EVALUATION ON ANTIUROLITHIATIC ACTIVITY OF *BRYOPHYLLUM PINNATUM* OF RATS

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ABSTRACT:

Urinary excretion of oxalate increases rapidly and significantly during chronic administration of ethylene glycol (EG) as a 0.75% v/v aqueous solution in drinking water to male Sprague-Dawley rats. Thirty inbred male Wistar Albino rats (180-200g body weight) were used in this study. Five groups of study were designed for 28 days. **Group 1:** was used as normal control & was given water only. **Group 2:** was given 0.75% v/v Ethylene glycol (EG) in drinking water; served as nephrolithiatic induced group. **Group 3** was given 0.75% v/v ethylene glycol (EG) in drinking water and 30 mg/kg body weight, i.p. of *Bryophyllum pinnatum* (Lam.) Oken. hydroethanolic leaf extract and served as Test-1 group. **Group 4:** was given 0.75% v/v ethylene glycol (EG) in drinking water and 50 mg/kg body weight, i.p. of *Bryophyllum pinnatum* (Lam.) Oken hydroethanolic leaf extract and served as Test-2group. **Group 5:** given standard drug Cystone (Himalaya health care Pvt. Ltd) 5 ml/kg body weight p.o. served as reference standard group. Concentraion of calcium, oxalate, phosphorous, creatinine and blood urea nitrogen was observed in each group. Histopathology of Kidney tissue was observed in neutral buffered formalin (10% formaldehyde in Phosphate buffered saline). On the basis of biochemical parameters and histopathological study it was confirmed that *Bryophyllum pinnatum* leaf extract protected the renal cells from oxidative stress and injury induce by calcium oxalate crystals.

Key words: Calcium oxalate, Bryophyllum pinnatum, Kidney stones and Zinc disk

INTRODUCTION:

The subject of urolithiasis has been reviewed in many recent literatures. Documented cases of spontaneous urinary stone formation in rats are rare, and there is no report of spontaneously formed CaOx stones in the rat upper urinary tract. Excess urinary excretion of crystallizable substances of choice with or without manipulation of urinary pH and/or deficient excretion of crystallization inhibitors have been the principal mechanisms used to induce experimentally crystallization in the urine and formation of stones in the kidneys. [1]

Calcium Oxalate Nephrolithiasis

CaOx kidney stones are produced in rats by the induction of acute or chronic hyperoxaluria using a variety of agents such as sodium oxalate, ammonium oxalate, hydroxy-L-proline, ethylene glycol, and glycolic acid. Hyperoxaluric agents have often been used in association with vitamin D or a magnesium-deficient diet and, sometimes, with a pH-reducing protocol of ammonium chloride administration.

Urinary excretion of oxalate increases rapidly and significantly during chronic administration of ethylene glycol (EG) as a 0.75% v/v aqueous solution in drinking water to male Sprague-Dawley rats.

Chronic hyperoxaluria induced by the administration of 0.75% v/v EG alone or with 2% w/v ammonium chloride (AC) to male rats produce crystalluria, which was followed by CaOx nephrolithiasis. Combined treatment with EG + AC resulted in persistent crystalluria in all rats by day 3 and in nephrolithiasis by day 7. It takes approximately 12 days of chronic administration of EG alone to show persistent crystalluria and about 3 weeks to start depositing crystals in the kidneys of rats. Initially, small dipyramidal crystals appear in urine. [2]

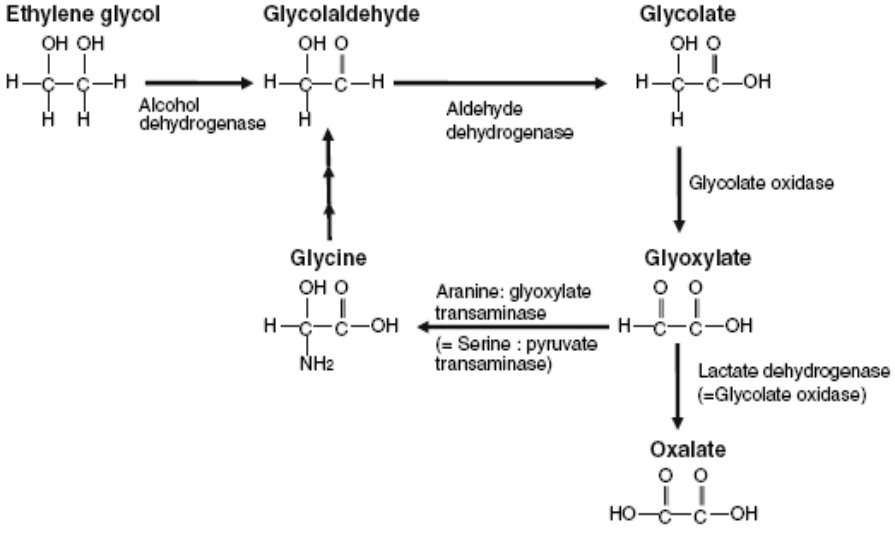

Fig 1: The major metabolic pathway of ethylene glycol to oxalate
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Foreign-body stones [2]

Vermeulen et al. developed a foreign-body model of production of urinary stones. Foreign bodies of a variety of substances were implanted in urinary bladders. The diet was modified or a lithogen was added to the drinking water to produce stone of desired composition.

The addition of EG to drinking water or a pyridoxine-deficient diet resulted in the formation of CaOx stones. Changing of the urinary ambient conditions by administration of EG for 2 weeks at 2-day or 2-week intervals resulted in the formation of urinary stones of mixed composition containing CaOx and struvite and/or CaP.

