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EVALUATION OF ANTI-UROLITHIACTIC ACTIVITY OF ETHANOLIC EXTRACT OF *PHYLLANTHUS URINARIA* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS

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ABSTRACT

Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–81% in males, and 47–60% in females. It is one of the third most common afflictions found in humans. The effect of oral administration of ethanolic extract of Phyllanthus urinaria has been studied in Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of serum calcium, creatinine, urea, uric acid, magnesium and phosphorus. Supplementation with ethanolic extract of Phyllanthus urinaria significantly reduced the elevated urinary oxalate, uric acid serum creatinine and serum calcium, creatinine, urea, uric acid, magnesium and phosphorus. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by ethanolic extract of Phyllanthus urinaria. The results indicate that the ethanolic extract Phyllanthus urinaria is endowed with antiurolithic activity.

KEYWORDS: Phyllanthus urinaria, Hyperoxaluria; Urolithiasis; Ethylene glycol.

INTRODUCTION

1. Introduction of Phyllanthus urinaria

1.1 Plant material

Phyllanthus urinaria is an annual plant with the main stem erect, unbranched or sparsely branched and seldom more than a foot tall. The branch at sides are with their two rows of

alternate leave resemble a compound leaf. The leaves themselves are finely hairy, nearly sessile (stalkless), oblong to narrowly obovate.

1.2 Ayurveda Properties

- Rasa (taste):bitter, astringent, sweet
- Virya (action):cooling
- Vipaka (post-digestive effect) : sweet
- Guna (quality) : dry light
- Dosha effect : pk-, V+
- Dhatu (tissue): plasma, blood, fat, reproductive
- Srota (channel): digestive, reproductive, urinary.

Phyllanthus urinaria (P. urinaria), one of the herbal plants belonging to the genus Phyllanthus (Euphorbiaceae), is widely distributed in China, Southern India and Southern America. It has long been used in folk medicine for the treatment of several diseases such as hepatitis B, nephrolithiasis and in painful disorders. The plant has traditional uses which include Diabetes, Dysentery, Flu, Headache, Fever, Conjunctivitis, Menstrual Disorder, Anticancer, Blenorrghia**,** Jaundice, Vaginitis and Dyspepsia. Urolithiasis, one of the most painful ailments of the urinary tract disorder, has beset humans from centuries. Calcium oxalate (CaOx) is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. The medical management of urolithiasis mainly involves techniques like extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy however; the prevention of recurrence of stone formation is not assured. Hence phytotherapy is being observed.

Kidney stones may contain various combinations of chemicals. The most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles. Kidney stone are composed of crystal and proteins that grow until they break loose and pass into the urine collection system. Stones containing calcium as oxalate, phosphate or both comprise about 80% of total. About 15% contain magnesium ammonium phosphate (Struvite; these are often associated with infection), and small numbers of pure cystine or uric acid stones are found. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events,

beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract.

Cystinuria and hyperoxaluria are two other rare, inherited metabolic disorders that often cause kidney stones. In cystinuria, too much of the amino acid cystine, which does not dissolve in urine, is voided, leading to the formation of stones made of cystine. In patients with hyperoxaluria, the body produces too much oxalate, a salt. When the urine contains more oxalate than can be dissolved, the crystals settle out and form stones. Hypercalciuria is inherited, and it may be the cause of stones in more than half of patients. Calcium is absorbed from food in excess and is lost into the urine. This high level of calcium in the urine causes crystals of calcium oxalate or calcium phosphate to form in the kidneys or elsewhere in the urinary tract.

Therefore, Phyllanthus urinaria became our study target and we prepared the drug with a standardized protocol under regulation and used it for further investigation.

MATERIALS AND METHODS

The evaluation of **anti-urolithiactic activity** was done on wistar albino rats. This research would need different material and equipment's explained as follows:-

2.1 Plant collection

Plant material: The plant of *Phyllanthus urinaria* was collected from the certified ayurvedic wholesaler. The plant was identified and authenticated by Asst. Prof. Dr. K. Madhava chatty, MSc, Head, Department of Botany, S.V. University-Tirupati.

