ANTIUROLITHIATIC ACTIVITY OF *BLEPHARIS BOERHAVIFOLIA* ON ETHYLENE GLYCOL INDUCED LITHIASIS IN ALBINO WISTAR RATS

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Abstract

Kidney stones affect approximately between 7% and 13% of the North American population, 5% to 9% in Europe, and 1% to 5% in Asia. Currently, there are no appropriate allopathic treatments for nephrolithiasis, and even surgery is insufficient to yield meaningful benefits. The best way for treatment is through the natural products. *Blepharis Boerhaviifolia* is said to be helpful in treating renal diseases in native medical system however, no comprehensive studies supporting antiurolithic activities have been reported. In order to prove the effectiveness in rat model of nephrolithiasis caused by ethylene glycol. This article was used to evaluate antiurolithiatic activity, with doses at 200 mg/kg and 400 mg/kg, administered orally. after conducting an acute toxicity through oral exposure study following OECD guidelines 423. During course of study curative and preventive doses of 200 mg/kg and 400 mg/kg were administered and Cystone 750 mg/kg was chosen. Levels of calcium, oxalate, and phosphate were determined from urine samples. Serum samples were subjected to BUN, Creatinine, Calcium, and Uric Acid using commercially available bioassay kits. Histopathological examinations were assessed for hemorrhage, glomerular congestion, peritubular inflammation, tubular congestion, necrosis and the existence of stones. As a result, remarkable recovery within short time period was evidently observed and recorded symptomatically as well as through investigations done during and after treatment. These findings confirmed the beneficial impact of Blepharis boerhavifolia in Urolithiasis while also demonstrating a significant diuretic effect, justifying its usage in Ayurveda as an antiurolithiatic medicine as well as additional pharmacological studies.

Keywords: Anti-urolithiatic activity**,** *Blepharis boerhavifolia***,** Ethylene glycol, Renal stones.

INTRODUCTION

Mineral deposits known as kidney stones can either float freely or be attached to the renal papillae within the renal calyces and pelvis. They are made up of both crystalline and biological elements[1]. Urolithiasis is brought on by a variety of reasons, including an imbalance in diet, nutrition, salt intake, and animal proteins, chelating agents like citrate, alkali foods, and alkali fiber[2]. The incidence of kidney stones is growing worldwide, as is their recurrence rate. Approximately 12% of people worldwide will experience urolithiasis at some point in their lives. It affects individuals of all ages, genders, and ethnic backgrounds. Even though the number of female cases of nephrolithiasis has lately increased, lifetime recurrence rates are higher in men[3,4]. Physical and chemical alterations, along with urine supersaturation, play a role in the formation of renal stones. In an environment with supersaturation, solutes begin to precipitate in the urine, leading to the formation of crystal aggregates and nucleation. The process of turning a liquid into a solid is affected by pH levels and specific amounts of surplus substances. The supersaturation of stone-forming materials such as calcium, phosphorus, uric acid, oxalate and cystine combined with a lack of adequate urine volume, increases the risk of crystallization in nephrolithiasis[5]. Calcium oxalate (CaOx) or CaOx combined with calcium phosphate stones account for more than 80% of urinary stones[6]. Chronic kidney illnesses, diabetes, cardiovascular conditions, end-stage renal failure, and hypertension all have higher risks when there is kidney stones present[7]. The body frequently expel little kidney stones on its own.

Currently, there are no effective or appropriate allopathic treatments for nephrolithiasis, and occasionally even surgery is insufficient to yield meaningful benefits. The best source in this case to evaluate a safe management of the lithiasis disease is probably natural products.

Numerous plants have been used around the world and are marketed as excellent urinary stone treatments. *Blepharis Boerhaviifolia* is said to be helpful in treating a variety of renal diseases in the native medical system[8,9]. However, no comprehensive studies supporting the antiurolithic activities of the ethanolic extract of *Blepharis Boerhaviifolia* (EEBB) have been conducted. In order to prove the effectiveness of the ethanolic EEBB's antiurolithiatic capabilities in a rat model of nephrolithiasis caused by ethylene glycol.

MATERIALS AND METHODS

Materials: All the chemicals were procured from SD fine Chemicals and the standard drug was taken from the Himalaya Drug Company and are of AR grade.

Collection of Plant Material: The whole plant of *Blepharis boerhaviifolia* was collected locally and was authenticated by Dr V Rama Rao, Research Officer (Botany) and the authentication no is **Ref. RRCBI-mus34**.

Preparation of the extract: The shade dried entire *Blepharis boerhaviifolia* plant was powdered and sieved through No.22, mesh. The petroleum ether was used to defat about 400 g (appx.) of coarse powder. The marc left over in the Soxhlet apparatus was subjected to 48 hrs of ethanol extraction. The marc was further subjected to hot air drying after ethanol extraction, and the rest was collected.[10]

Preliminary phytochemical investigation: The preliminary phytochemical investigation of the ethanolic extracts of *Blepharis boerhaviifolia* was performed following the method outlined by Khandelwal. The Phytochemical screening for the extract of *Blepharis boerhaviifolia* was performed for alkaloids, glycosides, bioflavonoid and volatile oils.[11]

Test for Alkaloids:

Blepharis boerhaviifolia extracts were separately dissolved and filtered into 5ml of distilled water or ethanol. The filtrates underwent various tests for alkaloid presences.

a. Mayer's Test

Blepharis boerhaviifolia filtrates were treated with Mayer's reagent (potassium mercuric iodide), and the formation of a yellow cream precipitate indicated the presence of alkaloids.

b. Wagner's Test:

Blepharis boerhaviifolia filtrates were treated with Wagner's reagent (potassium iodide iodine), and the production of a brown precipitate shows the presence of alkaloids.

c. Dragendroff's Test:

Blepharis boerhaviifolia filtrates were treated with Dragendroff's reagent solution (a potassium bismuth iodide solution); the presence of a red precipitate indicates the presence of alkaloids.

d. Hager's Test:

Blepharis boerhaviifolia filtrates were processed with Hager's reagent solution (a saturated picric acid solution), and the formation of a yellow precipitate signifies the presence of alkaloids.

