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ANTI UROLITHIATIC ACTIVITY OF VARIOUS EXTRACT OF *AZIMA TETRACANTHA* ON ETHYLENE GLYCOL INDUCED IN RATS

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ABSTRACT

Azima tetracantha is a plant commonly used as a traditional herbal medicine and possesses the wide range of pharmacological applications. The main objective is to investigate the anti-urolithiatic property of various extract of *Azima tetracantha* on ethylene glycol (EG) induced urolithiasis (0.75% v/v in drinking water for 28 days) in rats. Male wistar rats were divided into 7 groups as follows, Group I served as control treated with 4% w/v gum acacia 1.0 mL/kg/ orally for 28 days, Group II treated with ethylene glycol (0.75% v/v) for 28 days, Group III, IV, V, VI and VII received with cystone, various extracts ofethanolic, ethylacetate, chloroform and petroleum ether of *Azima tetracantha* respectively from 15-28th day along with EG for 28 days. After completion of the 28 days respective treatments, the level of various urolithiatic promoters in the biological samples such as urine, serum and kidney homogenate. The rats were sacrificed for isolation of kidney for histopathological evaluation and biochemical estimation. Biochemical markers like ACP, ALP, AST, ALT and LDH were evaluated in serum as well as in kidney homogenate. The ethanolic extract of *Azima tetracantha* showed significant anti-urolithiatic property when compared with other extracts by increased urine volume, decreased calcium, phosphate, oxalate levels and increased the magnesium levels in urine

significantly (P< 0.001), the serum levels of urea, uric acid, creatinine and BUN were significantly (P< 0.001) reduced, the biochemical markers were significantly (P < 0.001) reduced in both serum and kidney homogenate. From the above results it was concluded that *Azima tetracantha* having the antiurolithiatic property against ethylene glycol in rats. Hence this plant may be considered as one of the source for the isolation of new oral anti urolithiatic agent.

Keywords: Anti urolithiasis, ethylene glycol, *Azima tetracantha*, calcium, phosphate, oxalate, magnesium, uric acid, urea, creatinine

INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia **[1]**. It has an important effect on the health care system with a prevalence of >10% and an expected recurrence rate of ~50% **[2]**. It occurs both in men and women but the risk is generally higher in men and is becoming more common in young women **[3]**. A majority of urinary stones are composed of (CaOx) stones alone or CaOx mixed with calcium phosphate calcium oxalate. 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. In men, 70% to 80% of the calculi contain either calcium oxalate alone or in combination with appetite **[4]**.

Various techniques to investigate the urolithiasis such as radiological and other laboratory techniques are not sufficiently helpful to elucidate the exact cause and mechanism of stone formation. Other management of urolithiasis mainly involves surgical removal of stones, extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy are expensive, pose a threat of recurrence and have serious side effects such as hemorrhage, hypertension, tubular necrosis, and subsequently fibrosis of the kidney **[5]**. Various therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing **[6]**. Currently, there are no satisfactory drugs in modern medicine, which can dissolve the stone and therefore physicians remain to be depending on alternative systems of medicine for better relief.

In contrast, traditional medicines have offered a substitute for many diseases and also have provided some supplementary information about the pathogenesis of

various diseases. *Azima tetracantha*. (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit **[7]**. It occurs naturally in central, eastern and South Africa as well as in the Indian Ocean islands and extends through Arabia to tropical Asia. It is a spiny scrambling shrub. Spines are often in whorls of 4; branches 4-angled, leaves are elliptic to oblong, leathery, sharp-tipped at the apex **[8, 9]**. The plant has been documented diuretic, anti-rheumatism, anti ulcer, conjunctivitis, anti-inflammatory activity, good antibacterial activity against Staphylococcus aureus, Streptococci mutans, Salmonella typhi and good antifungal activity against Aspergillus niger, Candida albicans **[10-15]**. However, so far no scientific study has been reported regarding the anti-urolithiatic activity of *Azima tetracantha.* In this study, we investigated the antiurolithiatic activity of various extract of whole plant of *Azima tetracantha* against ethylene glycol induced - induced renal calculi and its possible underlying mechanisms using male wistar rats

MATERIALS AND METHODS

Identification, collection and extraction of plant material

Dried whole plant of *Azima tetracantha* Lam were collected from the forest around Panakkudi, Trinelveli District, Tamilnadu (India). The plants were authenticated by Prof. V. Chelladurai, Research Officer-Botany (Scientist-C), Central Council for Research in Ayurveda and Siddha, Govt. of India, Chennai. The powdered plant material was subjected to successive extraction by various solvent including ethanol, ethyl acetate, chloroform and petroleum ether in a soxhlet apparatus at 60° C. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath until it forms a thick paste. The extract was dried and stored in a refrigerator at 4 °C in a glass bottle throughout the study **[16]**.