Coating of the foreign body with organic material initiated the encrustation. The stone grew by confluent crystal growth and aggregation, and its fractured surface demonstrates characteristic concentric laminations and radial striations. The matrix consisted of amorphous and fibrillar elements mixed with cellular degradation products.

A: Ethylene Glycol Induced Urolithiasis Model [3, 4]

Material & Methods

Chemicals: Ethylene Glycol (EG) LR, Cystone (Himalaya health care Pvt. Ltd).

Animals: Thirty inbred male Wistar Albino rats (180-200g body weight) were used in this study. Animals were procured from Institutional Animal House (Reg no. 621/02/ac/CPCSEA) of Birla Institute of Technology, Mesra, Ranchi. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27^{0}C and humidity 60-65% with 12:12 light: dark cycles). Food was provided in the form of dry pellets (Celebrex, Monsanto health care Pvt. Ltd) and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

Instrument: Metabolic Cages

Preparation of the doses: Hydroethanolic leaf extracts of *Bryophyllum pinnatum* (Lam.) Oken. Were taken in two doses of 30 mg/kg & 50 mg/kg body weight and dissolved in distilled water.

Treatment Protocols: Following treatment given daily for 28 days:

Group 1: was used as normal control & was given water only.

Group 2: was given 0.75% v/v Ethylene glycol (EG) in drinking water; served as nephrolithiatic induced group.

Group 3 was given 0.75% v/v ethylene glycol (EG) in drinking water and 30 mg/kg body weight, i.p. of *Bryophyllum pinnatum* (Lam.) Oken. hydroethanolic leaf extract and served as Test-1 group.

Group 4: was given 0.75% v/v ethylene glycol (EG) in drinking water and 50 mg/kg body weight, i.p. of *Bryophyllum pinnatum* (Lam.) Oken hydroethanolic leaf extract and served as Test-2group. .
Group 5: given standard drug Cystone (Himalaya health care Pvt. Ltd) 5 ml/kg body weight p.o. served as reference standard group.

Method:

Ethylene glycol induced hyperoxaluria model by the method of Atmani et al; [5] was used to assess the antilithiatic activity in albino rats. Male wistar rats weighing about 200-250 g were housed in metabolic cages 3 days prior to the start of the experiment for acclimatization. The experiment was conducted in accordance to internationally accepted standard guidelines for use of animals. All animals were kept under a controlled 12 h light dark cycle and room temperature. They were fed with regular standard pellets food (Celebrex, Monsanto health care Pvt. Ltd) and had free access to tap water *ad libitum*. They were then divided into five groups comprising six animals each. EG was added in their drinking water for 28 days to induce a chronic low grade hyperoxalluria and generate CaOx deposition into kidneys. Treatment was given according to treatment protocols. Twenty-four hour urine samples from each rat were collected daily to measure the urinary volume and pH, and to analyze qualitatively crystalluria as well. At the end of the experimental study, all animals were sacrificed after ether anaesthesia. Blood was collected from heart puncture and the kidneys were excised for histopathological examination. [4,5]

ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY [6]

1: GENERAL OBSERVATION:

During the study period, body weight, water intake and animal health observed regularly, so that stressed and unhealthy animal were excluded from study.

2: MICROCOPIC URINALYSIS:

A sample of well-mixed urine (usually 10-15 ml) was centrifuged at relatively low speed (about 2,000 to 3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supernatant was decanted and a volume of 0.2 to 0.5 ml was left inside the tube. The sediment was resuspended in the remaining supernatant by flicking the bottom of the tube several times. A drop of resuspended sediment was poured onto a glass slide and cover slipped.

The sediment was first examined under low power to identify most crystals examination was carried out at high power to identify crystals. The number of crystal were counted and expressed in crystals/mm^3

3: URINE ANALYSIS:

All animals were kept in individual metabolic cages and urine samples of 24 h were collected till 28^{th} day. Animals had free access to drinking water during the urine collection period.

Volume and **pH** were measured immediately after collection. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4^0C. Urine was analyzed for **calcium, phosphate**, **oxalate** and **creatinine** contents.

A: Method for Oxalate Determination [7]

Direct Precipitation:

The quantitative precipitation of oxalate as its cacium salt, followed by permanganate titration, has attracted the attention of many workers because of its simplicity. The method presents little difficulty in aqueous solutions, and quantities of oxalic acid as low as 0.05 mg may be estimated with accuracy. Urine, however, contains many substances that either inhibit precipitation of calcium oxalate or carried down with the precipitate and react with permanganate. The former compounds include magnecium, polyphosphates, and other polyelectrolytes, while the latter include uric and citric acids.

The procedure described by Archer *et al.* has been widely used as a rapid screening test. The principle of the method is as follow: Samples of urine are adjusted to pH 5.0 to 5.2 by the addition of acetic acid or ammonia solution. Two millilitres of a calcium chloride solution (5g/ 100 ml) is added to each 50 ml sample and the mixtures are allowed to stand for 16 hrs at room temperature. The precipitated calcium oxalate is separated by centrifuging, the supernatant fluid is decanted, and the precipitate is washed with dilute ammonia solution. The washed precipitate is dissolved in 1N sulphuric acid and the solution is titrated with 0.01N KMnO4 at 60^0 to 70^0 C (1.0 ml of 0.01 N KMnO4 is equivalent to 0.45 mg of anhydrous oxalic acid).