Test for Glycosides:

Blepharis boerhaviifolia extracts were diluted and filtered separately into 5ml of distilled water/ethanol. The filtrates were subjected to several glycoside presence tests.

a. **Modified Borntrager's Test:**

Blepharis boerhaviifolia filtrates were individually treated with a solution of ferric chloride and heated for about 5-10 minutes using water bath. At room temperature the content was cooled and shaken with

equal volume of benzenes. The organic layer was separated from the solution and treated with ammonia (half its volume), pink colour formation in the ammonia layer indicates the presence of anthraquinone glycosides

b. **Legal's Test:**

Blepharis boerhaviifolia filtrates were treated with pyridine-treated sodium nitroprusside. Pink to red colour formation indicates cardiac glycoside presences.

c. **Keller Kilani Test:**

0.5 g of Blepharis boerhaviifolia was dissolved in 2ml of glacial acetic acid with one drop of ferric chloride solutions before being treated with 1ml of concentrated sulphuric acid. A brown ring obtained depicts the presence of cardenolide.

Test for volatile oil:

a. Sudan red III test:

Thin section of the drug is taken and Sudan III solution is added to get red colour due to oil globules

b. Tincture alkane test:

Thin section of drug taken to that few drops of tincture alkane is added to get red colour which means presence of volatile oil

Test for bioflavonoid:

a. Alkaline reagent test:

Apply a few drops of sodium hydroxide solution to the extract; an increase in colorlessness upon further addition of acid indicates the presence of flavonoids.

b. Lead acetate test:

Add a few drops of lead acetate solution to the extract; the appearance of a yellow precipitate suggests the presence of flavonoids.

EXPERIMENTAL ANIMALS:

42 Healthy Albino wistar rats (140- 180g) were procured from Vaarunya bio labs. The animals were kept under standard temperature conditions (22 ± 1°C). Relative humidity (55 ± 10%), 12 hours light and dark cycles and a standard diet of pellets, and water and libitum. The rats were adapted to the specified above-mentioned environmental conditions for a duration of 7 days following their random assignment into various groups and prior to the start of the experiment. The experimental protocol was approved by the Institutional Animals Ethics Committee, East West College of Pharmacy, and Bengaluru. Ref.No. **EWCP/CPCSEA/IAEC/VII/2023/01.**

ACUTE TOXICITY STUDIES[12]

The study on acute oral toxicity was performed in accordance with OECD guidelines 423 (Acute Toxic Method) for alcoholic extract of *Blepharis boerhaviifolia.* Initial dose of used was 2000mg / kg p.o. body weight of the extracts in ethanol. *Blepharis boerhaviifolia* alcoholic extracts was administered to 2 female mice, which was observed for 14 days. For the study, the extract doses were selected based on safety profile of 2000mg/kg dose and was selected as based on the safest dose ratio of 1/20th, 1/10th.

SCREENING MODELS:

Ethylene glycol induced Urolithiasis Model in rats[13].

The ethanolic extracts of whole plant of *Blepharis boerhaviifolia* was studied for their anti- urolithiatic activities.

Gp No.	Group	Treatment or Drugs Given	Duration
Group I	Control	Regular rat food and ad libitum water were provided.	28 Days
Group II	Toxic Control	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.	28 Days
Group III	Standard	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.+ Cystone group; received standard drug cystone (750 mg/kg, p.o.)	28 Days
Group IV	Preventive Group-I (200mg/kg)	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.	28 Days
		EEBB P.O (200mg/kg)	
Group V	Preventive Group-II (400mg/kg)	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.	28 Days
		EEBB P.O (400mg/kg)	
Group VI	Curative Group-I (200mg/kg)	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.	28 Days
		EEBB, P.O (200mg/kg)	15th to 28th
Group VII	Curative Group- II (400mg/kg)	Groups were fed with drinking water that contained 0.75% v/v ethylene glycol.	28 Days
		EEBB P.O (400mg/kg)	15th to 28th Day

Table no. 1. 42 animals were divided into seven groups randomly (n = 6)

Grouping of animals for Ethylene glycol induced Urolithiasis Model in rats.

All the animals were treated with suitable drugs/extracts for 28 days respectively as mentioned in above table. On 15th day & 28th day the rats were evaluated using the following parameters.

Evaluation parameter

Following parameters was evaluated:

I. Urine Analysis
   i. Volume
   ii. pH
   iii. Estimation of calcium, oxalate and phosphate
II. Urine Microscopy
III. Body weight
IV. Serum Estimation of
   i. Blood Urea Nitrogen
   ii. Creatinine
   iii. Calcium &
   iv. Uric acid
V. Kidney tissue histopathological changes was observed

URINE ANALYSIS[14]

Urine collection and analysis:

The animals were housed individually in metabolic cages, and urine was collected over a 24-hour period on the 15th and 28th days. The total volume of urine collected from each animal in all groups was recorded. Throughout the study, the animals had unrestricted access to drinking water. The urine samples were acidified with 3N HCl prior to being centrifuged at 1,500 rpm for 10 minutes. The samples were maintained at -20 degrees Celsius, and both the supernatant and the solid residues were discarded. Additionally, the urine volume and pH were quantitatively assessed. The presence of crystals in the urine was evaluated in the supernatant samples from both control and experimental rats using a light microscope at 40X magnification.

Figure 1: Metabolic Cages

BODY WEIGHT

The change in body weight of the animals was noted on 1st, 15th and 28th day during the experimental period.

Estimation of Calcium, Oxalate and Phosphate[14]

A . Calcium:

Principle: Calcium forms a bluish-purple complex with metal complexing dye Ocresolphtalein complex in alkaline solution, which is detected at 578 nm. The colour intensity is proportional to the calcium concentration in the sample. Hydroxyquinoline will operate as a masking agent, removing the magnesium interference.

Procedure: Take a 5 ml urine sample and add a few drops of NaOH, 1% acetic acid and 2 – 3 ml of ammonium oxalate solution observe the changes.

B . Phosphate:

Principle: The number of phosphates excreted through urine depends on diet. It is normally excreted 0.8 to 1.3 g of phosphate per day. In certain bone diseases excretion of phosphorus is increased while in hypoparathyroidism it is decreased. Phosphate is determined using a turbidimetric method. The reaction of phosphorus with sodium tetraphenyl boron produces insoluble turbid slurry. The amount of turbidity in a sample is measured at 630 nm and is proportional to the amount of phosphate present.

Procedure: 3 ml urine + 3 ml conc. HNO3 + 3ml of ammonium molybdate solution is boiled. Observe the changes.

URINE MICROSCOPY

The urine crystal deposition score was examined in the supernatant samples of both control and experimental rats using a light microscope set to 40X magnification. The quantity, dimensions, and morphology of CaOx crystals formed in the absence or presence of EEBB were assessed with a Leica DM 2500 LED microscope at 1000× magnification.

