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ANTI-UROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *Desmostachya bipinnata (L). stapf* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS

Syed Safiullah Ghori*, Kaab e Alam, Abid Hussain Abrar, M.A Rizwan Khan, Fouzia Fatima

Pharmacology Research Lab, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Telangana, India

Keywords:

EDB (Ethanolic extract of *Desmostachya bipinnata*), urolithiatic, EG (Ethylene glycol), MDA (Malondialdehyde), GSH (Glutathione reductase)

For Correspondence:

Syed Safiullah Ghori

Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Telangana, India

E-mail:

safiullahghori@gmail.com

ABSTRACT

The whole plant of Desmostachya bipinnata (L.) stapf belonging to the family poaceae, possess a wide range of medicinal properties. The present study was undertaken to investigate the beneficial effects of orally administered ethanolic extract of Desmostachya bipinnata (L.) stapf in experimentally induced urolithiasis in Wistar rats. Ethylene glycol was used for inducing urolithiasis and the Cystone was used as the standard drug. The various bio-chemical parameters like creatinine, uric acid, phosphorus, magnesium, calcium, oxalate were determined from serum,urine, and kidney . EDB and cystone in combination with the concentration of 5ml/kg 200 and 400 mg/kg respectively has significantly increased the urine output thus resulting in the prevention of stone formation in kidney. Serum and urine Bio-chemical parameters showed the increase in concentration when treated with ethylene glycol (0.75%). These groups when treatedwith EDB 200 and 400 mg/kg showed a significant decrease in all the serum and urine biochemical parameters. There was the enhancement in MDA levels of LPO when treated with the EG, and these groups when treated with EDB 200 and 400 mg/kg results in the decrease of kidney MDA levels. When treated with EG , catalase and GSH levels were decreased significantly for ten days.These groups when treated with the EDB 200 and 400 mg/kg showed an increase in kidney catalase and GSH levels. From these findings, it can be concluded that EDB administration to the rats with ethylene glycol induced urolithiasis prevented the formation of urinary stones, supporting the anti-urolithiatic activity of the plant.

INTRODUCTION

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.Occurrence of urolithiasis requires formation of a nidus, its retention and growth in the urinary tract which may cause obstruction of the ureter.[1]Urolithiasis, is one of the most painful ailments of the urinary tract disorders, 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. Besides, these treatments cause undesirable side effects such as hemorrhage,hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation. So It is worthwhile to look for an alternative treatment for the management of urolithiasis, therefore phytotherapy is being sought.[2] Men are three times more likely to be affected than women and the lifetime risk of developing a calculus in a Caucasian man is nearly 20%. 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 cysteine, respectively. The calcium-containing calculi consist of pure or various amount of calcium components such as calcium oxalate monohydrate, apetite, calcium hydrogen phosphate and calcium carbonate. In men, 70% to 80% of the calculi contain either calcium oxalate alone or in combination with apetite[3]

The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices. Increased rates of hypertension and obesity, which are linked to nephrolithiasis, also contribute to an increase in stone formation[4]**.** Plants are used as medicine since time immemorial. India is a rich source of medicinal plants. The medicinal plants are widely used in ancient systems of medicine.Desmostachya bipinnata (L.) stapf has shown anti-urolithiatic effect in combination with other plants. The plant contains alkaloids, sterols, glycosides, saponin, flavonoids, tannins and phenols. Hence in the present work an effort is made to explore the possible anti-urolithiatic activity of *Desmotachya bipinnata (L.)* stapf in rats.

MATERIAL AND METHODS

Drugs and Chemicals

Cystone (Himalaya Pharmaceutical, Banglore), Ethylene glycol (SRL Mumbai), Tween 80 (Merck Pvt Ltd, B, Mumbai)**,** Anaesthetic ether (SD Fine chem Ltd., Mumbai), Chloroform (SD Fine chem Ltd .Mumbai). Formalin (SD Fine chem Ltd., Mumbai) All chemicals and reagents were of analytical grade.

Diagnostic kits:

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

Instruments:

Auto-analyzer (Robonik), Refrigerator centrifuge (MPW-350R),UV-Spectro-photometer (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).

Plant material

The plant of *Desmostachya bipinnata* (L.) Stapf was collected from a certified ayurvedic wholesaler. The plant was identified and authenticated by Asst. Prof. Dr. K. Madhava shetty Department of Botany, S.V. University, Tirupati.

