistar rats for study[8].

A number of renal pathological diseases, including calcium oxalate kidney stones, have resulted due to the oxalate-induced damage to the renal cells. Elevated level of oxalate is responsible for the toxic effect of the renal epithelial cells via alteration in membrane integrity, generation of reactive oxygen species, and depleted source of antioxidant enzymes[9]. Ethylene glycol increase the risk of Urolithiasis by increasing urinary level of stone constituents (calcium, oxalate, phosphate and uric acid) and facilitated an optimal environment for stone growth like low citrate level. In view of its medicinal use as an Antiurolithiatic, *Trapa natans* L. fruits extract was studied to evaluate its potential to prevent calcium oxalate Urolithiasis. To our knowledge this is the first study to show the Antiurolithiatic effect of *Trapa natans* L fruits in ethylene glycol-induced Urolithiasis.

In present study oxalate and calcium excretion progressively increased in calculi-induced animals (GB) since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stone than hypercalciuria[10]**.** The change in urinary oxalate level are relatively much more important than those of calcium[11]. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or calcium phosphate from urine and subsequent crystal growth however *Trapa natans* L extract lowered the levels of oxalate as well as calcium level.

Magnesium one of the inhibitors for stone formation, reduces the super saturation of calcium oxalate by reducing the saturation of calcium oxalate and the growth of calcium oxalate crystals[12]. *Trapa natans* L. extract elevated the urinary magnesium level, and thus, reduced the propensity of crystallize, thereby creating an ambience unfavourable for precipitation. Increased excretion of protein has been noted in hyperoxaluria acid and stone formers. A high urinary colloidal concentration favours crystal growth. Such a condition was observed with (GB) in this study. In, Urolithiasis the glomerular filtration rate decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as Creatinine and uric acid get accumulated. Uric acid known as promote calcium oxalate crystal growth and the observed that uric acid binding proteins are capable to binding to calcium oxalate and modulate its crystallization also suggest its primary role in stone formation[13]. In present study higher level of uric acid, creatinine was show that kidney damage observed in ethylene glycol induced urolithiasis rats. The uric acid, creatinine level was decrease after treatment with *Trapa natans* L extract.

Microscopic examination of kidney section derived ethylene glycol induced Urolithiatic rats showed polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitialinflammation that might be attributed

to oxalate. Treatment with Trapa natans L. extractdecrease the number and size of calcium oxalate deposit in different part of the renal tubules and also prevented damages to the tubules and also prevented damage to the renal tubules and also prevented damages to the tubules and calyxes.

CONCLUSION

In conclusion, the results indicate that administration of fruit extract of *Trapa natans* L. reduced and prevented the growth of urinary stones. The underlying mechanism could be due to its antioxidant, diuretic effect, anti-inflammatory effect and its analgesic effect help in reduces pain. Further experimental and clinical studies are required to elucidate the chemical constituents of the extract and the mechanism that are responsible for the pharmacological activities.

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Table 1: Effect of ethanolic extract of *Trapa natans L.* Fruits in experimental hyperoxaluria

Parameter (Unit)	Group I Control	Group II Calculi induced	Group III Plant extract treated	Group IV Cystone treated
1.1 Urine biochemical parameter on 14th day				
Protein(Gm. /dl)	10.31±0.55	16.52±0.96	12.96±0.46	11.64±0.47
Magnesium(mg/dl)	6.2±0.34	2.4±0.12	3.72±0.16	5.41±0.22
Calcium(mg/dl)	24.2±1.03	29.78±0.37	27.21±0.28	25.73±0.34
Uric acid(mg/dl)	13.83±0.71	29.26±1.31	18.34±1.45	15.33±0.32
Creatinine(mg/dl)	21.38±0.44	52.33±0.83	32.16±0.41	27.83±0.20
Oxalate(mg/dl)	14.46±0.27	40.33±0.68	27.82±0.83	21.09±0.54
Phosphate(mg/dl)	39.35±0.68	70.60±0.70	59.80±0.9	50.80±0.64
1.2 Urine biochemical parameter on 28th day				
Protein(Gm. /dl)	11.72± 0.78	17.3± 0.96	13.65± 0.47	12 .85± 0.52
Magnesium(mg/dl)	6.58± 0.37	2.6± 0.16	3.83± 0.12	5.54± 0.24
Calcium(mg/dl)	25.98±0.83	30.93±0.51	27.87±0.38	26.54±0.31
Uric acid(mg/dl)	14.13±0.73	30.94±1.34	20.02±1.48	16.3±0.37
Creatinine(mg/dl)	21.90±0.37	55.14±1.18	34.16±0.40	27.38±0.40
Oxalate(mg/dl)	15.49±0.51	41.53±0.64	29.4±0.81	22.24±0.75
Phosphate(mg/dl)	40.98±0.85	74.20±0.73	60.85±0.85	52.3±0.78

Table 2: Effect of ethanolic extract of *Trapa natans L.* fruits on serum Parameters on the 28th Day