SERUM ESTIMATION

On the 28th day of the study, a comprehensive serum analysis was conducted to assess the levels of creatinine, uric acid, and blood urea nitrogen (BUN). The analysis was performed using specific kits in accordance with established standard procedures. The serum samples were processed and analysed with

the assistance of a semi-automatic analyser, ensuring precision and accuracy in the measurements. This approach provided detailed insights into the biochemical markers, shedding light on the impact of *Blepharis boerhaviifolia* ethanolic extract (EEBB) on kidney function and metabolic balance. The use of standardized procedures and advanced analytical techniques reinforced the reliability of the obtained results, contributing valuable data to the overall findings of the study.

KIDNEY TISSUE HISTOPATHOLOGY

The histopathological examination of kidney samples was conducted by performing tissue fixation, dehydration, embedding, sectioning, staining, and subsequent microscopic analysis. Formalin fixation ensured the preservation of structural integrity, followed by dehydration and clearing processes. The tissues were embedded in paraffin for precision during sectioning, after which thin slices were stained using Haematoxylin and Eosin, along with special stains. The stained slides were observed and analysed under a microscope, allowing for detailed examination of cellular structures and abnormalities.

RESULTS

PREPARATION OF EXTRACT

Approximately 400 g of *Blepharis boerhaviifolia whole plant* was used for extraction using ethanol. The nature and the extractive value of the extract are as follows:

TABLE No. 2 Extractive value of *Blepharis boerhaviifolia*

S. No	Extract	Colour	% Yield
1.	Ethanol extract of *Blepharis boerhaviifolia*	Dark Brown and sticky	8.75 %

PHYTOCHEMICAL INVESTIGATION:

Various preliminary chemical tests were conducted for the ethanolic extract *Blepharis boerhaviifolia* to determine the chemical constituents present in the extract. The results are tabled below.

TABLE No. 3. Preliminary Phytochemical Analysis.

Sl.no	Chemical Tests	Ethanol extract of *Blepharis boerhaviifolia*			
1.	Tests for Alkaloids	Dragendroff's Test	Wagner's Test	Mayer's Test	Hager's Test

		+	+	+	+
2.	Tests for Glycosides	Legals Test	Brontrager Test	Keller Killani test	
		+	+	+	
3.	Test for volatile oil	Sudan red III test		Tincture alkane test	
		+		+	
4.	Test for bioflavonoid	Alkaline reagent test		Lead acetate test	
		+		+	

+ indicates presence of the particular chemical constituent.

Effect of ethanolic extract of *Blepharis boerhavifolia* on change in body weight on normal and treated animals.

Table No.4 Effect of ethanolic extract of *Blepharis boerhavifolia* on change in body weight on normal and treated animals.

Group name	Day 0 (g)	Day 15 (g)	Day 28 (g)
Control	182±4.47	185±3.24	189±7.34
Toxic control	180±10.57	176±11.05	171±10.64
Standard	200±5.95	205±5.84	208±6.35**
Preventive Group I EEBB 200mg/kg	197±9.72	199±10.17	202±9.56
Preventive Group I EEBB 400mg/kg	198±13.59	201±12.45	204±12.28*
Curative Group I EEBB 200mg/kg	199±5.95	195±5.84	200±6.35**
Curative Group II EEBB 400mg/kg	205±7.09	201±5.63	205±6.34

Values are Mean ± S.E.M. (n=6); Significance values are ***P is less than 0.01 and *P is less than 0.05. Control group vs all groups +++ P is less than 0.001, ++P is less than 0.01 and +P is less than 0.05. Comparison between normal and all groups.

The body weight decreased noticeably in the toxic control group whereas increased significantly in standard, preventive and curative groups when compared to control groups. All treatment groups exhibited an increase in body weight relative to the toxic control group.

Effect of EEBB on Urine Volume on 15th day and 28th day

Table No.5. Effect of EEBB on Urine Volume on 15th day and 28th day

Group name	Day 15 (ml)	Day 28 (ml)
Control Group	7.3 ± 0.80	7.3 ± 0.13
Toxic control	5.1 ± 0.92^{+++}	4.8 ±0.12^{+++}
Standard drug (Cystone)	7.7 ± 0.80***	9.5 ±0.11***
Preventive Group I (EEBB 200mg/kg)	6.9 ± 0.88***	7.6 ±0.15***
Preventive Group II (EEBB 400mg/kg)	7.4 ± 0,10***	8.4 ±0.15***
Curative Group I (EEBB 200mg/kg)	7.1 ± 0.19***	7.3 ±0.13***
Curative Group II EEBB (400mg/kg)	7.5 ± 0.10***	7.9 ±0.14***

Values are Mean ± S.E.M. (n=6); ***P is less than 0.001 and **P is less than 0.01. Control group vs all groups ++P is less than 0.01 and +P is less than 0.05. Comparison between normal and all groups.

The urine volume decreased noticeably (p is less than 0.01) in the toxic control group whereas increased significantly (p is less than 0.001) in standard, preventive and curative groups when compared to control groups. Every treatment groups showed significant increase (p is less than 0.001) in urine volume when in relation to toxic control group (Fig no.2 & 3).