Experimental 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 light cycle and given standard laboratory feed and water. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

Preparation of 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 made in to coarse powder with the use of grinder. The powder of *Desmostachya bipinnata* (L.) Stapf obtained was weighed separately and transferred to a round bottomed flask and then subjected 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 petriplate on electric water bath (40ºC) and then kept in an oven at 30ºC for an hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 6.29%.[5]

Acute toxicity study

Acute toxicity studies were performed according to OECD-423guidelines category IV substance (acute toxic class method).[6]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 *Desmostachya bipinnata* (L.) Stapf 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. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin, mortality and general behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Preliminary phytochemical studies [7]

Ethanolic extract of the plant of *Desmostachya bipinnata* (L.) Stapf were subjected to chemical tests for the identification of active constituents.

PHARMACOLOGICAL SCREENING MODELS

Ethylene glycol induced urolithiasis model [8]

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 diet and tap water ad libitum for 10 days.

Group 2: EG 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: test group treated with ethanolic extract of *Desmostachya bipinnata* (L.) Stapf 200 mg/kg with normal lab diet and drinking water containing 0.75% [v/v] EG.

Group 5: test group treated with ethanolic extract *Desmostachya bipinnata* (L.) Stapf 400 mg/kg of body weight and fed with normal diet and drinking water containing 0.75% [v/v] EG.

URINE AND BLOOD SAMPLING [9]

The crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. 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). The blood samples were collected from the animals under anaesthesia (ether) from retro orbital route before sacrificing. The

collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium, urea, uric acid, magnesium and phosphorus.

Estimation of serum and urine parameters:

Serum Creatinine was determined by Mod. Jaffe's kinetic method[10],uric acidand urea by teitz method[11, 12].Calcium was estimated by method described by Burtis and Ashwood[13] Phosphorus was estimated Niccans and Sannelson method[14] Oxalate by Hodgkinsons and William method [15] Magnesium was estimated by Niccans and Sannelson method[16]

HISTOPATHOLOGY

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were rinsed in an ice-cold physiological solution, after the extraneous tissues were removed. The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (H and E) for histopathological examination. The slides were examined under a light microscope to study the architecture of the kidney and calcium oxalate deposits.

ENZYME ASSAY

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). Lipid Peroxidation was done by the method described by Jollow et al[17]catalase as described by Aeibi[18]and glutathione (GSH) by method of Seirkowoski et al[19].

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 $P<0.05$

RESULTS

Preliminary phytochemical screening

The extracts of plant were analyzed for the presence of various constituents. The result of this preliminary phytochemical examination showed the presence of Carbohydrates Glycosides Fixed oils, fats, Saponins, Tanins, Phytosterols, Flavonoids and Alkaloids.

Acute oral toxicity

Acute oral toxicity was carried out according to OECD guideline 423. EDB was safe up to 2000mg/kg.

Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on urinary volume and pH against EG induced urolithiasis.

EG (0.75%) administration showed significant ($p<0.001$) alteration of the output of urine and pH as compared to normal group. Administration of Cystone 5ml/kg, EDB 200 and 400 mg/kg caused significantly increased ($p<0.01$, $p<0.05$) urine output and pH of the urine as compared to control (EG) group.**(Table 1)**

Treatment group	Urinary Volume (ml/24hr)	Urine pH
Normal	18.4±0.97	7.5±2.1
Control (EG)	6.96±0.69a	4.5±1.39a
Standard Cystone (5ml/kg)	14.93±0.57***	8.2±1.32**
EPU 200mg/kg	8.41±0.45*	5.9±1.21*
EPU 400mg/kg	9.9±0.64*	6.14±2.24**

Table 1 Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on urinary volume and pH against EG induced urolithiasis

Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf **on serum biochemical parameters against EG induced urolithiasis.(Figure 1)**

Serum Creatinine

Administration of EG (0.75%) for 10 days caused significant elevation ($p<0.01$) in serum creatinine concentration compared to normal . Standard cystone 5ml/kg causes significant reduction ($p<0.001$) in serum creatinine concentration when compared to EG alone treated group. Pretreatment with EDB 200 and 400mg/kg caused significant reduction ($p<0.05$ and $p<0.001$) in serum creatinine concentration when compared to EG alone treated group.

Serum Urea

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

Serum Uric Acid

Administration of EG (0.75%) for 10 days caused significant elevation ($p<0.01$) in serum uric acid

concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p<0.001$) in serum uric acid concentration when compared to EG alone treated group. Pretreatment with EDB 200 and 400mg/kg causes significant reduction ($p<0.05$ and $p<0.001$) in serum uric acid concentration when compared to EG alone treated group.