Drugs and chemicals

Ethanol, Ethyl acetate, Chloroform, Petroleum ether and Cystone were obtained from Sigma Aldrich laboratory, Mumbai, India. Ethylene glycol was purchased from Merck Ltd., Mumbai, India. All other chemicals were used of analytical grade.

Phytochemical Analysis

The various extract was subjected to qualitative analysis of the phytoconstituents test for alkaloids, flavanoids, tannins, phenolic compounds, terpenoids, glycosides,

saponins, protein, mucilage, carbohydrates and phytosterols.

Animals

Healthy adult male wistar rats (150-180 gm) were used for the present study. They were housed in polypropylene cages (six per cage) and maintained under temperature (27 ± 2 ◦C) and light (12 h light/dark cycles; lights on at 0700 h) controlled environment. The animals had free access to standard pellet diet and drinking water. Behavioral studies were carried out in a quiet room between 9.00 am and 11.00 am to avoid circadian variation. The study protocol was approved (Vide. 14/243/CPCSEA) by Institutional Animal Ethical Committee constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, India.

Ethylene glycol-induced urolithiasis model in rats

Ethylene glycol induced urolithiasis model was used to assessing the urolithiatic activity in male wistar rats **[17]**. Animals were divided into seven groups of six rats in each group. The animals of Group I served as normal control, received 4% w/v gum acacia 1.0 mL/kg , Ethylene glycol (0.75%v/v) in drinking water was fed to Group II to Group VII for induction of renal calculi till 28th day, Group III received standard drug Cystone 750mg/kg (p.o) from 15th to 28th day, Group IV to VII received Ethanolic extract (200mg/kg), Ethyl acetate extract (200mg/kg), Chloroform extract (200mg/kg) and Petroleum ether extract (200mg/kg) of *Azima tetracantha* from 15th to 28th day of the experiment **[18]**. On 29th day, blood was collected from retro-orbital puncture and serum was separated for analyze the biochemical parameters. Animals were sacrificed by cervical dislocation and kidneys were removed for histopathological evaluation.

Collection and analysis of urine

After 28 days of an experimental design, all animals were kept in individual metabolic cages urine samples of 24 h were collected. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4^{o}C. After urine collection was analyzed for urine volume, calcium, phosphate, oxalate and magnesium content **[19]**.

Serum analysis

After urine collection on 29th day blood was collected from retro-orbital under

anesthetic condition and animals were sacrificed by cervical dislocation for isolation of kidney for histopathological and biochemical estimation. The blood was collected and serum was separated by centrifugation at 2500 rpm for 10min. The serum supernatant was collected and then diluted within the ratio of 1:10. Aliquots of the diluted serum were used for the determination of serum constituents like creatinine, uric acid, urea, and blood urea nitrogen and serum enzyme activities **[21]**.

Prepartion of kidney homogenate and Biochemical estimation

Isolated kidneys were cleaned from extraneous tissue and rinsed with ice-cold physiological saline and dried at 80 °C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min, and the supernatant was separated. The biochemical enzymes like alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase were estimated in both serum and kidney homogenate **[21-25]**.

Statistical analysis

All values were expressed as mean ± SEM (standard error of mean) of six rats (n=6). The statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The value of $p < 0.05$ was considered as significant. The statistical software used was GraphPad Prism (version 5.0)

RESULTS

Effect of various extract of *Azima tetracantha* on phytochemical evaluation

The various extract of *Azima tetracantha* reveals the presence of phytochemical constituents as shown **(Table 1)**.