B: Method for Calcium Determination [8]

Calcium determined by using the Crest Biosystem diagnostic kit.

Principle

Calcium in an alkaline medium combines with O-cresolphthalein complexone (OCPC) to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Calcium + OCPC (O-cresolphthalein complexone) ======>Purple coloured complex

Reagents

L1: buffer reagent
L2: colour reagent
S: calcium standard (10 mg/dl)

Procedure

1. Reagents were pipetted into clean dry test tubes according to table and labelled as blank (b), standard (s) and test (t):

Table 1: Preparation of sample for Calcium determination

Addition Sequence	Blank (B) ml	Standard (S) ml	Test (S) ml
Buffer Reagent (L1)	0.5	0.5	0.5
Colour Reagent (L2)	0.5	0.5	0.5
Distilled Water	0.02	-	-
Calcium Standard (S)	-	0.02	-
Sample	-	-	0.02

2. They were mixed well and incubated at room temperature (R.T) for 5 minutes. The absorbance of the standard (abs.S), and test sample (abs.T) against blank, was measured within 60 minutes, at wavelength/filter: 570 nm.

Calculations

Calcium in mg/dl = (absorbance of test/ absorbance of standard) x 10

Linearity

This procedure is linear up to 18 mg/dl. If values exceed this limit, the sample is diluted with distilled water and repeated the assay. The value is calculated using the proper dilution factor.

C: Method for Phosphorus Determination [9]

Phosphorus determined by using the Crest Biosystem diagnostic kit.

Principle

Phosphate ions in an acidic medium react with ammonium molybdate to form a phosphomolybdate complex.
Phosphorus +Ammonium molybdate =====> Phosphomolybdate complex
Phosphomolybdate complex + Metol=====> MolybdinumBlue Complex

Reagents

L1: acid reagent, L2: molybdate reagent, L3: colour reagent
S: phosphorus standard (5 mg/dl)

Procedure

1. Reagents were pipetted into clean dry test tubes as given in table and labelled as blank (b), standard (s) and test (t):
2. They were mixed well and incubated at R.T for 5 minutes. The absorbance of the standard (abs. S), and test sample (abs.T) against the blank, was measured within 30 minutes, wavelength/filter: 650 nm.

Table 2: Preparation of sample for Phosphorus determination

Addition Sequence	Blank (B) ml	Standard (S) ml	Test (S) ml
Acid Reagent (L1)	1.0	1.0	1.0
Molybdate Reagent (L2)	1.0	1.0	1.0
Distilled Water	0.1	-	-
Phosphorus Standard (S)	-	0.1	-
Sample	-	-	0.1
Colour Reagent	1.0	1.0	1.0

Calculations

Phosphorus in mg/dl = (abs T/abs S) x 5

Linearity

This procedure is linear up to 15 mg/dl. If values exceed this limit, the sample is diluted with distilled water and the assay repeated. the value calculated using the proper dilution factor.

4: SERUM ANALYSIS

After the experimental period, blood was collected by cardiac puncture under aneasthetic conditions and the animals were sacrificed. Serum was separated by centrifugation at 10,000×*g* for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

A: Method for Blood Urea Nitrogen Determination [10]

Blood Urea Nitrogen was determined by using the Crest Biosystem diagnostic kit.

Principle

Urease hydrolyzes urea to ammonia and CO_2. The ammonia formed further reacts with a phenolic chromogen and 2 hypochlorite to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

Urease

Urea + H_2O ==========> Ammonia + CO_2

Ammonia + phenolic chromogen + hypochlorite ======> green coloured complex

Reagents

L1: buffer reagent

L2: enzyme reagent

L3: chromogen reagent

S: urea standard (40 mg/dl)

Procedure

1. Reagent were pipetted into clean dry test tubes as given in table and labeled as blank (b), standard (s) and test (t) (Table-3).

Table 3: Preparation of sample for BUN determination

Addition Sequence	Blank (B) ml	Standard (S) ml	Test (S) ml
Buffer Reagent (L1)	1.0	1.0	1.0
Enzyme Reagent (L2)	0.1	0.1	0.1
Distilled Water	0.01	-	-
Urea Standard (S)	-	0.01	-
Sample	-	-	0.01

2. They were mixed well and incubated for 5 minutes or 10 minutes at RT (25º c). The absorbance of the standard (abs. S) and test sample (abs. T) against the blank, was measured within 60 minutes, at wavelength/filter: 570 nm.

Calculations
Urea in mg/dl = (abs. T/ abs. S) x 40
Urea nitrogen in mg/dl = urea in mg/dl x 0.467

Linearity
The procedure is linear up to 250 mg/dl. Using the working chromogen reagent (1.0 ml) the linearity is increased to 400 mg/dl. If values exceed this limit, the serum is diluted with normal saline (NaCl 0.9%) and repeated the assay. The value is calculated using the proper dilution factor.

B: Method for Creatinine Determination [11]
Creatinine was determined by the method of Jaffe Picrate method [10] using the Crest Biosystem diagnostic kit.