Serum Calcium

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

Serum Oxalate

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

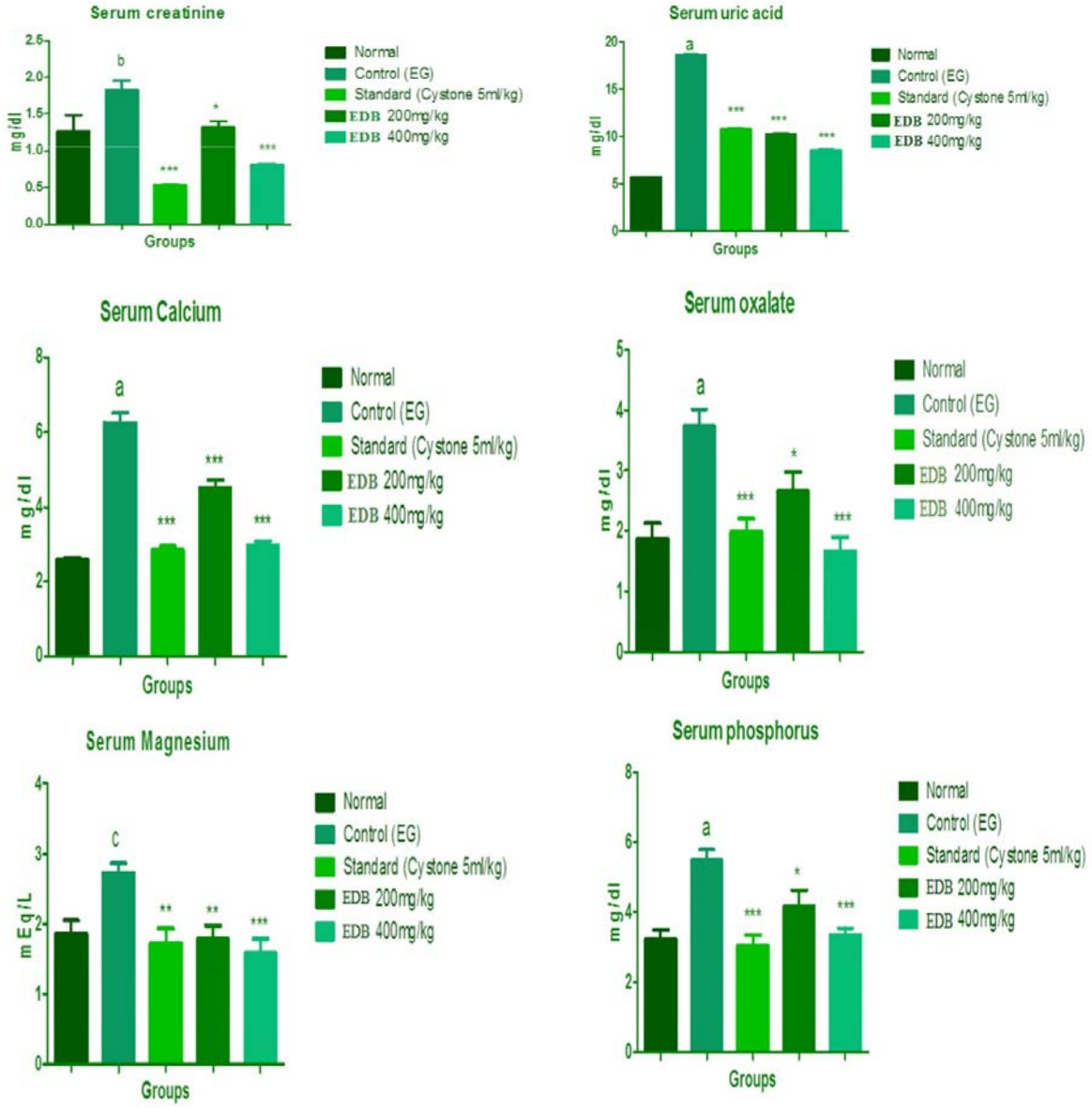
Serum Phosphorus

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

Serum Magnesium

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.05$) serum magnesium concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p<0.01$) in serum magnesium concentration when compared to EG alone treated group. Pretreatment with EDB 200 and 400mg/kg causes significant reduction ($p<0.01$ and $p<0.001$) in serum magnesium concentration when compared to EG alone treated group.