Effect of various extract of *Azima tetracantha* on urine volume

The ethylene glycol treated rats showed significantly ($p<0.001$) decreased the urine volume when compared with control rats. The treatment of various extract of *Azima tetracantha* and cystone treated rats were significantly ($p<0.001$, $p<0.001$, $p<0.01$ and $p<0.01$) increased the urine volume compared with ethylene glycol treated rats. The comparison between the various extract of *Azima tetracantha* along with ethylene glycol, the ethanolic extract treated rats showed more significantly ($p < 0.001$) increased in urine volume **(Figure 1)**.

Effect of various extract of *Azima tetracantha* on urine analysis

In the present study, chronic administration of ethylene glycol treated rats resulted in hyperoxaluria which reveals significantly ($p<0.001$) increased the level of oxalate, calcium and phosphate and significantly ($p<0.001$) decreased the magnesium level in urine when compared with control rats. Treatment with various extract of *Azima tetracantha* and cystone treated rats, significantly attentuates the elevated levels of oxalate, calcium and phosphate and magnesium level was significantly increased compared with EG treated rats. The ethanolic extract of *Azima tetracantha* was significantly ($p < 0.001$) decreased the calcium, oxalate and phosphate levels and significantly ($p<0.001$) increased the magnesium level in urinary excretion compared with other extracts of *Azima tetracantha* **(Table 2)**.

Effect of various extract of *Azima tetracantha* on serum analysis

In calculi-induced rats, renal damage was indicates that significantly ($p<0.001$) increased serum levels of creatinine, uric acid, urea and Blood Urea Nitrogen (BUN) compared with control rats. The treatment with various extract of *Azima tetracantha* and cystone treated rats significantly attenuates the serum levels of creatinine, uric acid, urea and BUN compared to ethylene glycol treated rats. Amongst the various extract of *Azima tetracantha* the ethanolic extract showed more effective in decreased the serum enzyme levels on renal calculi induced rats **(Table 3)**.

Effect of various extract of *Azima tetracantha* on biochemical estimation in serum as well as in kidney homogenate

In renal calculi-induced rats showed significantly ($p<0.001$) increased levels of the enzymes such as ACP, AST, ALP and ALT in serum as well as in kidney homogenate compared with control rats. Treatment with various extract of *Azima tetracantha* and cystone treated rats, various enzymes levels were significantly ($p<0.001$) decreased in the serum as well as kidney homogenate compared with ethylene glycol treated rats. Moreover, the ethanolic extract treated rats showed more effective in enzyme levels in serum as well as in tissue homogenates compared with various extracts of *Azima tetracantha* **(Table 4)**.

Effect of various extract of *Azima tetracantha* on LDH in serum as well as in kidney homogenate

The LDH levels were significantly ($p<0.001$) increased in serum as well as in kidney of ethylene glycol intoxicated rats

when compared with control rats. The treatment with various extract of *Azima tetracantha* and cystone treated rats along with ethylene glycol showed significantly decrease in LDH enzyme in serum as well as on kidney tissue compared with ethylene glycol treated rats. Amongst the various extracts of *Azima tetracantha* the ethanolic extract and cystone treated rats significantly ($p<0.001$) decreased the LDH level in both serum as well as kidney homogenate **(Table 5)**.

Effect of various extract of *Azima tetracantha* on histopathology evaluation

The histopathological observations of kidney showed normal structure and architectural intactness without any apparent damages in control **(Figure 2A)**. Ethylene glycol induced rats showed many calcium oxalate deposits inside the renal tubules and dilation of the proximal tubules along with interstitial inflammation were observed **(Figure 2B)**. The number of calcium oxalate deposits in the renal tubules of Groups III to VII rats **(Figure 2C, D, E, F & G)** was less than the Group II **(Figure 2B)**. Ethanolic extract of *Azima* tetracantha **(Figure 2C)** and cystone **(Figure 2D)** treated rats shows a considerable reduction in stone formation and restored the renal structure of the kidney when comparing the various extract and standard drug along with ethylene glycol treated rats **(Figure 2)**.