2.2 Preparation of Phyllanthus urinaria extract

The collected fresh plant material was dried in shade (2 days) and then dried in a hot air oven at 25ºC for three days and they were made in to coarse powder with the use of mixer. The powder of *Phyllanthus urinaria* obtained were weighed separately and transferred to a round bottomed flask and then to continuous heat extraction with soxhlet apparatus using 95% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petri plate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 6.29%.

2.3 Equipment

Autoanalyzer (Robonik), Refrigerator centrifuge (MPW-350R), UV-Spectro-phot ometer (UV-1601, Shimadzu Corporation, Kyoto, Japan), Mini Lyotrap (LTE Scientific Ltd.), Research centrifuge (Remi industries, Mumbai) and homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India).

2.4 Phytochemical screening of extract

Phytochemical screening of plant extract shows the presence of carbohydrate, protein, flavonoids, alkaloids and saponins.

2.5 Drugs and Chemicals

- Cystone (Himalaya Pharmaceutical, Bangalore)
- Ethylene glycol (SRL Mumbai)
- Tween 80 (Merck Pvt Ltd, B, Mumbai)
- Anaesthetic ether (SD Fine chem Ltd., Mumbai)
- Chloroform (SD Fine chem Ltd .Mumbai)
- Formaline (SD Fine chem Ltd., Mumbai) and all chemicals and reagents were of analytical grade

2.6 Diagnostic kits

Diagnostic kits used for estimation of Creatinine, Urea, Uric acid, Calcium, Phosphorus, Calcium oxalate were procured from **Robonik Diagnostic Ltd India.**

2.7 Preparation of Acute toxicity study

Procedure: Acute toxicity studies were performed according to OECD-423guidelines category IV substance (acute toxic class method). Swiss albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *Phyllanthus urinaria* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose.

2.8 Pharmacological screening for antiurolithic activity

2.8.1 Animals: Wistar albino male rats (180–220 g) were obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

2.8.2 Experimental Design:-Ethylene glycol induced urolithiasis model

Thirty healthy adult Wistar albino strain rats of either sex weighing 180-220g were randomly divided into five groups. Each group consisted of 6 animals. The treatment period was considered for 10 days.

Group 1: Normal rats were fed with standard rat chow diet and tap water ad libitum for 10 days.

Group 2: EG and ammonium chloride intoxicated rats were given normal lab diet + drinking water containing 0.75% [v/v] ethylene glycol (EG) for 10 days to induce urolithiasis.

Group 3: Standard group were fed with normal diet and drinking water containing 0.75% [v/v] EG and Cystone (5 ml/kg) for 10 days.

Group 4: the test groups treated with ethanolic extract of *Phyllanthus urinaria* 200 mg/kg with normal lab diet and drinking water containing 0.75% [v/v] EG.

Group 5: the test groups treated with ethanolic extract *Phyllanthus urinaria* 400 mg/kg of body weight were fed with normal diet and drinking water containing 0.75% [v/v].

2.8.3 Assessment of Anti-urolithic Activity

Collection and Analysis of Urine: The urine samples of the test animals in different groups were collected in their respective end day of the experiment (1%) EG model on 10th day in (0.75%) EG model. The collected urine sample volume and PH were measured followed by centrifugation at 3000 rpm for 10 minutes. After centrifugation the urine samples were examined under light microscope (LAICA, DME Germany 400X) to ensure the presence of oxalate microcrystal followed by biochemical analysis (urine oxalate, calcium and uric acid, creatinine, urea, magnesium and phosphorus).

Serum Analysis: The blood samples were collected from the animals under anaesthesia (ether) before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium, creatinine, urea, uric acid, magnesium and phosphorus

Kidney homogenate analysis: A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of protein, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA).

Histopathology: Normal rat's kidney showed normal cellular structure. The histopathology of kidney samples of rats treated with EG (0.75) **control** group showed loss of normal architecture with presence of white chalky coloured calcium oxalate crystals in several tubules and glomeruli in sections. The histopathology of kidney rats treated with **standard** drug cystone 5ml/kg and EG for 10 days showed normal architecture of the kidney. The histopathology of kidney samples of rats treated with **EPU 200mg/kg** and EG for 10 days showed mild colloidal cast inside tubules and **EPU 400mg/kg** showed cloudy changes and congestion of these glomeruli.

Statistical analysis: Results were indicated in terms of mean ± SEM. Statistical significance of data were assessed by analysis of variance (One way-ANOVA), followed by comparison between different groups using 'Dunnett's multiple comparison test. The significance was considered at the level of $P<0.05$.