Fig.no1.: Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on serum parameters



Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on urine biochemical parameters against EG induced urolithiasis.

Urinary Creatinine

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.001$) 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 EDB 200 and 400mg/kg causes significant reduction (not significant and $p<0.001$) in urine creatinine concentration when compared to EG alone treated group.

Urinary urea

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.001$) 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 EDB 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.

Urinary Calcium

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.001$) 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 EDB 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.

Urinary Oxalate

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

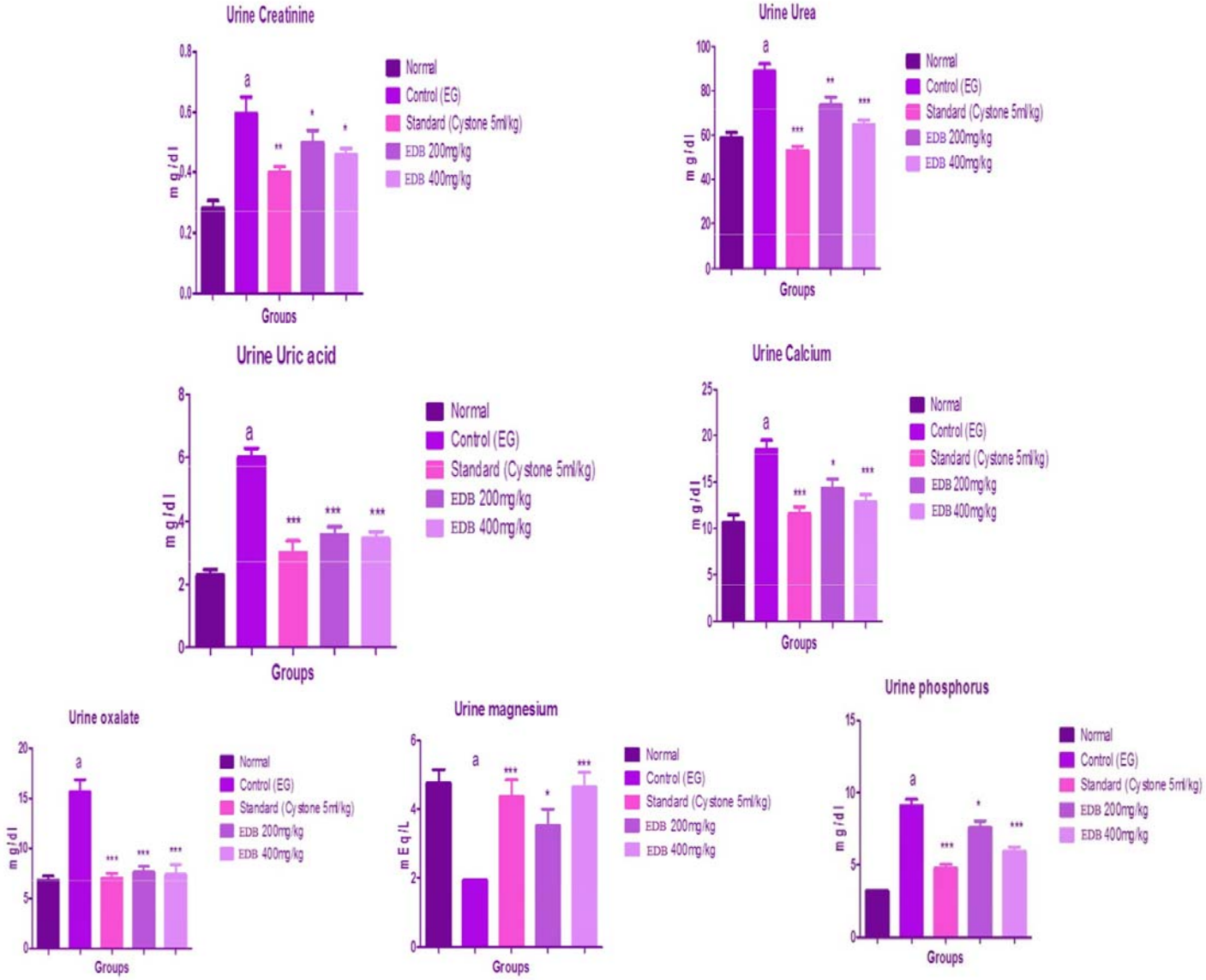
Urinary Phosphorus

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.001$) 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 EDB 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.