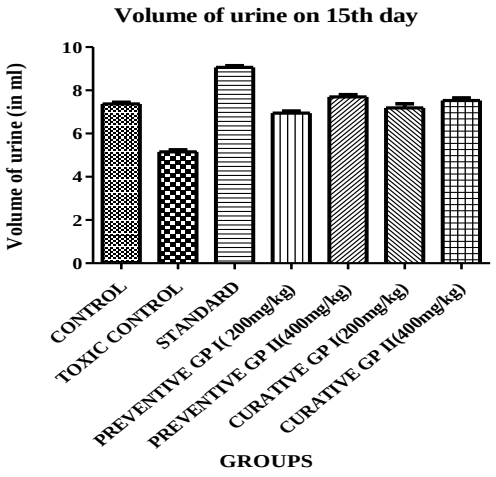


Figure no 2: Volume of urine on 15th day

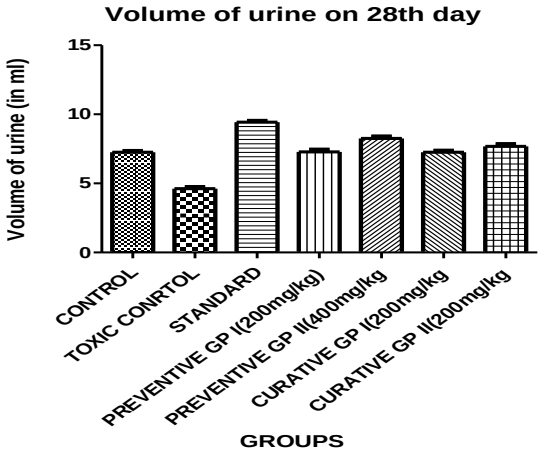


Figure no-3: Volume of urine on 28th day

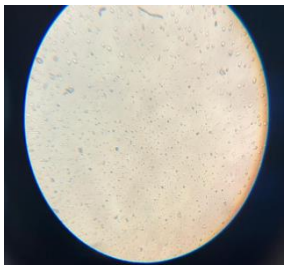
Urine pH

In the toxic control group, the urine volume and pH were observed to be acidic, indicating an environment conducive to kidney stone formation. In contrast, the standard, preventive, and

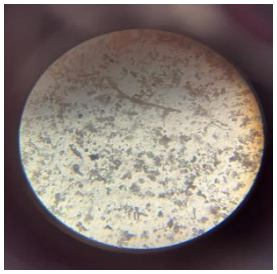
curative groups exhibited alkaline urine pH levels. This shift towards alkalinity in the treated groups suggests a significant alteration in the urinary environment, discouraging crystal nucleation and growth, and potentially hindering the formation of kidney stones.

URINE MICROSCOPY

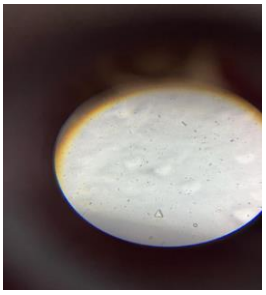
The control group primarily exhibited numerous large calcium oxalate monohydrate (COM) crystals, displaying either a rectangular shape or dendritic formations with sharp edges. Higher concentrations of EEBB and lower concentrations of Cystone promoted the development of tetrahedral-shaped calcium oxalate dihydrate (COD) crystals, which had a smoother surface morphology. Both EEBB and Cystone contributed to a reduction in the size and quantity of CaOx crystals. The percent reduction in size of CaOx crystals produced by EEBB was similar to what was observed with Cystone. However, EEBB significantly decreased the number of CaOx crystals compared to Cystone.



Control Group

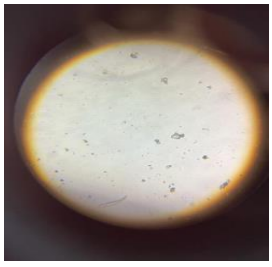


Toxic Conrol Group

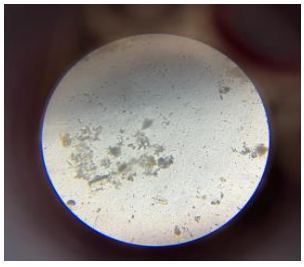


Standard

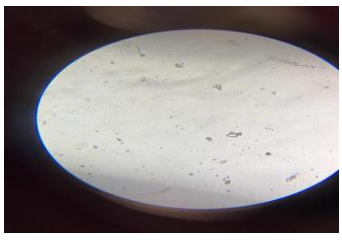
Preventive Group I



Preventive Group II



Curative Group I



Curative Group II

Figure 4: Microscopy of Urine

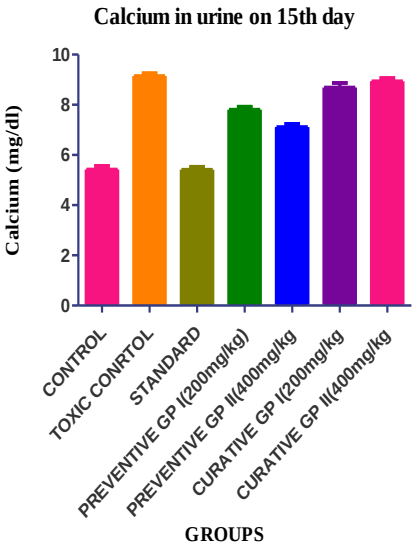
Effect of EEBB on Urine biochemical parameters on 15th day

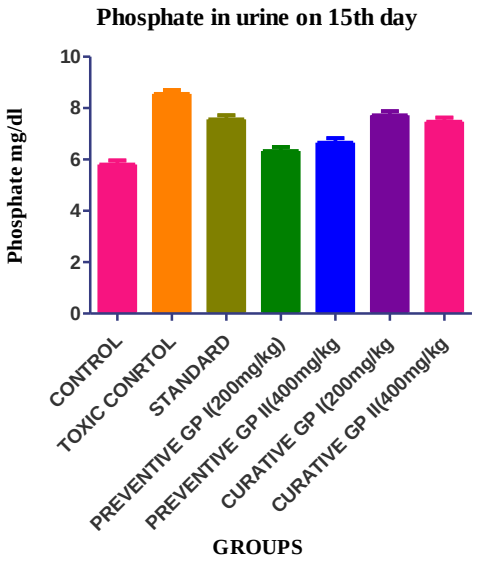
Table No.6. Effect of EEBB on Urine biochemical parameters on 15th day:

Parameters	Group 1 Normal	Toxic control	Standard	Preventive Group I EEBB 200mg/kg	Preventive Group II EEBB 400mg/kg	Curative Group I EEBB 200mg/kg	Curative Group II EEBB 400mg/kg
Calcium	5.64 ±0.15	9.02±0.13^{+++}	5.4±0.10***	7.8±0.11***	7.15±0.12***	8.8±0.15^{ns}	8.9±0.18^{ns}
Phosphate	5.85±0.16	8.57±0.15^{+++}	7.51±0.16**	6.29±0.15***	6.8±0.17***	7.80±0.16*	7.50±0.15***
Oxalate	1.15±0.05	3.06±0.07^{+++}	2.30±0.07***	1.87±0.09***	1.28±0.09***	2.09±0.07***	2.32±0.07***

Values are Mean ± S.E.M. (n=6); Significance values are ***P is less than 0.01 and *P is less than 0.05. Comparison between Control group and all groups +++ P is less than 0.001, ++P is less than 0.01 and +P is less than 0.05. Comparison between normal and all groups.

