



ASSESSMENT OF THE ANTILITHIATIC POTENTIAL OF METHANOL EXTRACT OF *TEPHROSIA PUMILA* AGAINST IN VITRO AND IN VIVO MODELS

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ABSTRACT

The current research was carried out to test anti-urolithiatic potentials of methanol extract of ariel parts of *Tephrosia pumila* (METP) against sodium oxalate produced kidney stones in albino wistar rats. The dried ariel parts of *Tephrosia pumila* was powdered, defatted and subjected to methanol extraction using soxhlet apparatus. The urolithiasis was induced in experimental animals by administering sodium oxalate (70 mg/kg, i.p) for 10 days and simultaneously animals were treated orally with METP at 100 mg/kg, 200 mg/kg and 400 mg/kg daily 1hour before the administration of sodium oxalate for 21 days. On 21st day, 18 hours urine and blood samples were collected and animals were sacrificed under light ether anesthesia. Both urine and serum samples were estimated for urea, creatinine, uric acid, sodium, potassium, chlorides, calcium and BUN. The kidney samples were examined for amounts of lipid peroxidation and antioxidant enzymes. Kidney tissue was also evaluated for the presence. In vitro anti-urolithiatic activity was determined for the extracts by nucleation, aggregation and oxalate depletion assay. The significant elevation of serum and reduction in urine parameters was observed in toxic control animals indicated the development of nephrolithiasis in the toxic control animals which were confirmed in the histopathological evaluation. But concomitant administration of METP has significantly enhanced the excretion constituents of through urine and reduced in serum in dose dependent manner. The histopathological evaluation of kidney samples from therapeutic animals has not shown the presence of stones. The results obtained from the present investigation confirmed the beneficiary effect of *Tephrosia pumila* in urolithiasis.

KEYWORDS: *Tephrosia pumila*, Sodium oxalate, urea, creatinine, BUN, Calcium, Serum ions.

INTRODUCTION

The formation of stones in the kidney or anywhere of urinary tract is known as urolithiasis which is the third most common disorder of the urinary tract. Stones can be composed of calcium (80% of all stones), oxalates, urates, cystine, xanthine, phosphate or all of these.^[1,2] Main risk factors that cause the urolithiasis are dietary factors, pre-existing diseases urinary tract infections and environmental factors (regions with a hot and dry climate). The worldwide incidence of nephrolithiasis is quite high and though extensive research studies have conducted on this complication, there is no truly satisfactory mode for the management of calculi. The

combination of surgical and medical approach using extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and antibiotics are employed for the treatment of urolithiasis.^[3,4] These methods are relatively costly, painful and require skilled hands and availability of appropriate instruments. In spite of tremendous advancements in the medicinal field, there is no truly satisfactory drug for the treatment of urolithiasis. Most patients still have to undergo medical surgery to get relieve from this painful disease. Ayurveda, a traditional system of Indian medicine, offers much scope for the successful management of urolithiasis using herbal drugs.^[5,6]

Natural substances such as plants have been used as the rich source of medicine since ancient time. All traditional medicinal systems have documented therapeutic uses of plants in their own ethno botanical texts. The list of drugs obtained from herbal source is fairly large and proved to be useful, though the rationale for their medicinal use is not scientifically proved except for a few medicinal plants and some proprietary composite herbal formulations.^[7-9] Hence there is a necessary to search for a treatment mode which overcomes all the demerits of the present approaches available and provides us a safe and effective relief for kidney stones.^[10-12]

Tephrosia pumila is the plant of *Fabaceae* family having very good medicinal value and extensively used in Ayurveda and folklore medicine for liver disease, renal disorders, diabetes mellitus, ulcers, cancers and etc. The plant also used as diuretic and for the treatment of kidney stones.^[13] However, there is lack of scientific data to support these medicinal activities, the present study was designed to evaluate the anti-urolithiatic activity of extracts of *Tephrosia pumila* against sodium oxalate induced urolithiasis in rats.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in present study were of analytical grade. The standard drug Cystone a polyherbal formulation was obtained from Himalya Dug Company, Bangalore. The diagnostic reagent kits for the estimation serum and urine parameters and sodium oxalate were obtained from SPAN diagnostics, Bangalore.