Principle
Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.
Creatinine + Alkaline picrate =======> Orange coloured complex

Reagents
L1: Picric acid reagent, L2: Buffer reagent, S: Creatinine standard (2 mg/dl)

Procedure
1. Reagents were Pipetted into clean dry test tubes as given in table and labelled as standard (s) or test (t):

Table 4: Preparation of sample for Creatinine determination

Addition Sequence	Blank (B) ml	Standard (S) ml	Test (S) ml
Picric Reagent (L1)	0.5	0.5	0.5
Buffer Reagent (L1)	0.5	0.5	0.5
Distilled Water	0.1	-	-
Creatinine Standard(S)	-	0.1	-
Sample	-	-	0.1

3. They were mixed well and read the initial absorbance at wavelength 520 nm A_1 for the standard and test after exactly 30 seconds. Read another absorbance A_2 of the standard & test exactly 60 seconds later. The change in absorbance dA was calculated for both the standard and test.

Wavelength/filter: 520 nm (hg 492 nm) / Green.
Change in absorbance for standard d As = $A_{1s} – A_{2s}$
Change in absorbance for test d At = $A_{1t} – A_{2t}$

Calculations
Creatinine in mg/dl = (d At / d As) x 2.0
Urine Creatinine in mg/dl = (d At/ d As) x 1.0
Urine Creatinine g / 24 hrs. = urine creatinine in g/l x vol. of urine in 24 hrs.

Linearity
This procedure is linear, upto 20 mg/dl of creatinine. If values exceed this limit, the sample is diluted with distilled water and repeated the assay. The value is calculated using the proper dilution factor.

5: KIDNEY HOMOGENATE ANALYSIS:

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80^0 C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000×*g* for 10 min and the supernatant was separated. The **calcium, phosphate**, **oxalate** & **creatinine** contents in kidney homogenate were determined. [12]

6: HISTOPATHOLOGICAL EXAMINATION [13]

Procedure:

1. After deep ether anaesthesia, animal was dissected by cutting on the ventral side.

2. Kidney tissue was fixed in neutral buffered formalin (10% formaldehyde in Phosphate buffered saline) over night.
3. After fixation, the tissues placed in 70% isopropyl alcohol for 3 hours and then in each ascending strength (80%, 90%, 100% isopropyl alcohol) for 2 hours each. The amount of alcohol used should be 15 times of the size of the tissue.
4. Then the tissue was dipped in acetone for a period of 1 – 2 h with periodical shaking.
5. After removing the acetone, xylene was added to check for the appearance of milkyness. If milkyness appears then repeat the dehydration procedure.
6. The dehydrated tissue was impregnated in paraffin wax (m.p. = 56 ^{0} C) for a period of 1h at 58 – 60 ^{0} C.
7. Molten paraffin poured into L-block along with the tissues and allowed it to become hard.
8. The tissue was sectioned into very thin (2–8 or 5 – 10 micrometer) sections using a microtome.
9. The tissue Mounted on the slides with Mayer's albumin solution (a mixture of equal parts of egg white and glycerin, beaten and filtered with the addition of 1% sodium salicylate) and incubated in warm oven for 2 h at 60ºC.
10. Slides containing paraffin sections were placed on a slide holder.
11. Slides were deparaffinized with Xylene for 30 minutes and the excess xylene blotted.
12. The tissue was rehydrate successively with 100%, 90%, 80% isopropyl alcohol for 2 – 3 min. each and put it into water for 3 min.
13. The excess water blotted; the tissue was kept into Haematoxylin stain for 1 – 2 min.
14. Then again kept into tap water for 1 – 2 min.
15. The slides containing tissue sections dipped into 1N HCl followed by Scott's water (Sodium Bicarbonate 3.5 g, Magnesium sulphate 20 g, distilled water 1 litre) for 1 min each.
16. The tissue was immersed in Eosin stain for 30 seconds.
17. Dehydrate the tissue successively with 80%, 90%, 100% isopropyl alcohol and finally with Xylene for 20 – 30 min.
18. Place cover slip on the slides using one drop of DPX (Dextrine-polystyrene xylene), taking care to leave no bubbles and dry overnight to make the permanent slide.

B: Implantation of Zinc disc in urinary bladder [11,13]

Material & Methods

Material: Zinc disc, Cystone (Himalaya health care Pvt. Ltd).

Animals: Thirty inbred male Wistar Albino rats (180-200g body weight) were used in this study. Animals were procured from Institutional Animal House (Reg no. 621/02/ac/CPCSEA) of Birla Institute of Technology, Mesra, Ranchi. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27^{0}C and humidity 60-65% with 12:12 light: dark cycles). Food was provided in the form of dry pellets (Celebrex, Monsanto health care Pvt. Ltd) and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

Preparation of the doses: Hydroethanolic leaf extracts of *Bryophyllum pinnatum* (Lam.) Oken. were taken in two doses of 30 mg/kg & 50 mg/kg body weight and dissolved in distilled water.

Treatment Protocols:

Group I, served as normal control and were given water only.

Group II, was implanted with Zinc disc in the Urinary bladder which served as untreated control group and were received water only.

Group III, was implanted with Zinc disc in the Urinary bladder and was given plant extract at a dose of 30 mg/kg body weight, i.p. and served as Test1group.

Group IV, was implanted with Zinc disc in the Urinary bladder and was given plant extract at a dose of 50 mg/kg body weight, i.p. and served as Test 2 group.

Group V, given standard drug Cystone (Himalaya health care Pvt. Ltd) 5 ml/kg body weight p.o. served as reference standard.