The calcium, oxalate and phosphate level increased noticeably (p is less than 0.001) in the toxic control group (Fig no. 4) whereas decreased significantly (p is less than 0.01) in standard, preventive and curative groups when in relation to control groups. All the treatment groups showed significant decrease (p is less than 0.01) in calcium and phosphate level when in relation to toxic control group.





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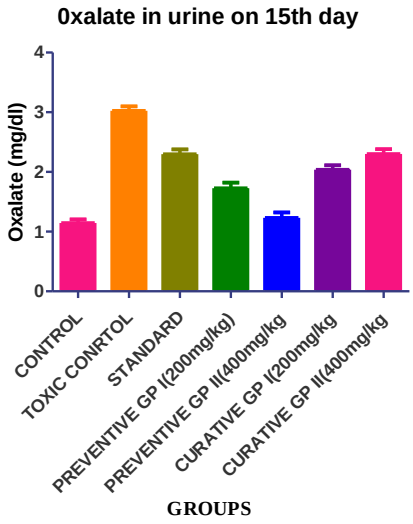


Figure 5, 6 & 7: Urine biochemical parameters on 15th day

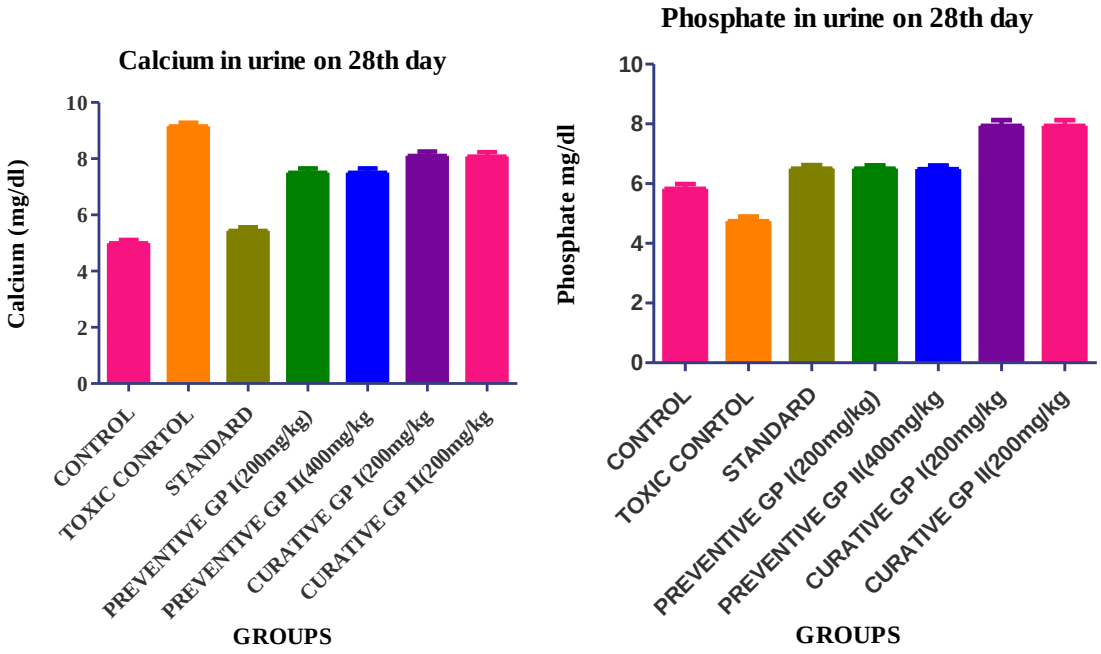
Effect of EEBB on Urine biochemical parameters on 28th day

Table No. 7 Effect of EEBB on Urine biochemical parameters on 28th day

Parameters	Control (Normal Saline)	Toxic Control (Ethylene Glycol)	Standard Cystone 750 mg/kg	Preventive Group-I EEBB 200 mg/kg	Preventive Group-II EEBB 400 mg/kg	Curative Group-I EEBB 200 mg/kg	Curative Group-II EEBB 400 mg/kg
Calcium	5.14±0.116	$9.35\pm0.12^{+++}$	$5.5\pm0.12^{***}$	$7.4\pm0.15^{***}$	$7.0\pm0.15^{***}$	$8.0\pm0.15^{***}$	$7.9\pm0.15^{***}$
Phosphate	5.99±0.16	$4.80\pm0.15^{+++}$	$6.55\pm0.12^{***}$	$6.09\pm0.10^{***}$	$6.6\pm0.11^{***}$	$7.9\pm0.19^{***}$	$7.6\pm0.19^{***}$
Oxalate	1.32±0.07	$3.47\pm0.11^{+++}$	$1.30\pm0.07^{***}$	$1.4\pm0.07^{***}$	$1.20\pm0.07^{***}$	$1.80\pm0.10^{***}$	$1.6\pm0.08^{***}$

Values are Mean ± S.E.M. (n=6); Significance values are ***P is less than0.001,**P is less than 0.01 and *P is less than 0.05. Comparison between Control group and all groups ^{+++}P is less than 0.001, $^{++}$P is less than 0.01 and $^{+}$P is less than 0.05. Comparison between Normal and all group.

The calcium, oxalate and phosphate level increased noticeably (p is less than 0.001) in the toxic control group (Fig no. 8,9 & 10) whereas decreased significantly (p is less than 0.001) in standard, preventive and curative groups when in relation to control groups. All the treatment groups showed significant decrease (p is less than 0.001) in calcium and phosphate level when in relation to toxic control group.



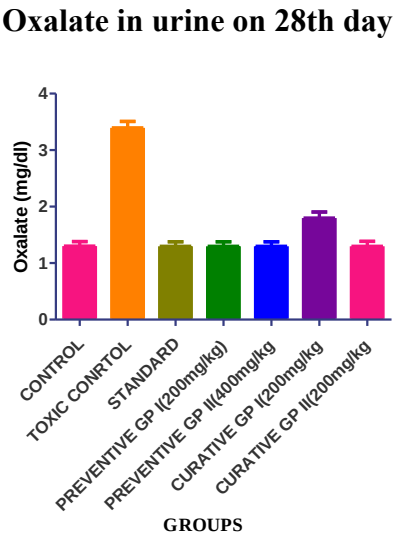


Figure no 8, 9 & 10: Urine biochemical parameters on 28th day

Effect of EEBB on Serum parameters on 28th day

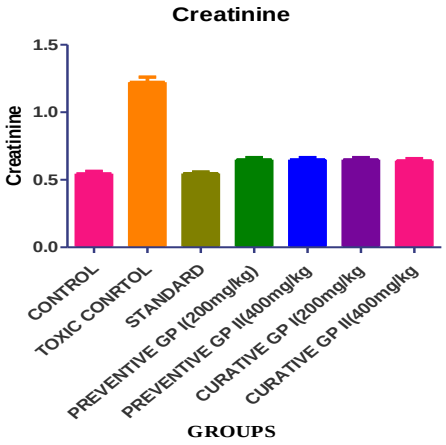
Table No. 8 Effect of EEBB on Serum parameters on 28th day