Preparation of plant extract

The ariel parts plant *Tephrosia pumila* Linn were collected in Sri Venkateswara university, Tirupati, Andhra Pradesh and authenticated by Dr.Madhava Chetty, Department of Botany, Sri Venkateswara university, Tirupati, Andhra Pradesh and authenticated by Dr.Madhava Chetty. The plant leaves were shade dried and powdered; the coarse powder was subjected to successive extraction with petroleum ether and methanol (70%). Then marc was subjected to extraction using chloroform water as solvent.^[14]

Preliminary phytochemical studies

The methanol extract of *Tephrosia pumila* was subjected to preliminary phytochemical investigation as per the procedure described by Khandewal. Dragondroff's reagent was used to detect presence of alkaloids. Neutral ferric chloride was used to detect phenolic compounds that appear in the form of blue spots. Folin Ciocalteau test and Fiegl' test was used to detect flavonoids and glycosides, respectively.^[15,16]

Animals

Healthy adult Wistar rats weighing 180-200 was purchased from the Venkateswara Enterprises,

Bangalore. The animals were housed in well ventilated cage and animals had 12 hours day and night schedule with temperature between $28 \pm 2^{\circ}\text{C}$. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were allowed free access to standard laboratory pellets and drinking water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (Ref.no.IJAHSM/IAEC/2014/003) with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Evaluation of *in vivo* anti-urolithiatic activity

Experimental procedure

The study was designed to find out the effect of methanol extract of *Tephrosia pumila* on therapeutic usage against sodium oxalate induced urolithiasis. The animal grouping and study design was as follows. The doses of METP were selected from previous studies and details of treatment protocol given below.^[17-20]

Group I-Normal control: Given with normal saline (2ml/kg, p.o) alone for 21 days,

Group II-Toxic control: Given with Sodium oxalate (70mg/kg, i.p) for first 10 days and 2% Tween20 as vehicle for 21 days.

Group-III Standard: Given with Sodium oxalate (70mg/kg, i.p) for first 10 days and Cystone 500mg/kg, p.o for 21 days.

Group IV-METP (100mg): Given with Sodium oxalate (70mg/kg, i.p) for first 10 days and methanol extract of *Tephrosia pumila* 100mg/kg, p.o for 21 days.

Group V METP (200mg): Given with Sodium oxalate (70mg/kg, i.p) for first 10 days and methanol extract of *Tephrosia pumila* 200mg/kg, p.o for 21 days.

Group VI METP (400mg): Given with Sodium oxalate (70mg/kg, i.p) for first 10 days and methanol extract of *Tephrosia pumila* 400mg/kg, p.o for 21 days.

All the albino rats except, Group I were administered with sodium oxalate (70 mg/kg, i.p.) one after extract or cystone treatment, daily for 10 days. All experimental animals were housed in specially designed metabolic cages individually for the entire experimental duration. The suspensions of standard drug Cystone and METP were prepared using tween20 as vehicle administered orally. The urine of each albino rat was collected on the 10th day after 6 h of sodium oxalate injection with thymol as a preservative. The blood samples were collected by puncturing retro-orbital vein under light ether anesthesia immediately after the collection of urine. The collected blood and urine samples were estimated for following various parameters.^[17-20]

Urine parameters: Volume of urine, pH of urine, concentrations of creatinine, urea, uric acid, sodium, potassium, chlorides, calcium and urine microscopy.

Serum parameters: Serum concentrations of creatinine, urea, blood urea nitrogen (BUN), uric acid, sodium, potassium, chlorides, calcium.

Histopathological evaluation

All the experimental animals were sacrificed by giving over dose of ether and autopsied to collect the kidney and liver samples. The right kidney was investigated for the presence of calcium oxalate crystals and stone formation by histological method.

Estimation of lipid peroxidation and antioxidant enzymes

The left kidneys of three experimental rats in each group were homogenized using Tris buffer and the homogenate obtained after centrifugation at 4000 rpm was used to estimate the concentration of calcium oxalate in the kidney. The three left kidneys of the remaining animals and the livers of all six animals of the respective group were perfused with KCl and homogenized with KCl (0.15 M) solution. The homogenate collected after centrifugation at 7000 rpm was used to determine the lipid peroxidase and liver antioxidants such as glutathione peroxidase, glutathione reductase, catalase peroxidase and superoxide dismutase.