Urinary Magnesium

Administration of EG (0.75%) for 10 days caused significantly increased ($p<0.001$) 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 EDB 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.: Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on urine parameters



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.

Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf 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 EDB 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.

Catalase

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 EDB 200 and 400mg/kg ($p<0.001$), a significant rise in the renal catalase levels was observed in treated groups (Table 3).

GSH

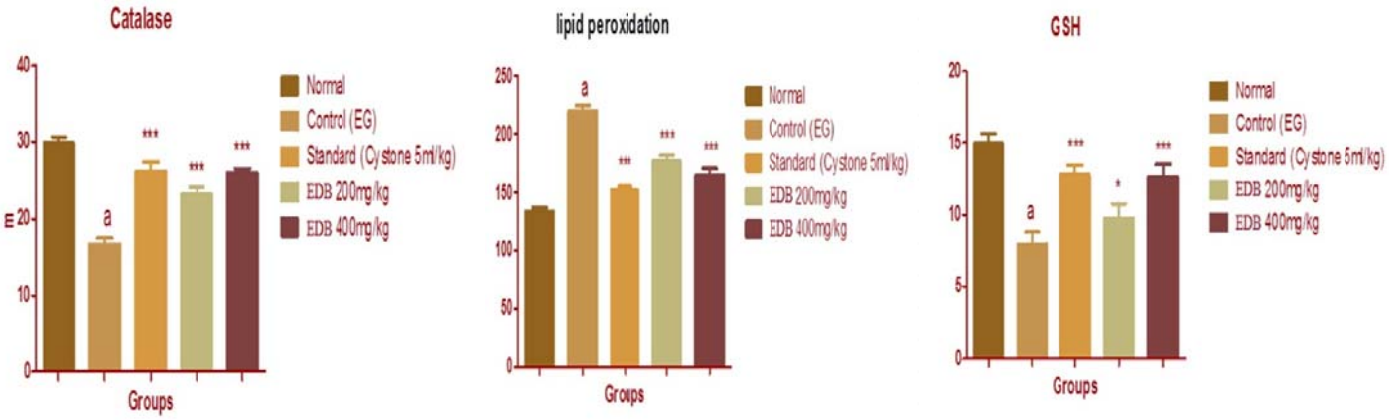
GSH levels of the kidney were significantly ($p<0.001$) decreased in the control groups on EG administration for 10 days, when compared to the normal group. On treatment with Standard cystone ($p<0.01$) and EDB 200 ($p<0.05$) and 400mg/kg ($p<0.01$), a significant rise in the renal catalase levels was observed in treated groups (Table 3)

Table.no.6: Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on antioxidant (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***
EDB 200mg/kg	23.29±0.92***	9.74±1.04*	176.7±5.05***
EDB 400mg/kg	26.00±0.56***	12.68±0.85**	164.9±5.59***

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.

Fig. Effect of ethanolic extract of *Desmostachya bipinnata* (L.) Stapf on Catalase (kidney enzyme) parameters against EG and induced urolithiasis.



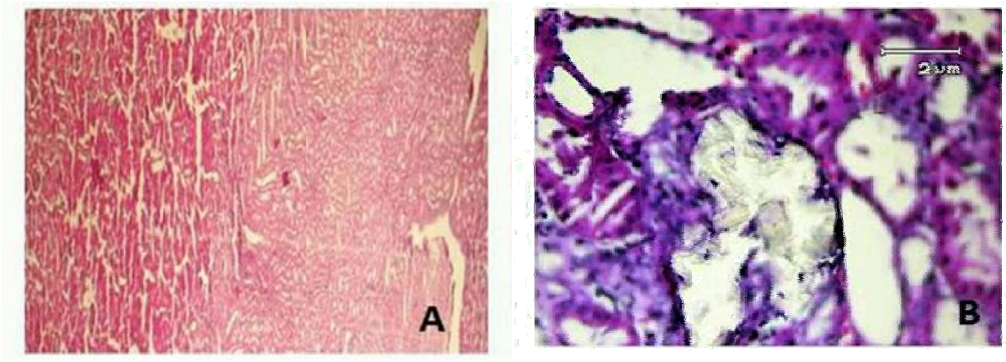
HISTOPATHOLOGY OF KIDNEY

The kidney of normal rat 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 polarizing microscope revealed presence of white chalky colored calcium oxalate crystals in several tubules and glomeruli. These groups also showed congestion of intersitium and inflammation of the pelvic calyceal systems.