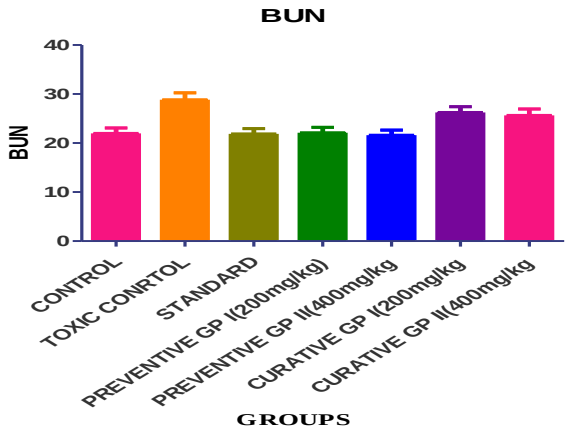
Parameters	Control (Normal Saline)	Toxic control (Ethylene Glycol)	Standard Cystone 750 mg/kg	Preventive Group-I EEBB 200 mg/kg	Preventive Group- II EEBB 400 mg/kg	Curative Group-I EEBB 200 mg/kg	Curative Group-II EEBB 400 mg/kg
Bun	22.5±1.10	28.0±1.42^{++}	23±1.08**	24.0±1.08**	24.53±1.11**	24.5±1.11**	24.0±1.08**

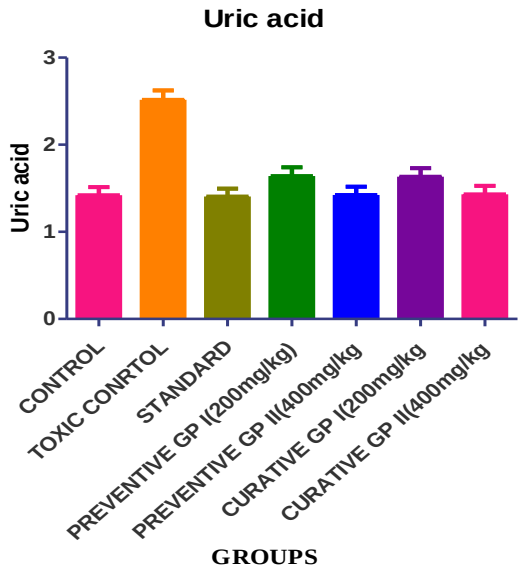
Creatinine	0.54±0.0	0.25±0.103^{+++}	0.58±0.01***	0.66±0.01***	0.62±0.01***	0.66±0.01***	0.61±0.01***
Uric acid	1.54±0.09	2.60±0.10^{+++}	1.45±0.08***	1.79±0.09***	1.62±0.09***	1.80±0.09***	1.66±0.09***
Calcium	5.65±0.16	6.4±0.18^{++}	5.5±17**	5.8±0.17**	5.7±0.16**	5.6±0.16**	5.55±0.16**

Values are Mean ± S.E.M. (n=6); Significance values are ******P* **is less than 0.001,P is less than 0.01 and *P is less than 0.05. Comparison between Control group and all groups $^{+++}$*P* is less than 0.001, $^{++}$P is less than 0.01 and $^{+}$P is less than 0.05. Comparison between Normal and all group.**

The serum parameters increased noticeably (p is less than 0.05) in the toxic control group (Fig no.11,12, 13 & 14) whereas decreased (p is less than 0.001) significantly in standard, preventive and curative groups when showed significant decrease (p is less than 0.01) in serum levels when in relation to toxic control group.







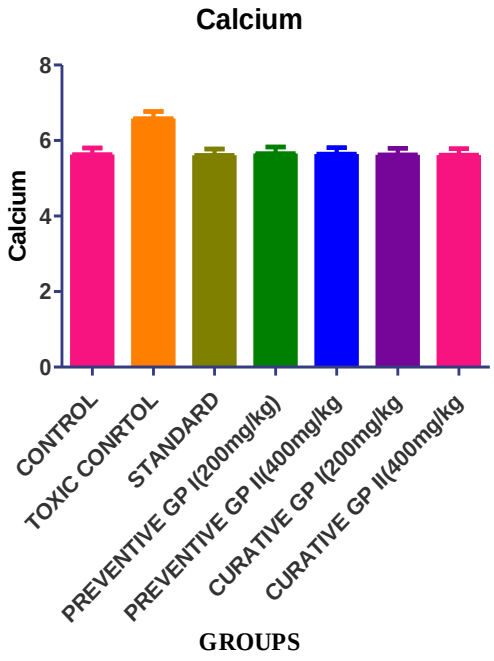


Figure 11,12,13 & 14: Serum Parameters on 28th day

HISTOPATHOLOGICAL STUDIES: CONTROL GROUP

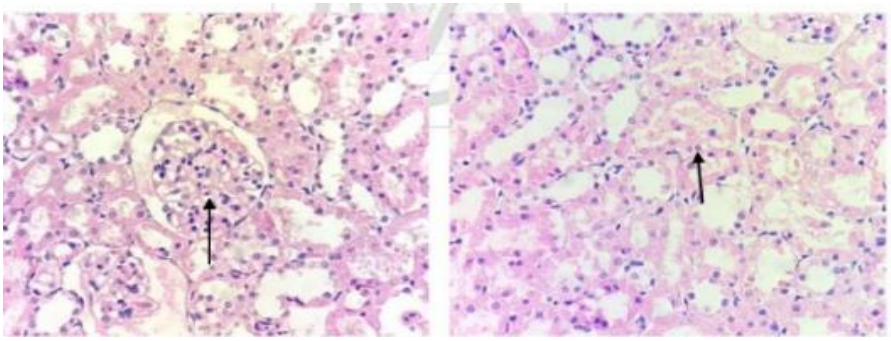


Figure 15: shows that the Glomerulus is having the normal cellularity (Fig 15.1. Arrow) and the tubules are intact (Fig 15.2 Arrow)