Table 1: Preliminary phytochemical analysis of various extract of *Azima tetracantha*

Phyto constituent	Ethanolic extract	Ethyl acetate extract	Chloroform extract	Pet. Ether extract
Flavanoid	+	+	+	+
Tannins	+	+	-	-
Saponins	+	+	-	-
Terpenoids	+	+	-	-
Glycosides	-	-	-	-
Phytosterols	+	+	+	+
Mucilage	-	-	-	-
Protein	+	+	+	+
Carbohydrates	+	+	+	-
Alkaloids	+	+	+	-

(-Absence); (+ Presence)

Table 2: Effect of various extracts of of *Azima tetracantha* on urinary calcium, magnesium, oxalates and phosphates (mg/24 hr) of different groups of rat

Treatments	Calcium (mg/dl)	Magnesium (mg/dl)	Oxalate (mg/dl)	Phosphates (mg/dl)
Control	8.56 ± 0.19	2.63 ± 0.08	1.36 ± 0.10	5.36 ± 0.10
Ethylene glycol 0.75%	11.6 ± 0.34 ^{c}	1.33 ± 0.12 ^{c}	5.5 ± 0.37 ^{c}	7.71 ± 0.17 ^{c}
Cystone (750mg/kg)	8.88 ±0.07 ^{z}	2.66 ± 0.22 ^{z}	1.44 ± 0.08 ^{z}	5.86 ± 0.05 ^{z}
Ethanolic extract	8.53 ± 0.13 ^{z}	2.66 ± 0.16 ^{z}	1.63 ± 0.08 ^{z}	5.46 ± 0.12 ^{z}
Ethyl acetate extract	9.7 ± 0.20 ^{y}	2.00 ± 0.23 ^{x}	2.88 ± 0.07 ^{x}	6.95 ± 0.25 ^{x}
Chloroform extract	9.06 ± 0.12 ^{y}	2.21 ± 0.17 ^{x}	3.43 ± 0.17	6.1 ± 0.24 ^{x}
Pet.ether extract	10.11 ± 0.48	1.76 ± 0.16	4.71 ± 0.11	6.7 ± 0.31^{x}

Each column represents the mean with SEM. The symbol denote the significance level: ^{a} $p<0.05$, ^{b} $p<0.01$ and ^{c} $p<0.001$ when compared with control groups: ^{x} $p<0.05$, ^{y} $p<0.01$ and ^{z} $p<0.001$ when compared with ethylene glycol group

Table 3: Effect of various extracts of of *Azima tetracantha* on serum urea, uric acid, creatinine and blood urea nitrogen (mg/24 hr) of different groups of rat

Treatments	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine	BUN
Control	9.9 ± 0.07	2.21 ± 0.17	0.38 ± 0.02	4.70 ± 0.05
Ethylene glycol 0.75%)	15.73 ± 0.18 ^{c}	4.78 ± 0.22 ^{c}	0.64 ± 0.03 ^{c}	7.5 ± 0.15 ^{c}
Cystone	10.15 ± 0.09 ^{z}	2.33 ± 0.19 ^{z}	0.42 ± 0.04 ^{z}	4.79 ± 0.21^{z}
Ethanolic extract	9.81 ± 0.16 ^{z}	2.43 ± 0.13 ^{z}	0.43 ± 0.03 ^{z}	4.61 ± 0.09 ^{z}
Ethyl acetate extract	12.4 ± 0.10 ^{x}	3.73 ± 0.19 ^{x}	0.49 ± 0.05 ^{y}	4.93 ± 0.18 ^{z}
Chloroform extract	13.86 ± 0.05	4.05 ± 0.12	0.51 ± 0.02 ^{x}	4.98 ± 0.20 ^{z}
Pet.ether extract	14.28 ± 0.14	4.46 ± 0.13	0.58 ± 0.04 ^{x}	5.47 ± 0.15 ^{x}

Each column represents the mean with SEM. The symbol denote the significance level: ^{a} p<0.05, ^{b} p<0.01 and ^{c} p<0.001 when compared with control groups: ^{x} p<0.05, ^{y} p<0.01 and ^{z} p<0.001 when compared with ethylene glycol group

Table 4: Effect of various extracts of of *Azima tetracantha* on serum and kidney parameter of different groups of rat