Method: Male rats of the Wistar strain weighing about 200-250 g were used for experiment. Rats were anaesthetized with sodium pentobarbitone (40 mg/kg body weight, i.p.). A suprapubic incision was given and the urinary bladder was exposed. A small cut was made at the top of the bladder, the urine was aspirated aseptically into a sterile vial for bacteriological examination and pH of the urine was determined using narrow range pH paper. A previously weighed sterile zinc disc (50 mg/kg) was inserted into the bladder and the incision was closed with a single suture using absorbable 4-0 chromic catgut (Ethicon) according to method of Komer et al., 1951; Vermeulen, 1962. The abdomen was closed in layers. The rats were allowed to recover for 1 week. Rats were divided into five groups of six animals each and put on different treatment schedule according to treatment protocol. All the parameters are evaluated as in EG induced urolithiasis for assessment of antiurolithiatic activity. [13]

Statistical calculations:
The data expressed are mean ± standard error of mean (SEM) and the median inhibitory concentration (IC_{50}value) with 95% confidence intervals. All statistical comparisons between the groups are made by means of One Way Analysis of Variance with post hoc Dunnett's test or by Student's t-test. The *p* value less than 0.05 is regarded as significant. The concentration-response curves were analyzed by non-linear regression using Graph Pad Instate 3 (Trial version).

RESULTS

GENERAL OBSERVATIONS:
The results of body weight, water intake and urine volume, urinary pH and composition were recorded before the commencement of treatment & found that these were not significantly different amongst the groups. The parameters recorded from groups of animals at the end of 28

days of treatment period have been summarized in Table 19. Weight of untreated group of animals where nephrolithiasis has been induced decreased significantly ($p<0.01$) comparison to normal and treated groups. However the treatment groups showed little change in body weight (Fig.18). Water intake significantly ($p<0.01$) increased in all groups compared to control group.

Table 5: Effect of treatments on Body weight, water-intake & Crystalluria

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Change in Body weight (gms)	6.34 ±1.56	-8.23 ±4.51[b]	3.27 ±1.23[b]	3.42 ±1.39[b]	5.47 ±1.46[b]
Daily Water Intake (ml/24hr)	15.51 ±0.23	22.51 ±0.28[b]	25.32 ±0.47[b]	32.72 ±1.28[b]	37.68 ±1.31[b]
Crystalluria (No of Crystals/mm3)	2.43 ±1.45	185.00 ±6.60[b]	35.00 ±1.41[d]	16.37 ±1.25[d]	9.00 ±1.26[d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Microscopic Urinalysis:

The Change in body weight, Daily water intake, & Crystalluria (No. of Crystals/mm3) levels in normal control, nephrolithiatic induced control, Cystone (standard drug) treated and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups have been tabulated in Table-19. Only a few CaOx crystals were seen in the 24 h morning urine of the vehicle control animals, whereas, hyperoxaluric treatment group induced significant ($p< 0.01$) CaOx crystalluria. At 30 & 50 mg/kg body weight i.p. *Bryophyllum pinnatum* leaf extract visibly reduced the crystal size with significantly decreased ($P<0.01$) number of crystals but at 50 mg/kg body weight i.p. there was a highly significant decrease in urine crystal count as well as crystal size (Fig.2.)

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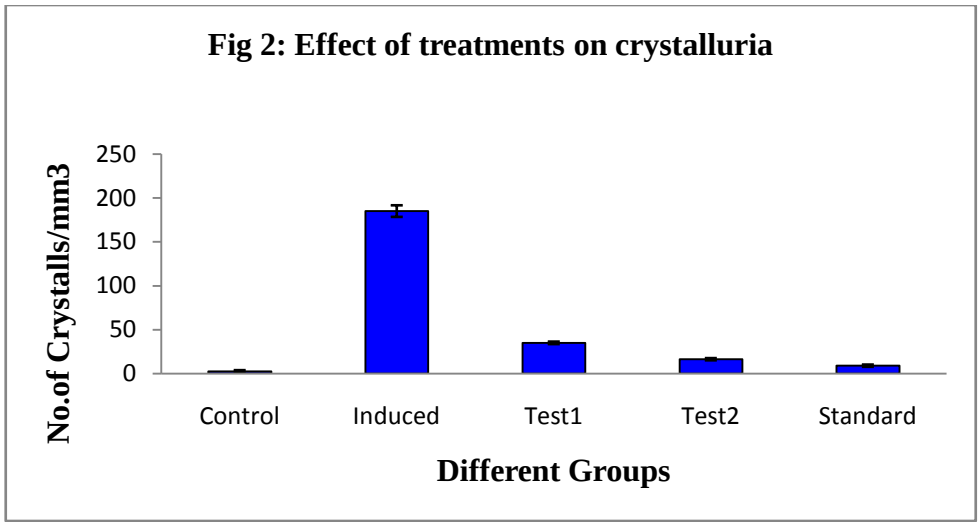


Fig. 2: The effect of treatment on body weight. (Weight of induced group decreased significantly ($p < 0.01$) in comparison to normal and treated group. The treatment groups show little change in body weight)