TOXIC CONTROL GROUP

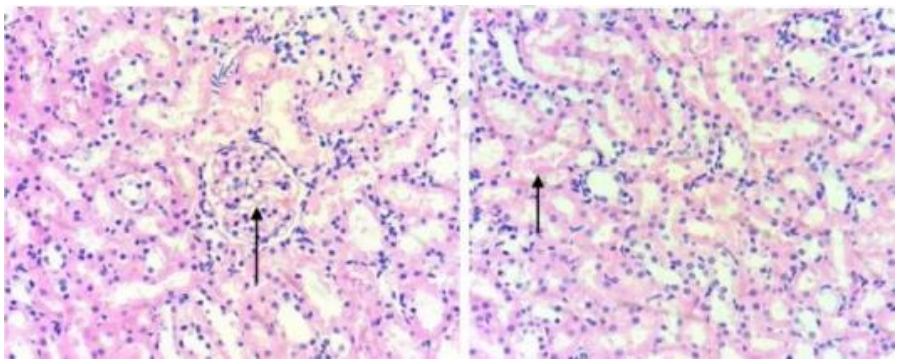


Figure 16: shows that the Glomerulus is having hypercellularity (Fig 16.1 Arrow)

and the tubules have necrosis (Fig 16.2 Arrow)

STANDARD GROUP (Cystone treated)

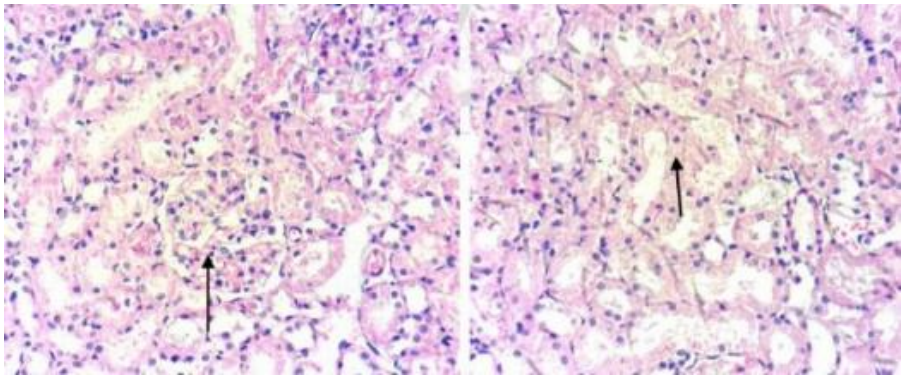


Figure 17: shows that the Glomerulus is having normal cellularity (Fig 17.1 Arrow) and the tubules have mild necrosis (Fig 17.2Arrow)

PREVENTIVE GROUP I (EEBB-200 mg/kg)

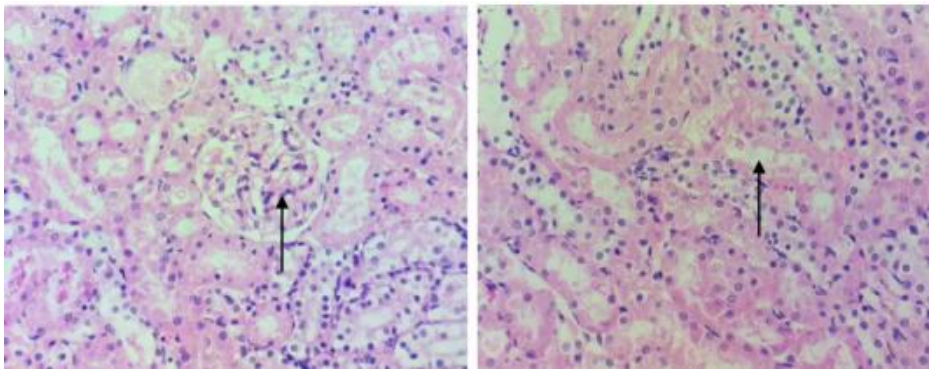


Figure 18: shows that the Glomerulus is having normal cellularity (Fig 18.1 Arrow) and the tubules are having moderate degenerative changes (Fig 18.2 Arrow)

PREVENTIVE GROUP II (EEBB-400 mg/kg)

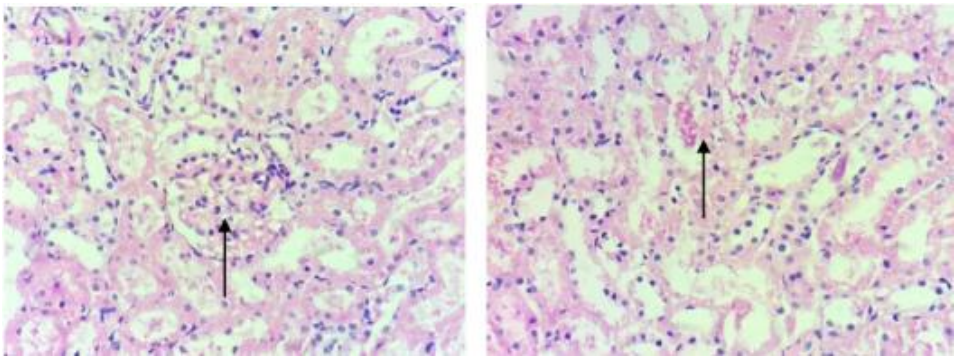


Fig 19: shows that the Glomerulus is having normal cellularity (Fig 19.1 Arrow)

and the tubules are having mild degenerative changes (Fig 19.2 Arrow)

CURATIVE GROUP I (EEBB-200 mg/kg)

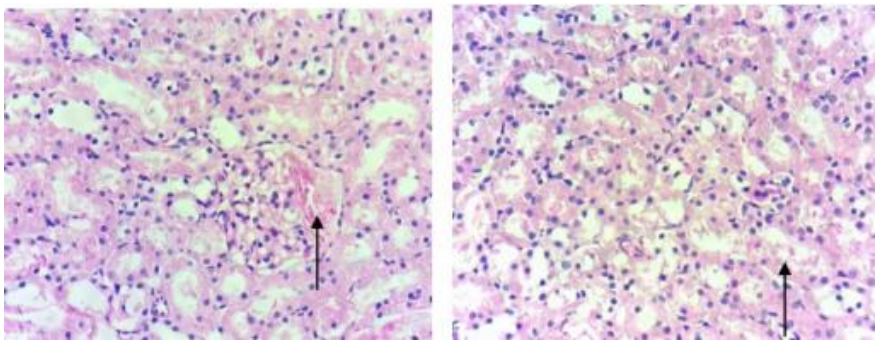


Fig 20: shows that the Glomerulus is having focal segmental sclerosis (Fig 20.1 Arrow) and the tubules are having necrosis. (Fig 20.2 Arrow)