Treatments	ACP		AST		ALP		ALT	
	serum	kidney	serum	Kidney	serum	kidney	serum	Kidney
Control	66.6 ± 1.36	12.5 ± 0.87	32.6 ± 1.63	11.7 ± 0.70	73.3 ± 1.63	6.66 ± 0.51	31 ± 0.89	13.01 ± 0.33
Ethylene glycol	113.8 ± 3.06 ^{c}	8.5 ± 0.44 ^{c}	87.5 ± 4.32 ^{c}	6.78 ± 0.51^{c}	153.6 ± 2.8 ^{c}	3.5 ± 0.35 ^{c}	95.1 ± 0.75 ^{c}	9.48 ± 0.33 ^{c}
Cystone	73.0 ± 1.67 ^{z}	13.2 ± 0.87 ^{z}	37.1 ± 1.60 ^{z}	12 ± 0.89 ^{z}	75.6 ± 1.96 ^{z}	6.67 ± 0.24 ^{z}	32.6 ± 2.06 ^{z}	12.83 ± 0.26 ^{z}
Ethanolic extract	71.6 ± 2.73 ^{z}	13.1 ± 0.83 ^{z}	37.8 ± 1.32 ^{z}	11.9 ± 0.93 ^{z}	76.0 ± 2.52 ^{z}	6.51 ± 0.31 ^{z}	35 ± 1.41^{z}	12.81 ± 0.26 ^{z}
Ethyl acetate extract	77. 0 ± 2.09 ^{z}	11.3 ± 0.85 ^{z}	40.3 ± 1.36 ^{z}	10.6 ± 0.45 ^{z}	79.6 ± 1.50 ^{z}	6.15 ± 0.28 ^{z}	42.1 ± 2.22 ^{z}	11.9 ± 0.08 ^{y}
Chloroform extract	81.0 ± 0.89 ^{z}	10.6 ± 0.58^{y}	43.6 ± 1.50 ^{z}	9.7 ± 0.38 ^{y}	88.3 ± 1.50 ^{z}	5.5 ± 0.32 ^{y}	41.8 ± 1.72 ^{z}	11.3 ± 0.32 ^{y}
Pet.ether extract	87.1 ± 2.78 ^{y}	9.8 ± 0.68 ^{x}	51.8 ± 2.31^{y}	8.66 ± 0.40	98 ± 1.78 ^{y}	4.8 ± 0.20 ^{x}	50.8 ± 3.48 ^{y}	10.73 ± 0.47 ^{x}

Each column represents the mean with SEM. The symbol denote the significance level: ^{a} p<0.05, ^{b} p<0.01 and ^{c} p<0.001 when compared with control groups: ^{x} p<0.05, ^{y} p<0.01 and ^{z} p<0.001 when compared with ethylene glycol group

Table 5: Effect of various extracts of of *Azima tetracantha* on LDH serum and kidney parameter of different groups of rat

Treatments	LDH in Serum (µmoles/L)	LDH in Kidney (µmoles /mg)
Control	145.9 ± 0.70	3.71 ± 0.02
Ethylene glycol	205.4 ± 0.49 ^{c}	7.58 ± 0.07 ^{c}
Cystone	146.8 ± 0.67 ^{z}	3.77 ± 0.02 ^{z}
Ethanolic extract	147.9 ± 0.50 ^{z}	3.74 ± 0.03 ^{z}
Ethyl acetate extract	152 ± 0.40 ^{z}	4.18 ± 0.11^{z}
Chloroform extract	154.5 ± 0.8 ^{z}	4.5 ± 0.12 ^{z}
Pet.ether extract	160.75 ± 0.27 ^{y}	4.9 ± 0.16 ^{y}

Each column represents the mean with SEM. The symbol denote the significance level: ^{a} p<0.05, ^{b} p<0.01 and ^{c} p<0.001 when compared with control groups: ^{x} p<0.05, ^{y} p<0.01 and p<0.001 when compared with ethylene glycol group