Parameter (Unit)	Group I Control	Group II Calculi induced	Group III Plant extract	Group IV Cystone treated
Protein(Gm. /dl)	12.06±0.08	18.56±0.20	15.04±0.22	12.78±0.14
Magnesium(mg/dl)	5.25±0.02	2.14±0.14	3.65±0.17	4.45±0.12
Calcium(mg/dl)	9.30±0.1	21.38±0.55	16.06±0.09	12.23±0.01
Uric acid(mg/dl)	6.60±0.06	9.18±0.12	8.02±0.09	7.23±0.06
Creatinine(mg/dl)	7.35±0.06	9.30±0.06	9.02±0.05	8.14±0.06
Oxalate(mg/dl)	3.38±0.31	14.07±0.07	8.03±0.31	7.18±0.41
Phosphate(mg/dl)	7.79±0.1	18.4±0.48	14.15±0.17	12.41±0.15

Value of Urine parameters are assessed in 24h urine sample
All values are expressed as mean ± SD for six animals each group
Statistical significances P < 0.05

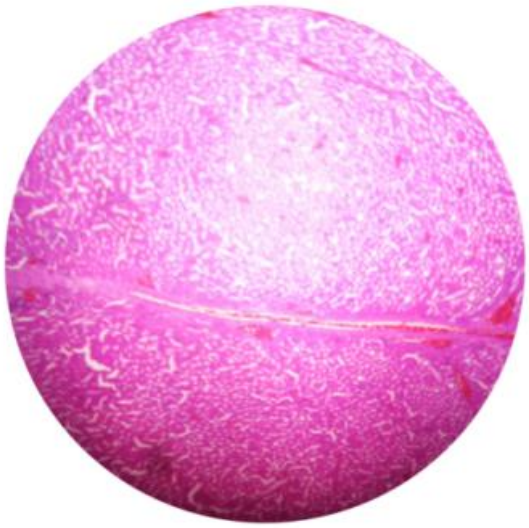

Fig. 1: Kidney section of GA (Normal control) rat

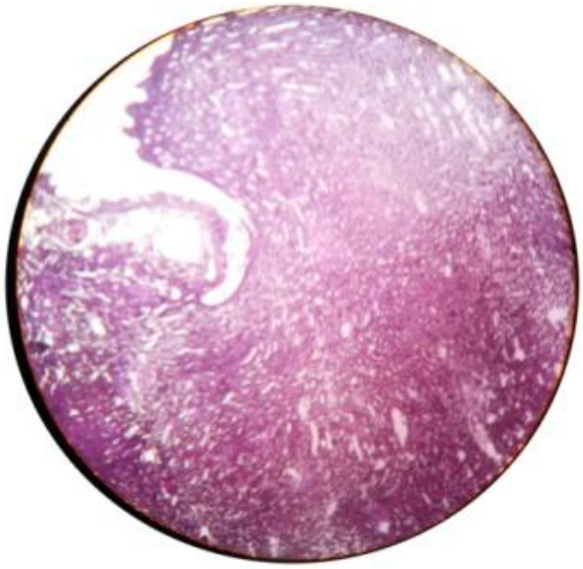

Fig. 2: Kidney section of GB (Urolithiatic control)

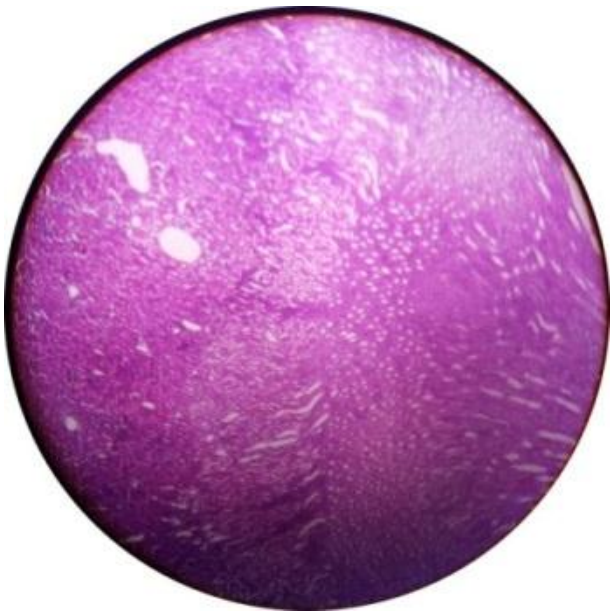

Fig. 3: Kidney section of GC (
Treated with *Trapa natans L.* 300 mg)

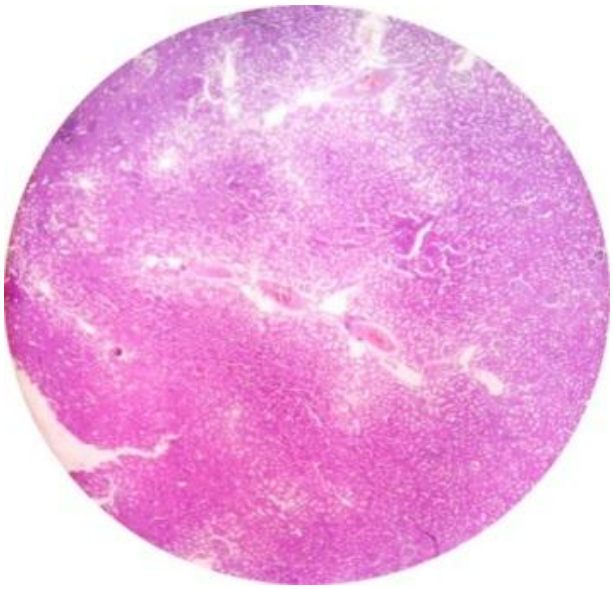

Fig. 4: Kidney section of GD
(Treated with Cystone, 750mg)

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