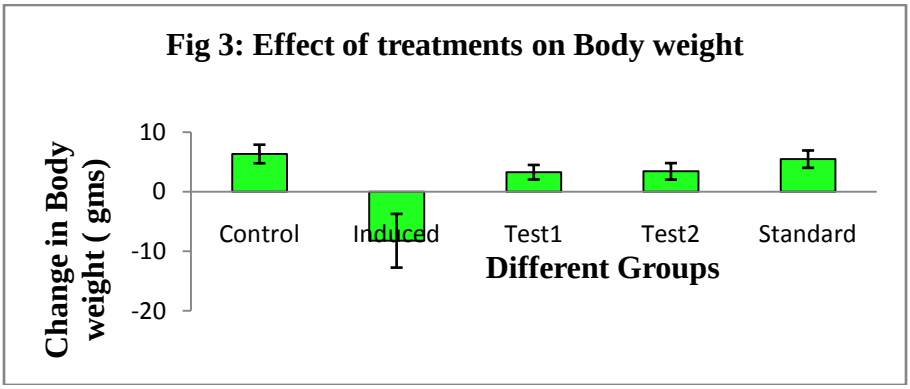
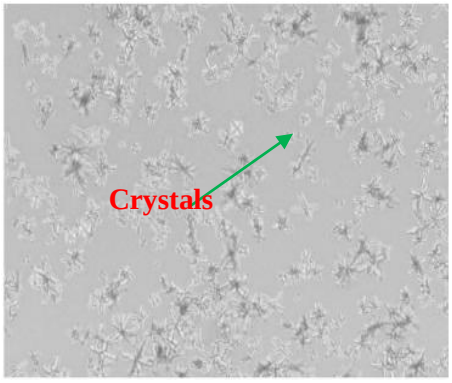


Fig 4: Presence of Calcium oxalate crystals in the urine of different treatment groups seen under microscope (Leica DME) at 40x10X magnification

1.Normal Control



2. EG control

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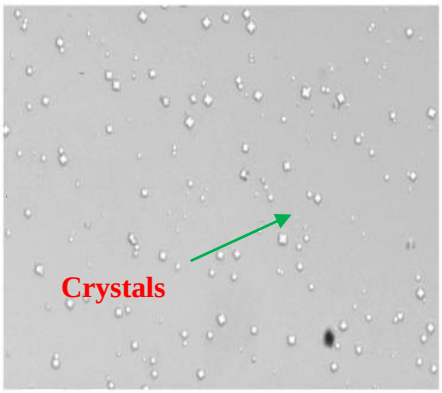
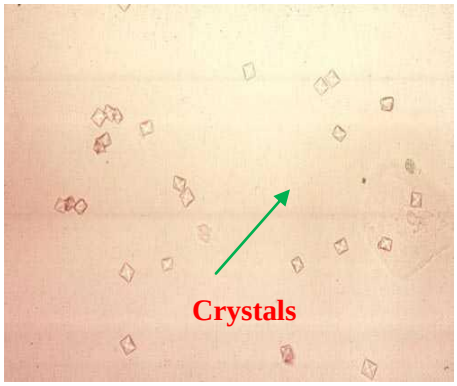
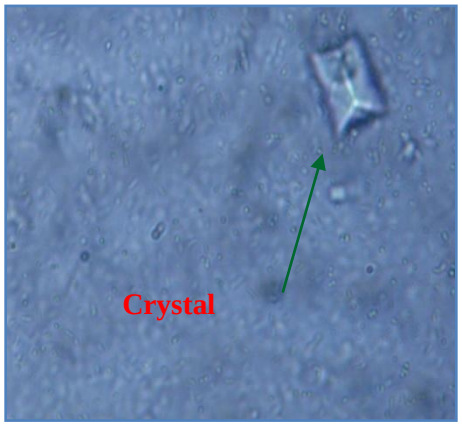


3. BPE 30mg/kg Treatment



4. BPE 50 mg/kg Treatment



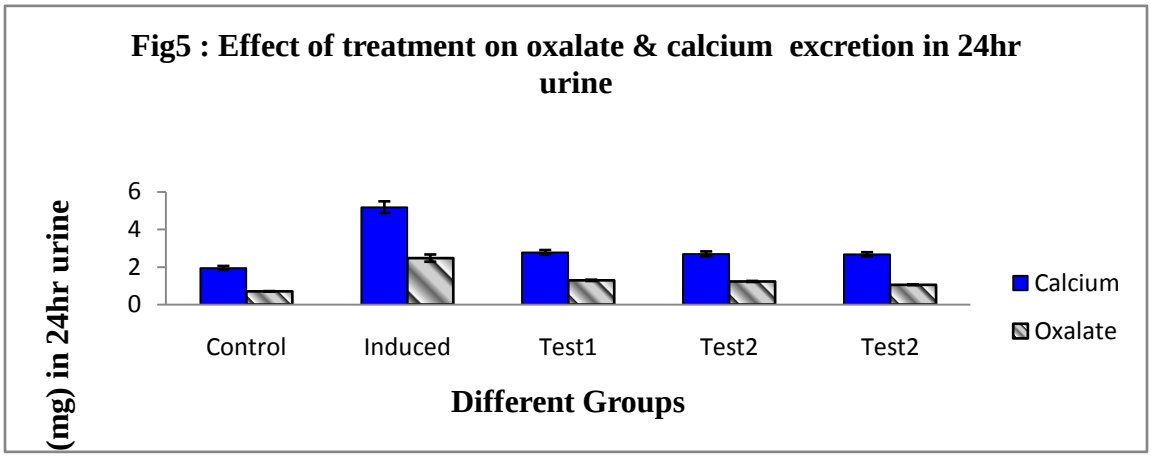
5. Standard treatment

Urine Analysis: The results of urine analysis in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table 20. Lithogenic induced treatment with EG significantly reduced ($p<0.01$) the pH of urine in the induced group as compared to that of the control group. A co-treatment with *Bryophyllum pinnatum* leaf extract at both the doses (30 mg/kg body weight i.p. and 50 mg/kg body weight i.p.) increased urine volume in a dose-dependent manner, although these parameters remained higher than those of the induced animals even at 30 mg/kg body weight i.p. ($p < 0.05$). In parallel with crystalluria, there was an increased calcium and oxalate concentrations of the urine collected from the induced animals. *Bryophyllum pinnatum* leaf extract at 30 & 50 mg/kg body weight i.p. prevented the change in urinary calcium and oxalate ($p < 0.05$) and ($p < 0.01$) respectively as compared to induced group (Table6 & Fig.6)