Biochemical parameters: The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyzer.

The results of preliminary phytochemical studies of the plant extract are presented in **Table follows:**

Phytoconstituents	Presence or Absence
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Gums & mucilage	-
Potein & amino acids	-
Saponins	++
Tannins	+
Phytosterols	+
Flavonoids	+++
Alkaloids	+++

Presence: +, Absence: -

3. RESULTS

3.1. Urinary output determination- Effect of ethanolic extract of *Phyllanthus urinaria* on urine biochemical parameters against EG induced urolithiasis.

Urinary Creatinine

Administration of EG (0.75%) for 10 days caused significant increased ($p<0.001$) in urine creatinine concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p<0.01$) in urine creatinine concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction (not significant and $p<0.001$) in urine creatinine concentration when compared to EG alone treated group.

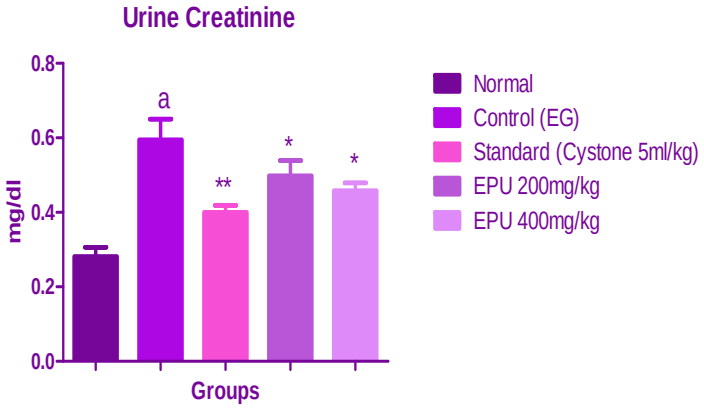


Fig.no.1: Effect of ethanolic extract of *Phyllanthus urinaria* on urine creatinine parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, **p<0.01 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Urea

Administration of EG (0.75%) for 10 days caused significant increased ($p<0.001$) in urine urea concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p<0.001$) in urine urea concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction ($p<0.01$ and $p<0.001$) in urine urea concentration when compared to EG alone treated group.

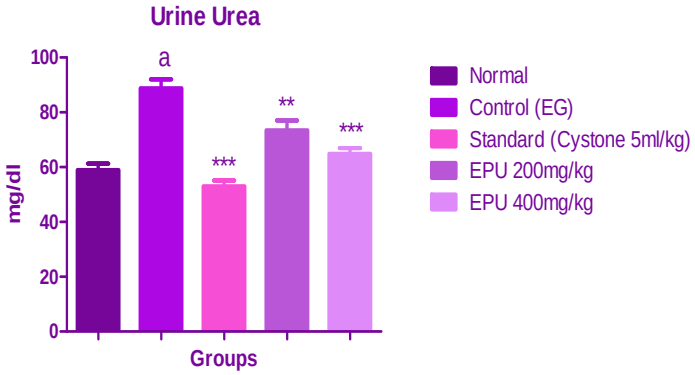


Fig.no.2: Effect of ethanolic extract of *Phyllanthus urinaria* on urine urea parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Calcium

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine calcium concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine calcium concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine calcium concentration when compared to EG alone treated group.

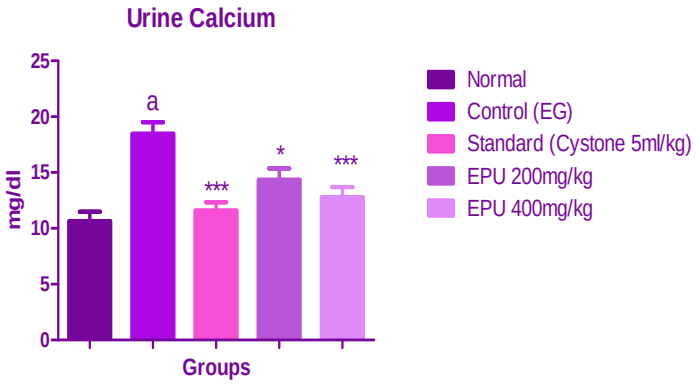


Fig.no.3: Effect of ethanolic extract of *Phyllanthus urinaria* on urine calcium parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Oxalate

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine oxalate concentration compared to normal one. Standard cystone5ml/kg causes significant reduction (p<0.001) in urine oxalate concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction (p<0.001 and p<0.001) in urine oxalate concentration when compared to EG alone treated group.