CURATIVE GROUP II (EEBB-400 mg/kg)

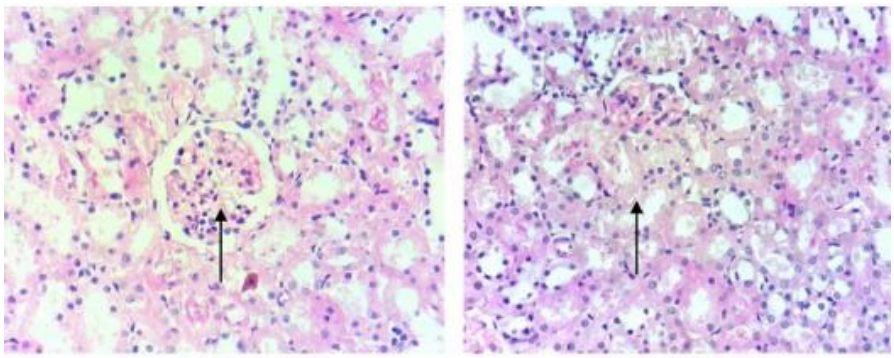


Figure 21: shows that the Glomerulus is having normal cellularity (Fig 21.1 Arrow)

And the tubules are having mild necrosis (Fig 21.2 Arrow)

DISCUSSION:

The conducted acute toxicity study confirmed the safety of the ethanolic extract of *Blepharis boerhaviifolia* (EEBB) administered at a maximum dose of 2000 mg/kg body weight, indicating its potential for further studies. The study focused on evaluating the antiurolithiatic properties of EEBB using an ethylene glycol-induced urolithiasis model in rats, mimicking human physiological system. In the urolithiasis-induced rats, elevated levels of urine calcium, phosphate and oxalate were observed, indicating the formation of kidney stones. However, treatment with EEBB at preventive and curative doses led to a notable decrease in these stone-forming components, suggesting the extract's potential to prevent stone formation. The increased urine output in the treated groups further supported the preventive and curative effects of EEBB, as reduced urine output is indicative of stone obstruction.

Serum analysis revealed elevated levels of BUN, creatinine, and uric acid in the toxic control group, indicating kidney damage. Treatment with EEBB led to a notable reduction in these values, suggesting renal safeguarding and enhanced kidney function. Analysis of kidney homogenates also revealed lower concentrations of stone-forming elements in the groups treated with EEBB, further confirming its antiurolithiatic properties. Histopathological examination revealed the presence of calcium oxalate crystal deposits and associated abnormalities in toxic control group. Treatment with EEBB led to a significant decrease in crystal depositions and abnormalities, demonstrating its ability to prevent these pathological changes in the kidney tissues.

The observed reduction in body weight in the toxic control group, likely due to physiological imbalance and mental stress caused by urolithiasis, was mitigated in the EEBB-treated groups. This suggests that EEBB not only prevents kidney stone formation but also helps maintain normal physiological functions and mental well-being. Microscopic studies confirmed the absence of stones in the groups treated with EEBB, indicating its potential to prevent the nucleation and aggregation of stones or dissolve pre-formed crystals. The decreased urine excretion in the toxic control group, caused by crystal deposition and reduced glomerular filtration rate, was countered by EEBB, leading to increased urine output and avoiding the buildup of nitrogenous waste substances in the blood.

The noted reduction in oxalate levels, lower calcium excretion, and stable phosphate levels in urine further reinforce the anti-urolithiatic properties of EEBB. The prevention of hyperoxaluria, a crucial factor in urolithiasis, indicates the extract's efficacy in hindering the process of stone formation. Additionally, the buildup of irregularly shaped polymorphic crystals within the tubules, seen in the histopathological analysis of Renal sections, was notably decreased in the groups treated with EEBB.

This study provides substantial evidence for antiurolithiatic properties of EEBB properties through multiple mechanisms. Its active constituents, yet to be fully identified, likely contribute to its efficacy. EEBB inhibits crystal aggregation by preventing calcium oxalate crystals from clumping together and dissolves pre-formed crystals in the urinary system, facilitating their passage. The extract reduces oxalate levels in urine, hindering crystal formation, and maintains a balanced urinary pH, discouraging crystal nucleation and growth. EEBB's diuretic properties increase urine production, preventing saturation of urine with stone-forming compounds. Additionally, EEBB exhibits antioxidant activity, protecting kidney tissues from oxidative stress, a factor in kidney stone formation. The extract also modulates specific metabolic pathways and likely influences enzymes involved in the conversion of ethylene glycol into oxalate, disrupting the process leading to oxalate stone formation. Further research and clinical studies could explore the extract's potential for human application, providing a natural and effective alternative for the prevention and treatment of urolithiasis.

CONCLUSION:

In this study, the EEBB demonstrated significant antiurolithiatic properties, indicating its potential as a natural remedy for urolithiasis. The EEBB effectively reduced elevated levels of urine calcium, oxalate and phosphate, essential components in Renal stone formation, both preventively and curatively. Additionally, it enhanced urine output, preventing stone obstruction. The observed decrease in BUN, creatinine and uric acid levels in serum indicated its renal protective effects, suggesting the preservation of kidney function. Microscopic studies revealed EEBB's ability to prevent crystal nucleation and aggregation, and histopathological examinations confirmed its efficacy in reducing calcium oxalate crystal depositions and associated abnormalities in kidney tissues. These findings are indicative of EEBB's potential to inhibit crystal aggregation, dissolve pre-formed crystals, regulate urinary pH, enhance diuresis, and provide antioxidant protection. Furthermore, the extract likely modulates metabolic pathways involved in stone formation. Although the specific active constituents remain to be fully identified, this comprehensive study lays a strong foundation for further research and clinical studies, potentially paving the way for EEBB's application as a natural and effective alternative for both prevention and treatment of urolithiasis in patients.

ACKNOWLEDGMENT: The authors thankful to the Oxbridge college of pharmacy, Bangalore, India. Vidya Siri College of Pharmacy, Bangalore, and King Faisal University, Al-Ahsa, Saudi Arabia for their support in carrying out this research work.

FUNDING: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU250526]

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