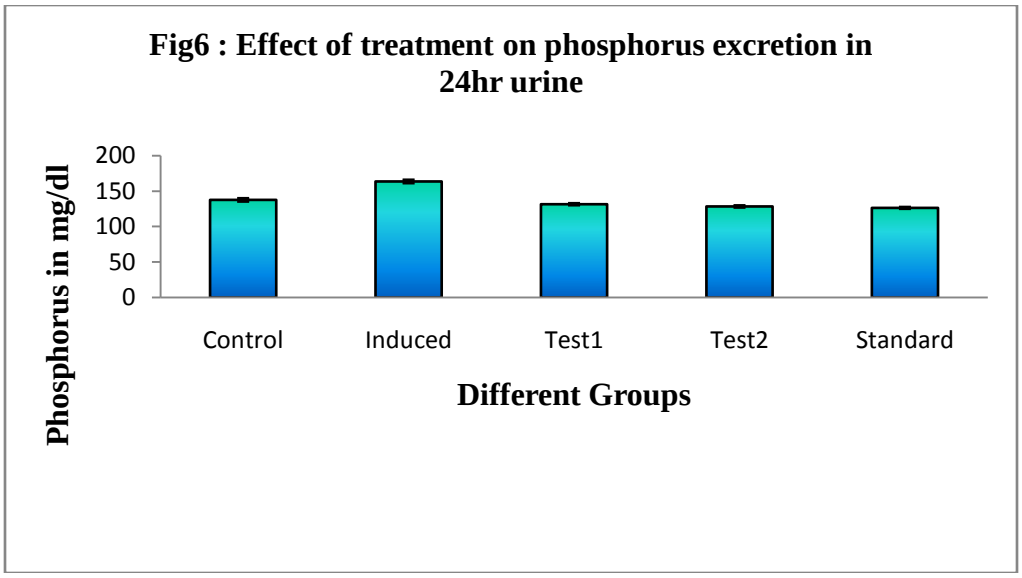
Table 6: Effect of treatments on twenty four Hours Urine Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Volume (ml/24hr)	8.77 ±0.53	13.25 ±0.27[b]	18.07 ±0.22[b,d]	23.06 ±1.33[b,d]	28.24 ±1.23 [b,d]
pH	7.4 ±0.03	6.3 ±0.06[b].	7.3 ±0.07[c]	7.4 ±0.06[d]	7.4 ±0.05[d]
Calcium (mg/dl)	1.94 ±0.105	5.17 ±0.311[b]	2.77 ±0.125 [a,d]	2.69 ±0.129 [a, d]	2.67 ±0.108[b, d]
Oxalate (mg)	0.697 ±0.012	2.465 ±0.193 [b]	1.282 ±0.034[a,d]	1.223 ±0.027 [a,d]	1.043 ±0.016 [a,d]
Phosphorus (mg/dl)	137.786 ±2.318	163.755 ±2.386 [b]	131.655 ±1.387[c]	128.522 ±1.502 [d]	126.487 ±1.253 [b, d]

All values are expressed as mean **±** S.E.M. for six animals in each group.
a p<0.05 compared with control group; **b** p<0.01 compared with control group;
c p<0.05 compared with Induced group; **d** p<0.01 compared with Induced group.



However the treatment groups show the little change in phosphorus excretion in urine (Fig. 7).



Serum Analysis:

The results of serum analysis for the measurement of renal function in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaves extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table 21. Lithogenic treatment caused impairment of renal functions of the induced animals as evident from the markers of glomerular and tubular damage, raised BUN and serum Creatinine (Fig.22 & 23) and reduced creatinine clearance ($p < 0.01$), which were dose-dependently prevented in the animals receiving a simultaneous treatment with *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. Oxalate serum level were also significantly elevated ($p<0.01$) in induced animals due to lithogenic treatment (Fig. 23).

Table 7: Effect of Treatments on Serum Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Blood Urea Nitrogen(BUN)	50.831 ±6.575	102.952 ±13.028 [b]	65.881 ±8.094[d]	43.431 ±4.034[c,d]	44.621 ±3.245 [d]
Creatinine (mg/dl)	0.713 ±0.047	0.952 ±0.027 [b]	0.603 ±0.031[c]	0.507 ±0.083 [c,d]	0.571 ±0.055 [d]
Phosphate (mg/dl)	25.245 ±0.368	39.015 ±1.025 [c]	31.133 ±0.443 [c]	29.699 ±0.236[c]	29.494 ±0.469 [b]
Oxalate (mg/dl)	0.337 ±0.022	1.957 ±0.067[b]	1.202 ±0.039 [d]	0.750 ±0.030 [b, d]	0.720 ±0.025 [b, d]
Calcium (mg/dl)	1.738 ±0.045	3.493 ±0.177 [b]	2.768 ±0.088 [d]	2.122 ±0.116 [b, d]	2.115 ±0.147 [b, d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Fig 7: Effect of Treatments on Blood Urea Nitrogen Level in Serum