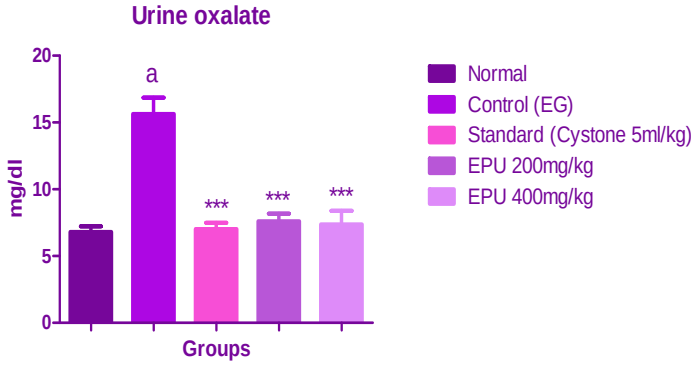


Fig.no.4: Effect of ethanolic extract of *Phyllanthus urinaria* on urine oxalate parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Phosphorus

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine phosphorus concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine phosphorus concentration when compared to EG alone treated.

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine phosphorus concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine phosphorus concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine phosphorus concentration when compared to EG alone treated group.

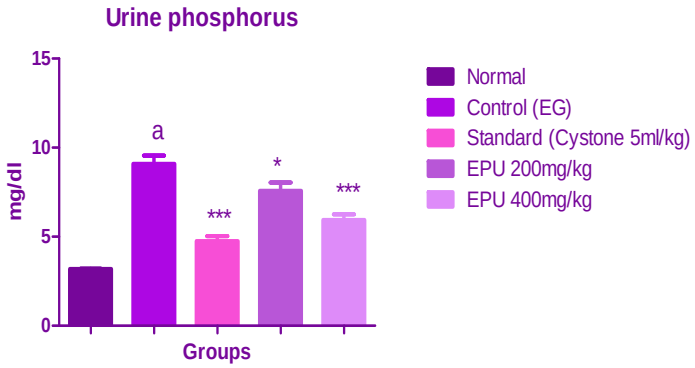


Fig.no.5: Effect of ethanolic extract of *Phyllanthus urinaria* on urine phosphorus parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Magnesium

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine magnesium concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine magnesium concentration when compared to EG alone treated group. Pretreatment with EPU 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine magnesium concentration when compared to EG alone treated group.

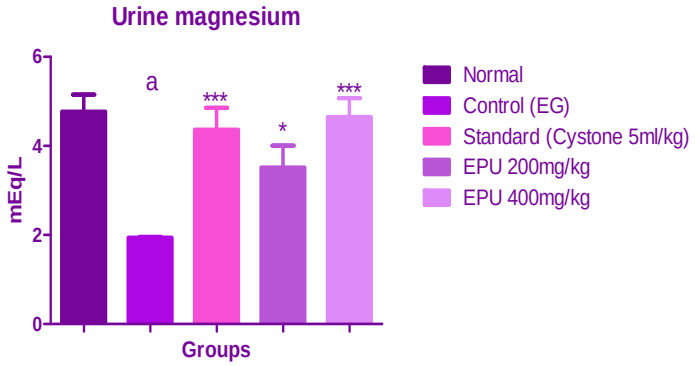


Fig.no.6: Effect of ethanolic extract of *Phyllanthus urinaria* on urine magnesium parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal*

Effect of ethanolic extract of *Phyllanthus urinaria* on urine biochemical parameters against EG induced urolithiasis.