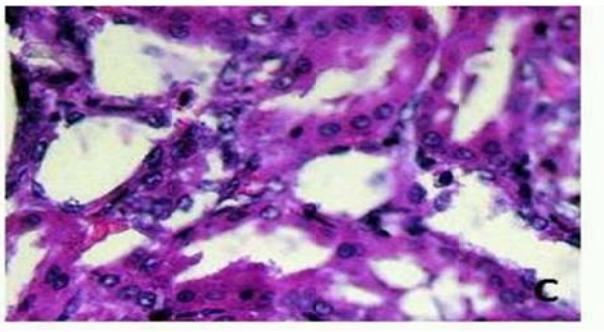
The histopathology of kidney of rats treated with standard drug cystone 5ml/kg and EG for 10 days showed normal architecture of the kidney.

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

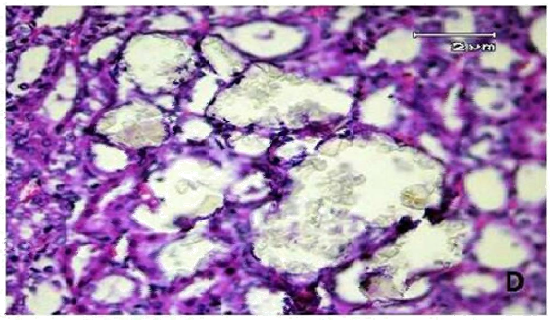
Fig. Histopathology of kidney



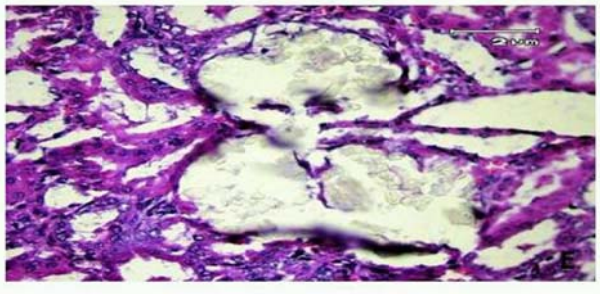
A: Normal group **B: EG group**



C: Standard (Cystone 5ml/kg) group



D: group EDB, 200mg/kg



E: group EDB 400mg/kg

DISCUSSION

Urinary stone disease is a common, painful and expensive medical condition[20]. Though extracorporeal shock wave lithotripsy has facilitated the stone removal and reduced the morbidity associated with urinary stone, recurrence is common[21] 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.[22]Rats are commonly used to study the pathogenesis of human CaOx kidney stone disease, as the metabolism is regarded almost similar in rats and humans[23] 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[24] thus favors adhesion and retention of CaOx particles within the renal tubules[25].Hence, in the present study, EG in drinking water was employed to induce hyperoxaluria in rats. Urinary supersaturatio3n in relation to stone forming constituents, mainly urinary oxalate is important in renal calculi formation[26] insoluble CaOx crystals[27].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 EDB, the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinary excretion in control groups implies the potential of EDB. 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[28]. 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, EDB 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[29]Mucoproteins have significant affinity for CaOx surface and promote the growth of crystals and cement them.[30]

Flavonoids act by disintegrating the mucoprotiens, thereby prevent calcium and oxalate deposition and excretion[31]In the present study also, preliminary phytochemical screening of EDB revealed the presence of flavonoids. Thus, in the EDB 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. [32]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.[33]Daily consumption of tea reduced the risk of kidney stone formation in women by

8%. [Moreover, low concentration of renal cellular glutathione favors LPO and subsequent retention of calcium and oxalate in the kidneys[34]Health benefits of beverages like tea are due to its antioxidant properties of flavonoids [35] which act by quenching ROS and also by chelating metal ions like iron and copper. Lupeol and betulin were proposed to act by scavenging oxalate promoted free radicals and enhancing body antioxidant status, thus reducing oxalate induced renal peroxidative tissue damage. In the present study, lowered levels of MDA and enhanced levels of antioxidant enzymes, GSH and catalase in the kidneys of the EDB 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 *Desmostachya bipinnata* might have been responsible for the inhibition of CaOx crystal aggregation and stone formation. The results support the use of *Desmostachya bipinnata* 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.

CONCLUSION

In conclusion, the presented data indicates that administration of the EDB to rats with ethylene glycol induced urolithiasis prevented the formation of urinary stones, supporting anti-urolithiatic 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 anti-urolithiatic property of *Desmostachya bipinnata* (L.) Stapf.

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