Histopathological examination of kidney samples

After exsanguinations, the right kidney was swiftly removed and washed with ice-cold saline. The kidney samples were fixed with 10% formaldehyde, dried in a graduated series of alcohol, and embedded in paraffin wax prior to sectioning. After being dewaxed and rehydrated, the tissue was divided into sections that were each about 5 m thick. The sections were then stained with haematoxylin-eosin dye and checked for histological changes using a light microscope. A 100X magnification was used to analyse each sample.^[21,22]

Determination of *in vitro* anti-urolithiatic activity

Nucleation assay

The effect of *Tephrosia pumila* ethanol extract on calcium oxalate (CaOx) crystal formation was investigated using the nucleation assay, as described by a prior study by Patel et al. (2012). Calcium chloride (CaCl₂) (5 mM) and sodium oxalate solution (Na₂C₂O₄) (7.5 mM) were generated in Tris-HCl (0.5 M) and NaCl (0.15 M) buffer (pH 6.5). The methanol extract and Cystone (Himalaya Herbal Healthcare, India) were diluted in distilled water at concentrations of 100–1000 g/mL. In each millilitre, solutions of METP and Cystone were combined in concentrations of 100, 200, 400, 600, 800, and 1000 mg/mL. The next step was to add 3 mL of calcium chloride and 3 mL of sodium carbonate solution. The mixtures were baked for 30 minutes at 37 °C, then chilled to room temperature. The optical density (OD) at 620 nm (INESA L6S) of the mixtures was then measured using a spectrophotometer.^[23,24] The extract and Cystone's % inhibition of nucleation were calculated using the procedure below.

$$\% \text{ of Inhibition} = 1 - \frac{\text{OD test}}{\text{OD control}} \times 100$$

Aggregation assay

The aggregation assay was performed to assess how our methanol extract and Cystone affects CaOx crystal aggregation (2018). 50 mM solutions of CaCl₂ and Na₂C₂O₄ were combined, heated to 60 °C in a water bath for 1 hour, and then incubated at 37 °C overnight. After drying, a 0.05 M Tris-HCl and 0.5 M NaCl buffer containing a CaOx crystal solution (0.8 mg/mL) was created (pH 6.5). Three millilitres of CaOx solution were mixed with one millilitre of each dilution (100–1000 g/mL) of the Cystone® and Boldoa extract before being vortexed and incubated for 30 minutes at 37 °C. The percentage inhibition of aggregation was computed in the same way as for the nucleation experiment after the OD of the final mixtures was read at 620 nm.^[23,24]

Oxalate depletion assay

The oxalate depletion experiment was used to assess how the METP and Cystone affected the development of CaOx crystals. METP and Cystone were made at different quantities (100 g/mL, 500 g/mL, and 1000 g/mL) in distilled water. In a 50 mM sodium acetate buffer, a 1.5 g/mL CaOx crystal slurry was created (pH 5.7). In 1.5 mL of Tris-HCl (10 mM) and NaCl (90 mM) buffer (pH 7.4), 4 mM CaCl₂ and 4 mM Na₂C₂O₄ solutions were added, followed by 30 L of CaOx crystal slurry. The rate of oxalate depletion from the solution was then measured at 214 nm for 10 min to determine the development of CaOx crystals. By adding 1 mL of the TVME and Cystone at concentrations of 100 g/mL, 500 g/mL, and 1000 g/mL to the reaction mixture, variations in OD were once more noted to assess the influence of each concentration on crystal development. Then, according to the instructions for the nucleation assay, the percentage inhibition of crystal growth was computed.^[23,24]

RESULTS AND DISCUSSION

Preliminary phytochemical study

The study revealed that, consist of glycosides, alkaloids, flavonoids, tannins and phenolic compounds (Table 1).

Table 1: List of primary phytoconstituents present in METP.

Sl. No	Phyto-constituent	Presence
1.	Carbohydrates	++
2.	Glycosides	+++
3.	Poly phenols	+++
4.	Proteins	++
5.	Alkaloids	+
6.	Flavonoids	+++

Evaluation of *in vivo* anti-urolithiatic potentials of METP

Effect of METP on urinary output and pH

The pH and volume of urine belongs to control animals were significantly (P<0.05) decreased due to sodium

oxalate induced nephrolithiasis in comparison with normal rats but pH and urine volume were significantly ($P<0.05$) reduced in therapeutic animals given with

cystone and METP at 200mg/kg dose and 400mg/kg dose. But significant changes were not seen animals treated with low dose of METP (Table 2).