Treatment group	Urine Biochemical Parameters						
Normal	**Creatinine mg/dl**	**Urea mg/dl**	**Uric Acid mg/dl**	**Calcium mg/dl**	**Oxalate mg/dl**	**Phosphorus mg/dl**	**Magnesium mg/dl**
Control (EG 0.75%)	0.28±0.02	58.94± 2.35	2.31± 0.16	10.65± 0.83	6.81± 0.41	3.195± 0.01	4.77± 0.37
Standard Cystone (5ml/kg)	0.59±0.05a	88.82± 3.19a	6.01± 0.28a	18.49± 1.03a	15.65± 1.22a	9.093± 0.46a	1.94± 0.01a
EPU 200mg/kg	0.40±0.01**	53.05± 2.10***	2.99± 0.36***	11.62± 0.72***	7.03± 0.46***	4.758± 0.27***	4.36± 0.48***
EPU 400mg/kg	0.49±0.04ns	73.48± 3.53**	3.56± 0.25***	14.37± 0.98*	7.60± 0.57***	7.58± 0.45*	3.51± 0.48*

3.2 Effect of ethanolic extract of *Phyllanthus urinaria* on LPO (kidney enzyme) parameters against EG induced urolithiasis.

Oxidative stress-In-vivo LPO In control animals, EG induced lithogenesis produced a significant enhancement in the renal MDA levels ($p<0.001$) respectively, when compared to the normal group. After treatment with standard cystone 5ml/kg and EPU 200 and 400mg/kg significant ($P<0.001$) reduction in the kidney MDA levels was observed in the treated groups, when compared to their respective control groups.

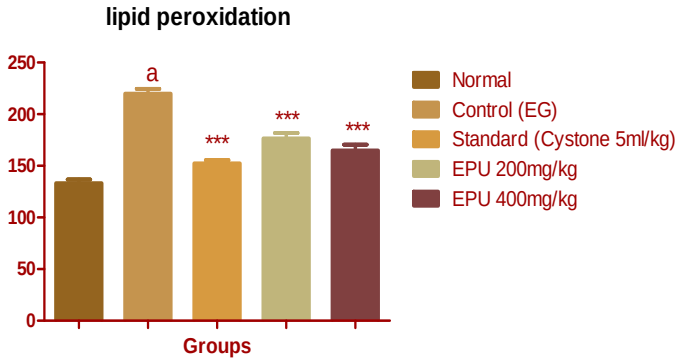


Fig.no.1: Effect of ethanolic extract of *Phyllanthus urinaria* on Lipidperoxide (kidney enzyme) parameters against EG induced urolithiasis.

*All the values are Mean±SEM, One way ANOVA followed by multiple comparison of Dunnett's test, ***$p<0.001$ as compared to control and $^{a}p<0.001$, as when compared to normal.*

Catalase: levels of the kidney were significantly ($p<0.001$) decreased in the control groups on EG administration for 10 days, when compared to the normal group. On treatment with Standard cystone and EPU 200 and 400mg/kg ($p<0.001$), a significant rise in the renal catalase levels was observed in treated groups.

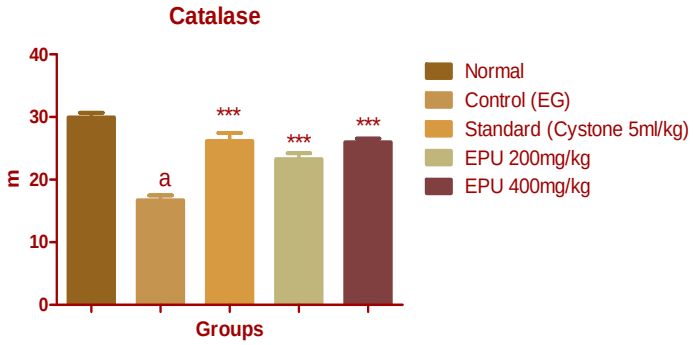


Fig.no.2: Effect of ethanolic extract of *Phyllanthus urinaria* on Catalase (kidney enzyme) parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.* **GSH** levels of the kidney were significantly ($p<0.001$) decreased in the control groups on EG administration for 10 days, when compared to the normal group. On treatment with Standard cystone ($p<0.01$) and EPU 200 ($p<0.05$) and 400mg/kg ($p<0.01$), a significant rise in the renal catalase levels was observed in treated groups.