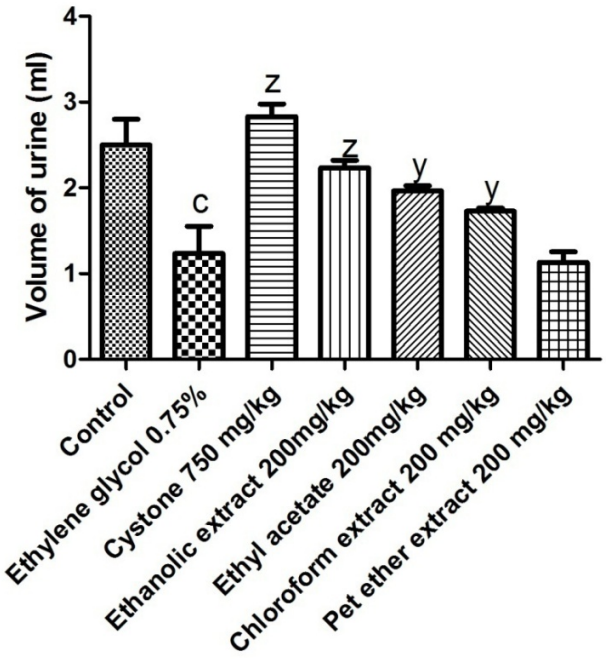

Figure 1: Effect of various extracts of *Azima tetracantha* on urine volume (ml)
The values were expressed as mean with SEM. The symbol denote the significance level: [a] p<0.05, [b] p<0.01 and [c] p<0.001 when compared with control groups: [x] p<0.05, [y] p<0.01 and [z] p<0.001 when compared with ethylene glycol group

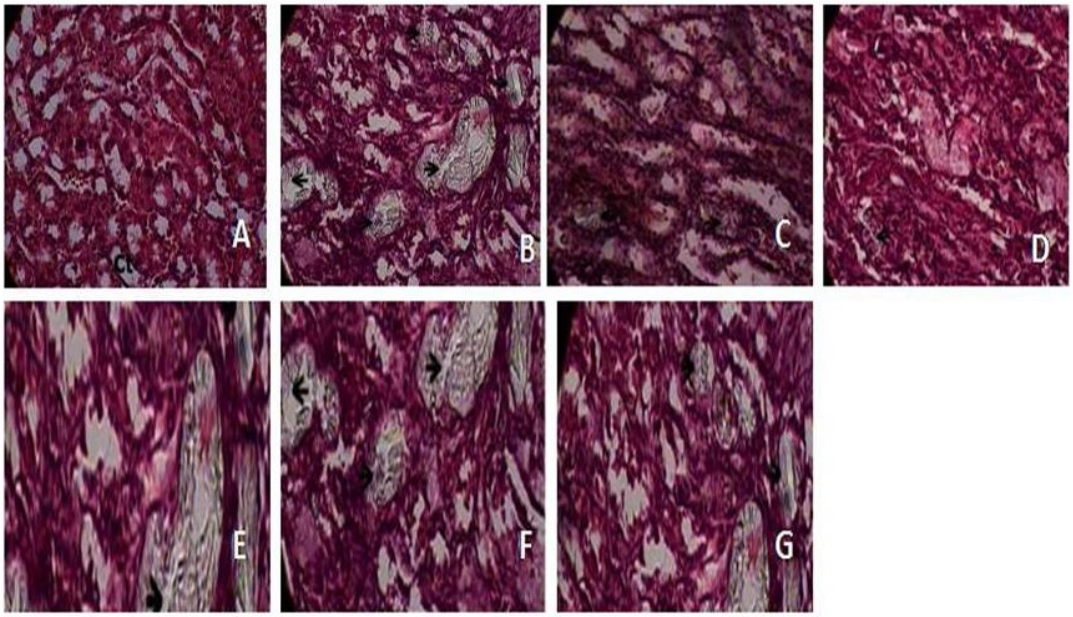

Figure 2: Histopathology of Kidney tissue
Figure 2A: Vehicle control,
Figure 2B: Ethylene glycol induced urolithiatic rat,
Figure 2C: Cystone on ethylene glycol induced rat
Figure 2D: Ethanolic extract of *Azima tetracantha* on ethylene glycol induced rat
Figure 2E: Ethyl acetate extract of *Azima tetracantha* on ethylene glycol induced rat
Figure 2F: Chloroform extract of *Azima tetracantha* on ethylene glycol induced rat
Figure 2G: Petroleum ether extract of *Azima tetracantha* on ethylene glycol induced rat