Table No 2: Effect of METP on urine pH and volume against sodium oxalate induced urolithiasis in rats.

Treatment	pH	Volume of urine (ml)
Normal Control	3.452±0.2	7.61±0.54
Toxic Control	1.635 ^a ±0.1	4.65 ^a ±0.39
Standard (Cystone 500mg/kg)	7.448 ^b ±0.28	7.39 ^b ±0.33
METP 100 mg/kg	1.99±0.05	4.64±0.35
METP 200 mg/kg	3.01 ^c ±0.06	5.94 ^c ±0.19
METP 400 mg/kg	2.450 ^c ±0.09	7.33 ^c ±0.49

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^ap<0.05, Toxic control vs Normal control

^bp<0.05, Standard vs Toxic control and ^cp<0.05, Extract treated vs Toxic control

Effect of METP on urinary parameters

The investigation of urine revealed the significant ($P<0.05$) decline in amount of various components creatinine, urea, uric acid, sodium, potassium, chlorides but increased concentrations of total oxalate and calcium in disease control animals when compared to normal rats. While animals pretreated with cystone and METP (100mg/kg and 200mg/kg) have exhibited significantly ($P<0.05$) higher ranges of creatinine, urea, uric acid, potassium, sodium and chlorides and decreased concentration of calcium and total oxalate in urine. There were no considerable changes in urine parameters in animals given METP at with low doses (Table 3).

Effect of METP on Serum parameters

The sodium oxalate produced urolithiasis caused significant ($P<0.05$) elevation of blood constituents creatinine, urea, BUN, uric acid, sodium, potassium, chlorides and calcium in experimental animals of vehicle control group when compared with normal animals. Besides the animals treated with cystone, medium dose and high dose of METP have significant ($P<0.05$) exhibited decline in plasma concentrations of above mentioned parameters creatinine, urea, BUN, uric acid, sodium, potassium, chlorides and calcium when collate with disease control animals. But there was significant alteration was not observed animals treated with low dose of METP (Table 4).

Table No 2: Effect of METP on urine parameters against sodium oxalate induced urolithiasis in rats.

Treatment	Urine parameters						
	Creatinine	Urea	Uric acid	Sodium	Potassium	Chlorides	Calcium
Normal Control	0.76±0.04	15.37±0.92	4.45 ±0.2	138.2 ±0.8	48.68±1.9	73.19±2.9	5.733±0.25
Toxic Control	0.33 ^a ±0.02	6.89 ^a ±0.1	1.7 ^a ±0.1	77.05 ^a ±2.0	23.8 ^a ±0.8	33.36 ^a ±1.2	9.03 ^a ±0.3
Standard Cystone 500mg	0.71 ^b ±0.02	13.08 ^b ±0.7	4.36 ^b ±0.2	142.0 ^b ±2.4	47.50 ^b ±1.4	70.09 ^b ±3.6	5.64 ^b ±0.3
METP 100 mg/kg	0.35±0.02	8.322±0.3	1.77±0.7	71.56 ±2.8	26.87 ±1.3	34.95±0.4	8.585±0.3
METP 200 mg/kg	0.48 ^c ±0.025	9.88 ^c ±0.4	2.8 ^c ±0.3	139.0 ^c ±0.788	34.91 ^c ±0.7	40.86 ^c ±0.6	8.35 ^c ±0.4
METP 400 mg/kg	0.7 ^c ±0.014	13.9 ^c ±0.5	4.19 ^c ±0	138.31 ^c ±1.1	44.6 ^c ±0.9	65.14 ^c ±1.6	6.37 ^c ±0.5

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^ap<0.05, Toxic control vs Normal control

^bp<0.05, Standard vs Toxic control and ^cp<0.05, Extract treated vs Toxic control

Table No 3: Effect of METP on serum parameters against sodium oxalate induced urolithiasis in rats.