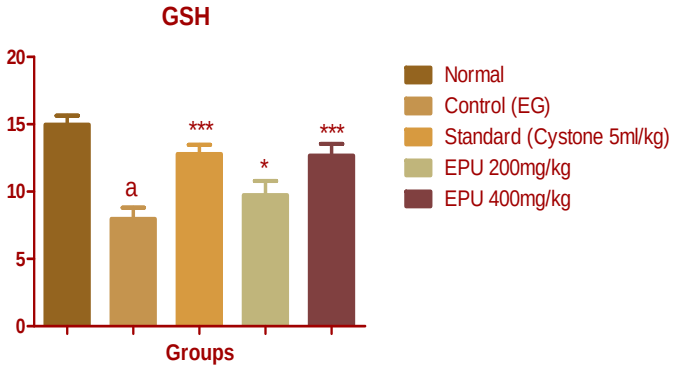


Fig.no.3: Effect of ethanolic extract of *Phyllanthus urinaria* on GSH (kidney enzyme) parameters against EG induced urolithiasis.

Treatment group	*In-vivo* Antioxidant parameters		
	CATALASE µm/mg tissue	GSH µm/mg tissue	LPO µm of /H_2O_2/mg tissue
Normal	29.95±0.72	14.97±0.67	133.1±3.60
Control (EG 0.75%)	16.73±0.74a	7.970±0.83a	219.8±4.65a
Standard Cystone (5ml/kg)	26.19±1.25***	12.80±0.68**	152.3±3.29***
EPU 200mg/kg	23.29±0.92***	9.74±1.04*	176.7±5.05***
EPU 400mg/kg	26.00±0.56***	12.68±0.85**	164.9±5.59***

*All the values are Mean±SEM, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Effect of ethanolic extract of *Phyllanthus urinaria* on antioxidant (kidney enzyme) parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, ns= not significant, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

4. HISTOPATHOLOGY OF KIDNEY

Normal rat's kidney showed normal cellular structure. The histopathology of kidney samples of rats treated with EG (0.75) **control** group showed loss of normal architecture with presence of crystalline structure in dilated collecting tubules. The same section when viewed under polarising microscope revealed presence of white chalky coloured calcium oxalate crystals in several tubules and glomeruli. These groups also showed congestion of intersitium and inflammation of the pelvic calyceal systems.

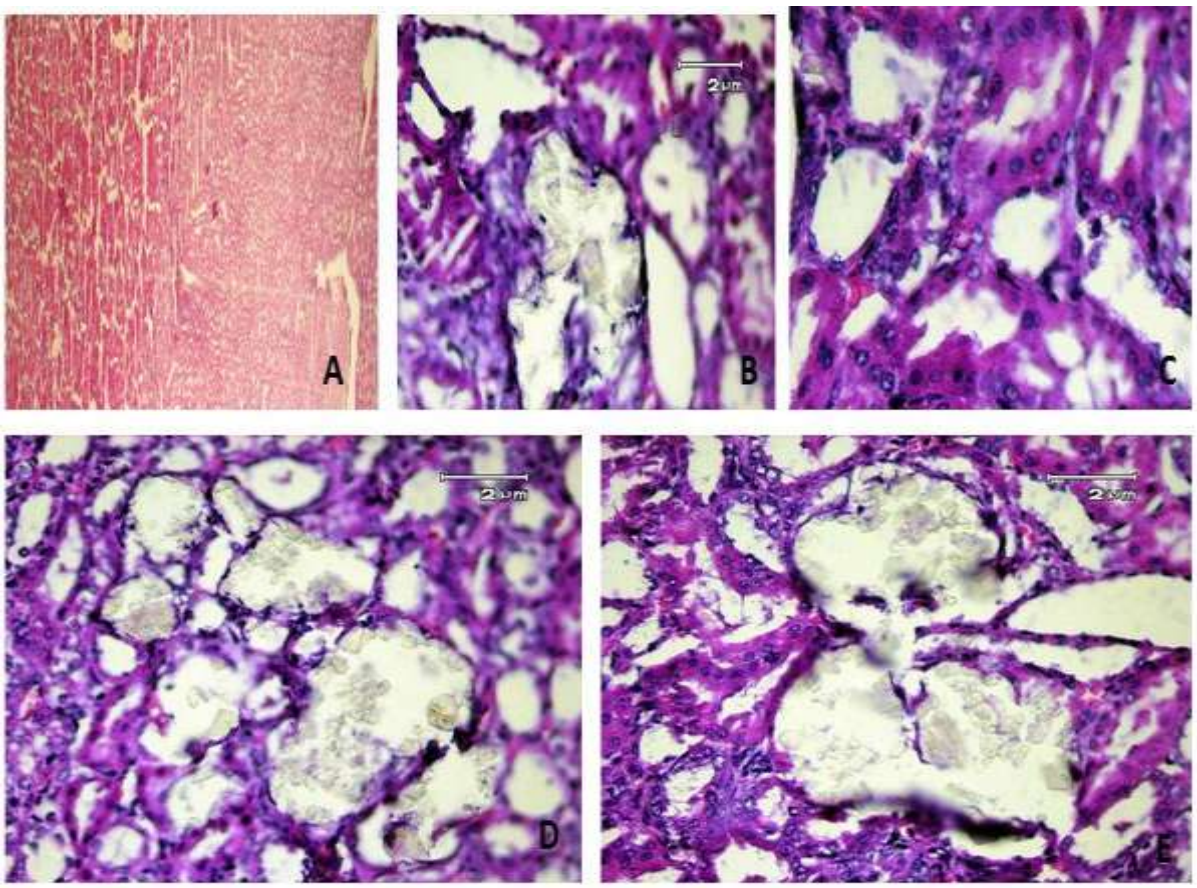
The histopathology of kidney rats treated with **standard** drug cystone 5ml/kg and EG for 10 days showed normal architecture of the kidney.