DISCUSSION

In the present study, we investigate the anti urolithiatic activities of various extract of *Azima tetracantha* in male wistar rats. Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies have shown that kidney stone formation in female rats was significantly less than male rats **[26]**. The preliminary phytochemical evaluation of *Azima tetracantha* confirmed the presence of phenolic compounds, flavonoid, alkaloids and tannins. Previous study supports for our present study the plant extract enriched with phenolic compounds, flavonoids, and isoflavonoids can lead to relaxation of smooth muscle of the urinary and biliary tract which could enable the ejection of stones from the kidney and reduced the size of calculi in rats **[27]**. Tannins, saponins and flavonoids these constituents may be responsible for the reduction in super saturation of oxalate in tissue by diuretic or protection of cells **[28]**. The glomerular filtration rate (GFR) is an important parameter for ensuring renal function and it gets decreased in urolithiasis due to the obstruction to the outflow of the urine by stones in urinary system, which leads to a rise in nitrogenous waste products like urea, creatinine, and uric acid in blood **[29]**. In calculi-induced rats, elevated serum levels of creatinine, urea, uric acid, BUN and decreased in urinary volume were observed. The treatment with extracts of *Azima tetracantha* restores the urine volume and nitrogenous waste products urea, creatinine and uric acid levels to normal thus reducing the risk of kidney stone formation.

Previously reported studies have proved that repeated administration of ethylene glycol (0.75% v/v) for 28 days cause generation of kidney stone and the most important cause for it was found to be presence of calcium oxalate **[30, 31]**. In the present study, oxalate and calcium excretion are progressively increased in calculi induced animals. Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria **[32]**. The rats were treated with of *Azima tetracantha* reduced both calcium and oxalate levels, which are known to prove beneficial in preventing the calculi formation due to supersaturation of these substance. An increase in urinary phosphate was observed in calculi induced rats. Increased urinary phosphate excretion along with oxalate stress helps in stone formation by generating calcium phosphate crystals, which ultimately induces calcium

oxalate deposition **[33]**. Treatment of extracts of *Azima tetracantha* decreased the phosphate levels, thus reducing risk of stone formation. Normal constituents of urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well- known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats **[34]**. Urinary magnesium was significantly diminished in ethylene glycol induced urolithic rats. The magnesium levels restore to normal level when treated with *Azima tetracantha*.

The ethylene glycol treated rats showed increased the AST and ALT levels were observed in serum as well as in kidney tissue. This can be attributed to the damaged structural integrity of the renal cells causing the enzymes which are located in the cytoplasm to be released into the circulation **[35]**. The results are in the agreement with the finding if serum and renal ACP and ALT levels were increased due to administration of EG might be due to leakage of the enzyme into the general circulation from the collateral circulation **[36]**. The administration of extracts *Azima tetracantha* decreased the elevated levels of AST and ALT compared to EG treatment group. LDH, a renal cytoplasmic enzyme, is one of the enzymes for various diseases of the urinary tract. Under lithogenic condition, its activity has been reported to increase in urine **[37]**. In the present investigation, LDH being an oxalate-synthesizing enzyme is found to be inhibited by the external milieu of oxalate reaching the kidney, leading to its increased excretion. Treatment with the extracts of *Azima tetracantha* reduces the LDH level in both serum and kidney, thereby relieving the kidney of the high oxalate content.

Histopathological evaluation of kidney through microscopic examination showed accumulation of calcium oxalate deposits inside the tubules in caliculi induced animals. Marked changes such as dilation of proximal tubule along with interstitial inflammation were observed in urolithiatic rats. The rat treated with various extracts of *Azima tetracantha,* significantly attenuates the number and size of calcium oxalate deposits in different parts of renal tubule and also reduced damage to the renal tubules. Therefore with the reference of above parameters, the plant *Azima tetracantha* has proven the anti-urolithiatic property. Moreover, the ethanolic extract shows more protective against ethylene glycol induced renal calculi. From the above results indicates administration of *Azima tetracantha* reduced and prevented the growth of urinary

stones. The mechanism underlying this effect is possibly mediated through the nephroprotective properties of chemical constituents present in *Azima tetracantha*. However, it requires more investigation to clarify the exact mechanism of this action.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest to reveal.

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