Treatment	Serum parameters							
	Creatinine	Urea	BUN	Uric acid	Sodium	Potassium	Chlorides	Calcium
Normal Control	0.67±0.03	30.46±1.5	14.233±0.73	2.568±0.16	133.8±1.4	5.212 ±0.42	79.92 ±1.0	2.228±0.1
Toxic Control	2.2 ^a ±0.1	54.89 ^a ±2.09	25.649 ^a ±0.9	4.615 ^a ±0.2	234.4 ^a ±3.6	12.23 ^a ±0.6	140.0 ^a ±1.5	4.48 ^a ±0.1
Standard (Cystone 500mg/kg)	0.69 ^b ±0.0	26.9 ^b ±1.5	12.6 ^b ±0.7	2.9 ^b ±0.07	134.5 ^b ±2.0	5.018 ^b ±0.1	80.58 ^b ±0.4	2.26 ^b ±0.2
METP 100 mg/kg	2.1 ±0.0	51.80 ±1.	24.205±0.6	4.375±0.1	223.4±3.8	11.77±0.19	136.3±1.6	4.595±0.1
METP 200 mg/kg	1.7 ^c ±0.04	40.3 ^c ±0.73	18.8 ^c ±0.34	3.7 ^c ±0.09	221.6 ^c ±3.1	11.34 ^c ±0.27	135.3 ^c ±1.4	3.49 ^c ±0.07
METP 400 mg/kg	1.1 ^c ±0.01	32.51 ^c ±1.	15.1 ^c ±0.5	3.1 ^c ±0.04	207.8 ^c ±1.9	10.53 ^c ±0.1	134. ^c ±2.1	2.3 ^c ±0.1

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

^ap<0.05, Toxic control vs Normal control

^bp<0.05, Standard vs Toxic control and ^cp<0.05, Extract treated vs Toxic control

Effect METP on antioxidant activity

It was found that, there was significant ($P<0.05$) reduction in concentration of antioxidants GPX, CAP, GSD, GRD in toxic control animals treated with sodium oxalate alone compare to normal animals. But animals of treated with sylimarine and METC (200 & 400 mg/kg), have exhibited significant ($P<0.001$) rise in liver antioxidant enzyme compare to toxic animals (Table 5).

Effect METP on antioxidant enzymes

There was significant ($P<0.05$) rise concentration of lipid peroxidation in toxic control animals treated with sodium oxalate alone compare to normal animals. While animals of therapeutic groups treated with scystone and METP (200mg/kg and 400 mg/kg), have shown significant ($P<0.05$) rise in liver antioxidant enzyme compare to toxic animals (Table 5).

Administration of TPME reduced the formation of lipid peroxidation and increased the concentrations of antioxidant enzymes in the renal tissue which indicates its ability scavenge free radicals which is also an important mechanism for antilithiatic properties.

Histopathological evaluation

The histopathological examination of sample of kidney samples from urolithiatic control rats treated with sodium oxalate alone have exhibited moderate crystals (++) in 2 out of 6 and maximum crystals deposition (+++) in 4 out of 6 animals with severe hydrophobic degeneration of the proximal convoluted and dilated collecting tubules. These kidney samples have also showed edema and congestion of interstitial tissue and inflammation of the pelvis. There was severe congestion of the glomeruli and

blood vessels. While therapeutic group animals treated with standard drug cystone and METP (at 100 and 200mg/kg) have exhibited only congestion of blood vessels and mild hydropic degeneration with no deposition of oxalate crystals (Figure 1).

Evaluation of *in vitro* anti-urolithiatic potentials

In the nucleation assay, the formation of many calcium oxalate crystals was caused by the addition of sodium oxalate solution to the reaction mixture containing calcium chloride. The control group was found to have a substantial number of calcium oxalate monohydrate (COM) crystals that were either rectangular in shape or dendrites with sharp edges. Calcium oxalate dihydrate (COD) crystals with a smoother morphology were produced when the methanol extract was used at greater concentrations and the reference standard cystone at lower concentrations (Fig. 3). Both Cystone and the methanol extract decreased the quantity and size of calcium oxalate. Calcium oxalate crystal size reduction obtained for the METP (72.38%) was equivalent to that produced by standard medication (71.654%). In the aggregation assay, the aggregation of preexisting calcium oxalate crystals was significantly reduced by the METP ($P 0.05$). In comparison to the conventional treatment, the METP produced a reduction in aggregation that was 76.82% higher at 1000 g/mL (Fig. 4). In the oxalate depletion assay, METP reduced calcium oxalate crystal formation by 61.38% at a concentration of 1000 μ g/mL, while Cystone showed a 60.84% reduction. At all tested concentrations, METP demonstrated an inhibitory effect on calcium oxalate crystal formation comparable to that of Cystone. (Figure 2 and 3).