The histopathology of kidney samples of rats treated with **EPU 200mg/kg** and EG for 10 days showed mild colloidal cast inside tubules and **EPU 400mg/kg** showed cloudy changes and congestion of these glomeruli. However the architecture of kidney was almost normal.

Histopathology of kidney

- A: Normal group
- B: EG group
- C: Standard (Cystone 5ml/kg) group

- D: group EPU 200mg/kg
- E :group EPU 400mg/kg



5. DISCUSSION

Urinary stone disease is a common, painful and expensive medical condition. Though extracorporeal shock wave lithotripsy has facilitated the stone removal and reduced the morbidity associated with urinary stone, recurrence is common. Several experimental and clinical studies on some of the plants used in the Indian traditional system of medicine proved their efficacy in the management of renal stone disease. Therefore, it is advisable to evaluate plants used in the traditional medicine to treat kidney stone disease for Antiurolithiatic activity, which might be also useful in reducing stone recurrence rate.

Rats are commonly used to study the pathogenesis of human CaOx kidney stone disease, as Ox metabolism is regarded almost similar in rats and humans. Ingestion of EG has been found to be a reliable inducer of Ox lithiasis in rats. EG is converted to endogenous oxalic acid by the liver enzyme glycolate oxidase and AC induces urinary acidification, is supposed to upset the enzyme sorting mechanism in the tubular cells in the kidney, thus favors adhesion and retention of CaOx particles within the renal tubules. Hence, in the present

study, EG in drinking water was employed to induce hyperoxaluria in rats. Urinary super saturation in relation to stone forming constituents, mainly urinary oxalate is important in renal calculi formation, as urinary oxalic acid complexes with calcium and forms insoluble CaOx crystals. Enhanced deposition and urinary excretion of calcium and oxalate in the preventive and curative control group animals indicate that administration of EG induced hyperoxaluria. An increase in the kidney weight and enhanced urinary creatinine excretion in the control group animals also substantiated these results.

On administration of EPU, the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinary excretion in control groups implies the potential of EPU in preventing the formation and dissolving the preformed CaOx stones.

On treatment with the extract and standard cystone, the significant reduction in the elevated urinary creatinine, urea, uric acid, calcium, phosphorus, oxalate and magnesium in the treated groups reflects the improvement in hyperoxaluria induced renal impairment. Dissolution of calculi can be achieved by alteration in urinary pH. If the pH is 5.0 or below, the stones likely to form are of uric acid type, if 5.0-6.5, calcium oxalate type and if above 7 indicates crystals of magnesium ammonium phosphate. In the present study, a decrease in the normal urine pH of 7.0-7.5 to 5.5-6.0 in the control groups, indicates hyperoxaluria induced CaOx stone formation. In the treated groups, EPU and cystone 5ml/kg administration restored the pH to 6.5-7.5, supporting the decrease in the deposition and excretion of calcium and oxalate.