Table No 4: Effect of METP on liver antioxidant enzymes and lipid peroxidation sodium oxalate induced urolithiasis in rats in rats.

Treatment	Liver Enzymes (mg/G)					
	LOP	GPX	SOD	GRD	GST	CAP
Normal	7.42 $\pm$ 0.2	8.77 $\pm$ 0.31	9.32 $\pm$ 0.85	4.11 $\pm$ 0.31	7.22 $\pm$ 0.33	58.22 $\pm$ 2.2
Toxic Control	16.94 ^a $\pm$ 0.3	4.81 ^a $\pm$ 0.24	5.52 ^a $\pm$ 0.68	2.32 ^a $\pm$ 0.19	3.51 ^a $\pm$ 0.18	30.75 ^a $\pm$ 1.3
Standard	10.19 ^b $\pm$ 0.21	8.21 ^b $\pm$ 0.22	8.59 ^b $\pm$ 0.77	3.78 ^b $\pm$ 0.15	6.64 ^b $\pm$ 0.39	50.25 ^b $\pm$ 1.4
METP 100 mg/kg	15.32 $\pm$ 0.28	4.33 $\pm$ 0.21	5.66 $\pm$ 0.67	2.59 $\pm$ 0.23	4.11 $\pm$ 0.11	33.62 $\pm$ 2.8
METP 200 mg/kg	8.15 ^c $\pm$ 0.18	6.85 ^c $\pm$ 0.20	6.94 ^c $\pm$ 0.48	4.37 ^c $\pm$ 0.25	5.68 ^c $\pm$ 0.23	42.82 ^c $\pm$ 2.1
METP 400 mg/kg	7.76 $\pm$ 0.26	8.42 ^c $\pm$ 0.24	8.84 ^c $\pm$ 0.27	4.76 ^c $\pm$ 0.19	7.67 ^c $\pm$ 0.31	53.55 ^c $\pm$ 1.9

Values are mean $\pm$ S.E.M, n=6 symbols represent statistical significance.

^a $p<0.05$, Toxic control vs Normal control

^b $p<0.05$, Standard vs Toxic control and ^c $p<0.05$, Extract treated vs Toxic control