Mucoproteins have significant affinity for CaOx surface and promote the growth of crystals and cement them. Flavonoids act by disintegrating the mucoprotiens, thereby prevent calcium and oxalate deposition and excretion. In the present study also, preliminary phytochemical screening of EPU revealed the presence of flavonoids. Thus, in the EPU treated groups, flavonoids might have reduced calcium and oxalate deposition by pre-coating CaOx crystals and disintegrating the mucoproteins. The stone forming effects of EG are also ascribed to its hyperoxaluria induced oxidative damage. Oxalate has been reported to induce LPO and to cause renal tissue damage. As kidney is rich in polyunsaturated fatty acids, is susceptible to ROS attack. Excessive generation of ROS and/or a reduction in cellular antioxidant levels results in the development of OS.

MDA is one of the most common by products of ROS induced OS. In the present study, increased levels of MDA, diminished levels of GSH and catalase in the control groups

indicate that EG administration promoted extensive generation of ROS. The resultant ROS may have consumed GSH and catalase excessively and impaired antioxidant protection. Thus, the unquenched ROS may have provoked cellular damage and resulted in enhanced OS, which might have further favoured the accumulation and retention of oxalate and subsequent deposition of CaOx. Studies show that treatment with antioxidants prevents CaOx deposition in the kidney and reduce Ox excretion. Daily consumption of tea reduced the risk of kidney stone formation in women by 8%. Moreover, low concentration of renal cellular glutathione favours LPO and subsequent retention of calcium and oxalate in the kidneys.

Health benefits of tea are due to its antioxidant properties of flavonoids which act by quenching ROS and also by chelating metal ions like iron and copper. In the present study, lowered levels of MDA and enhanced levels of antioxidant enzymes, GSH and catalase in the kidneys of the EPU treated animals indicate attenuation of hyperoxaluria induced LPO and oxidative damage. Flavonoids may have minimized ROS by free radical scavenging and prevented further generation, by metal chelating property. Thus, the flavonoid principles of *Phyllanthus urinaria* might have been responsible for the inhibition of CaOx crystal aggregation and stone formation. The results support the use of *Phyllanthus urinaria* plant as an effective alternative in treating CaOx urolithiasis. Disintegration of the mucoproteins and pre-coating of CaOx crystals by antioxidant effect of flavonoid principles may be responsible for the possible antiurolithiatic activity of *Phyllanthus urinaria*. Further studies are necessary to find out the chemical components responsible for the antiurolithiatic activity of *Phyllanthus urinaria.*

6. CONCLUSION

In conclusion, the presented data indicate that administration of the EPU plant to rats with ethylene glycol/Ammonium chloride induced lithiasis and prevented the formation of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentration of stone forming constituents. The protective effect against oxalate induced lipid peroxidation may be contributing to the recovery of renal damage. These effects could conclude the antiurolithiatic property of *Phyllanthus urinaria.*

7. SUMMARY

In the present study, dried powder of *Phyllanthus urinaria* was subjected to extraction using 90% ethanol for the extraction. Some extract was reserved for preliminary phytochemical investigation and rest was utilized for pharmacological screening.

Preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, glycosides, saponins, and flavonoids. The pharmacological screening included evaluation of antiurolithiatic activity using 0.75% ethylene glycol induced urolithiasis model in male Wistar albino rats. The kidney stone formation induced in rats, as a result of 10 days chronic administration of 0.75% was significantly inhibited by oral administration of EPU.

Administration of EPU and Cystone 5ml/kg caused significantly increased urine output and pH of the urine as compared to control (EG) group.

Pretreatment with standard cystone 5ml/kg, EPU 200 and 400mg/kg causes significant reduction in serum creatinine, urea, uric acid, calcium, oxalate, phosphorus and magnesium concentration when compared to EG alone treated group.

Pretreatment with standard cystone 5ml/kg, EPU 200 and 400mg/kg causes significant reduction in urinary creatinine, urea, uric acid, calcium, oxalate, phosphorus and magnesium concentration when compared to EG alone treated group. After treatment with standard cystone 5ml/kg and EPU 200 and 400mg/kg significant reduction in the kidney MDA levels was observed in the treated groups, when compared to their respective control groups.

Catalase and GSH levels of the kidney were significantly decreased in the control groups on EG administration for 10 days, when compared to the normal group. On treatment with Standard cystone and EPU 200 and 400mg/kg a significant rise in the renal catalase and GSH levels was observed in treated groups.

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