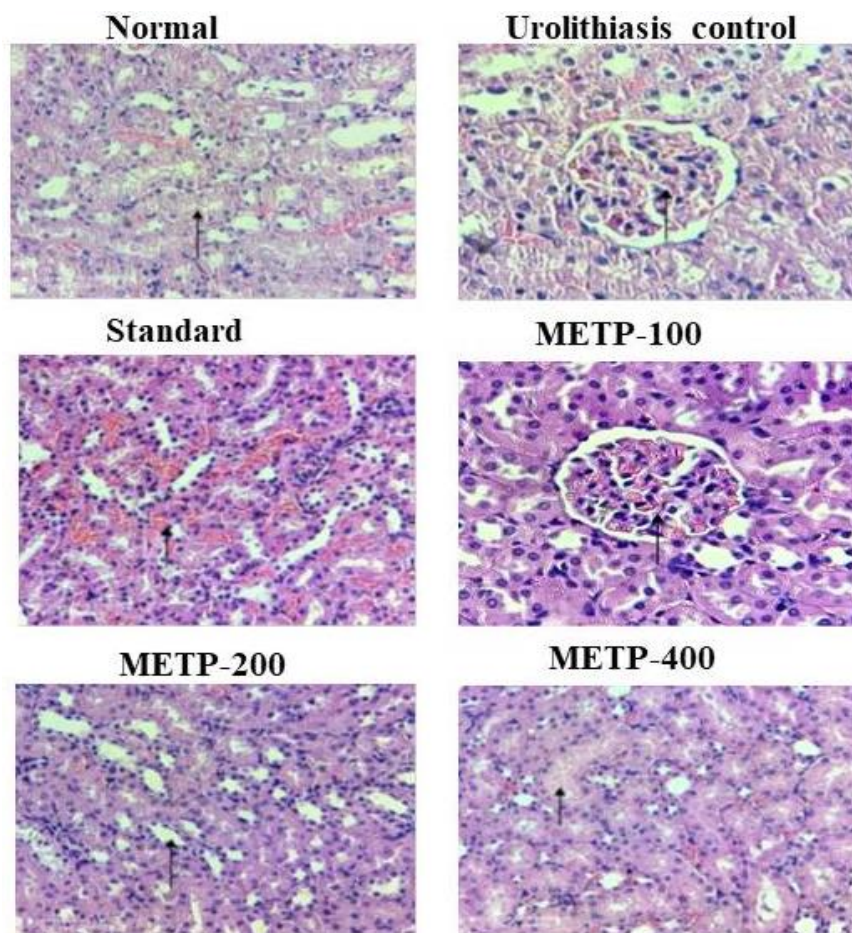


Figure No. 1: Histopathological Examination of kidney.

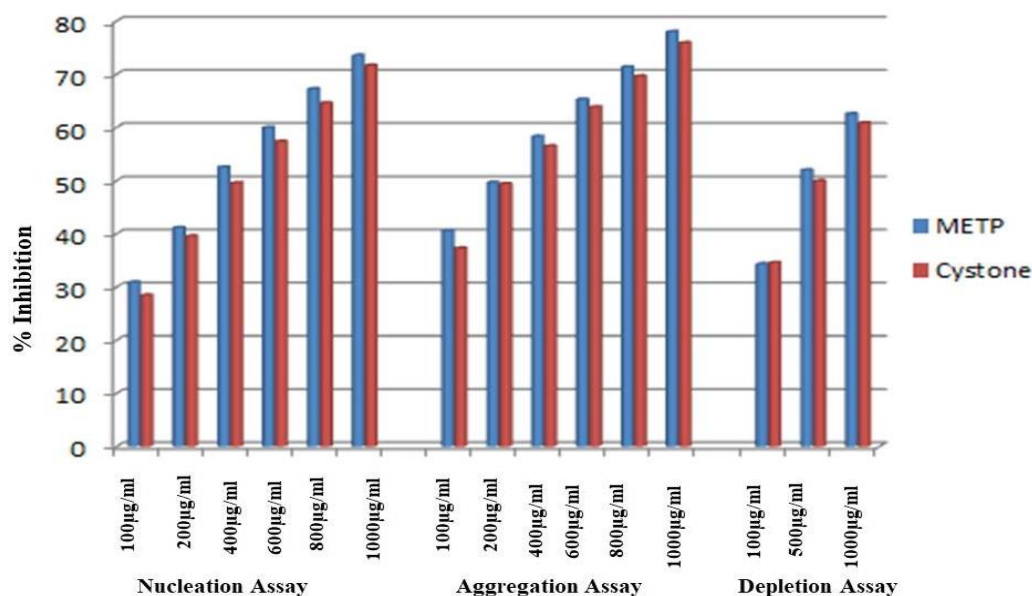


Figure 2: Percentage of inhibition of crystal formation in presence of METP on in vitro anti-urolithiatic activity.

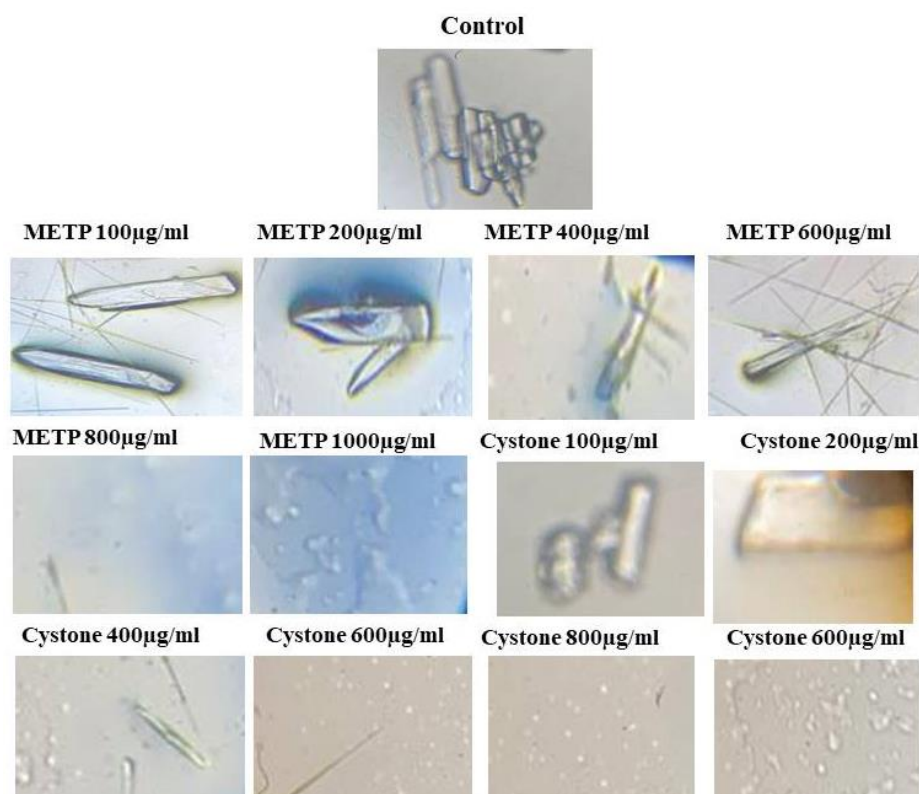


Figure 3: Inhibitory effect of METP on in vitro anti-urolithiatic activity showing crystals.

DISCUSSION

Urolithiasis is a common renal disorder characterized by the formation of calcium oxalate stones due to urinary supersaturation, oxidative stress, and crystal aggregation. Experimental induction using sodium oxalate mimics human nephrolithiasis. Herbal extracts with diuretic and antioxidant properties may prevent stone formation by inhibiting crystallization and protecting renal function.^[25,26]

In the present investigation, we evaluated the protective effect of the methanolic extract of *Tephrosia pumila* (METP) against sodium oxalate-induced nephrolithiasis in albino Wistar rats. Sodium oxalate administration (70 mg/kg) increases systemic oxalate levels, which readily combine with calcium ions to form insoluble calcium oxalate crystals. Excess oxalate excretion in urine leads to supersaturation, crystal nucleation, aggregation, and subsequent deposition in renal tissues. Accumulation of calcium oxalate crystals disrupts normal renal architecture and impairs kidney function.

In the disease control group, sodium oxalate caused a significant reduction in urine output along with elevated serum levels of urea, creatinine, uric acid, and blood urea nitrogen (BUN), indicating renal dysfunction and azotemia. These alterations are attributed to impaired glomerular filtration and decreased elimination of nitrogenous waste products. Treatment with METP significantly improved urine volume and enhanced the excretion of nitrogenous metabolites, thereby reducing

their serum concentrations and ameliorating renal impairment.

Nephrolithiasis is primarily associated with increased urinary concentrations of calcium and oxalate, leading to supersaturation and stone formation. In the present study, disease control animals exhibited elevated urinary calcium and oxalate levels, promoting crystal formation. Administration of METP significantly reduced urinary calcium and oxalate concentrations compared to toxic control animals. This effect may be attributed to increased urine output and consequent dilution of lithogenic constituents, thereby preventing supersaturation and crystal deposition.^[27,28, 29]

Furthermore, METP treatment enhanced renal antioxidant status and significantly reduced lipid peroxidation, suggesting that its renoprotective effect may be mediated through attenuation of oxidative stress. Histopathological examination of kidney sections from extract-treated animals revealed preservation of normal renal architecture with minimal or no crystal deposition, whereas sodium oxalate-treated control animals showed extensive calcium oxalate crystal accumulation and tissue damage.

The extract was also evaluated for its in vitro antilithiatic activity using nucleation, aggregation, and crystal growth (depletion) assays. In all three models, METP significantly inhibited calcium oxalate crystal formation and aggregation, demonstrating its potential to interfere with key steps involved in stone formation. Overall, the

findings suggest that the methanolic extract of *Tephrosia pumila* possesses significant in vivo and in vitro antilithiatic activity, possibly mediated through diuretic, antioxidant, and crystal growth inhibitory mechanisms.

CONCLUSION

The data obtained from the current investigation suggesting that the methanol extract of *Tephrosia pumila* possess significant anti-lithiatic potentials against sodium oxalate induced urolithiasis in wistar rats and also *in vitro*.

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Conflict of Interest

We are hereby declaring that there is no conflict of interest with respect to above research work.

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