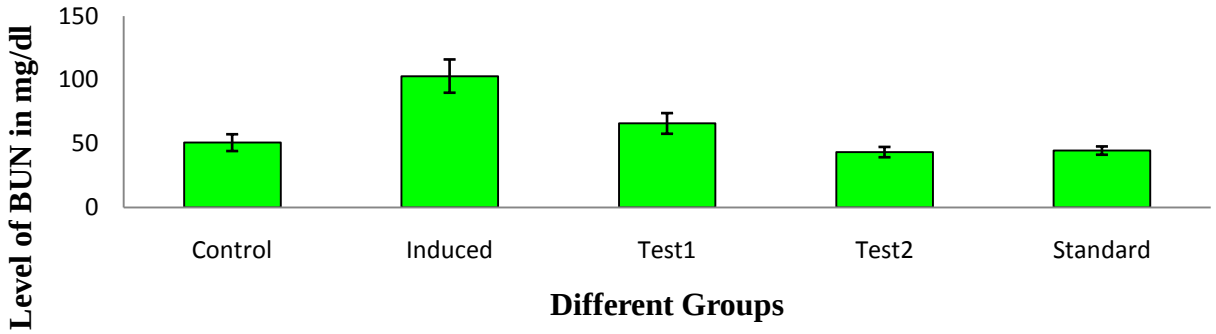
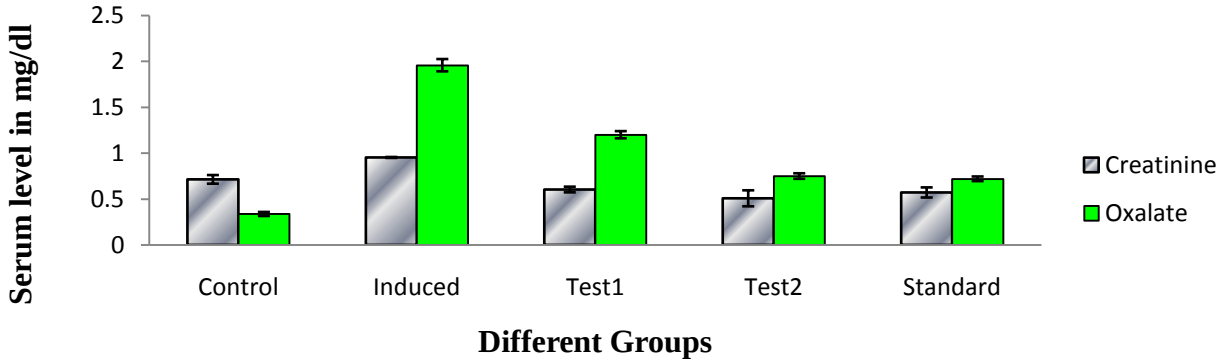


Fig 8: effect of Treatments on Creatinine & Oxalate level in serum



Kidney homogenate analysis:

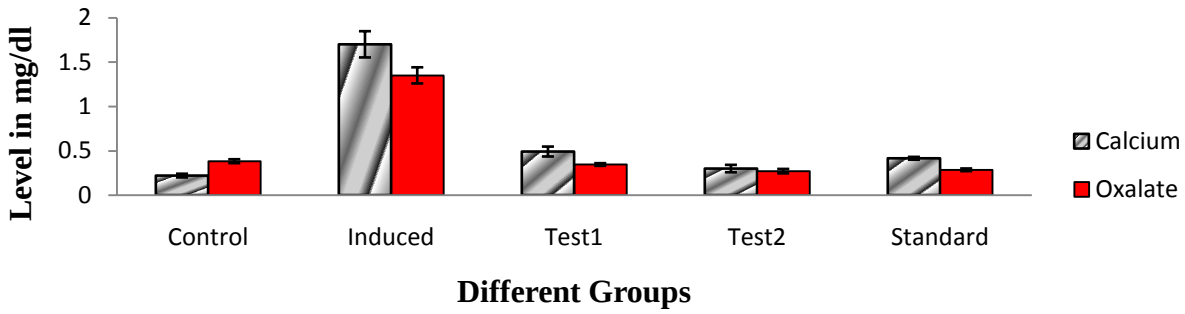
The results of kidney homogenate analysis for the measurement of renal function in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table 22. Kidneys excised from induced group were larger and heavier than the control animals ($p < 0.01$), whereas in *Bryophyllum pinnatum* leaf extract treated at 50 mg/kg body weight i.p. group kidneys were not significantly different from those of the control animals. Calcium and oxalate content of kidney significantly ($p<0.01$) increased in induced rats but in *Bryophyllum pinnatum* leaf extract treated groups these levels were not significantly different from control (Fig. 24).

Table 8: Effect of treatments on Kidney Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Kidney wt (gms)	0.651 ±0.031	1.625 ±0.052 [b]	0.722 ±0.021 [d]	0.633 ±0.026 [d]	0.591 ±0.023 [d]
Calcium (mg/dl)	0.220 ±0.020	1.707 ±0.148 [b]	0.491 ±0.056 [b, d]	0.299 ±0.042 [d]	0.415 ±0.016 [d]
Oxalate (mg/dl)	0.382 ±0.022	1.350 ±0.090 [b]	0.345 ±0.015 [d]	0.270 ±0.025 [b, d]	0.285 ±0.015 [d]
Phosphorus(mg)	22.868 ±1.271	30.306 ±2.316	23.059 ±0.366 [c]	20.969 ±0.698 [d]	21.693 ±0.476 [d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Fig 9: Effect of Treatment on Calcium & Oxalate level in Kidney Homogenate



Histopathological examination of Kidneys:

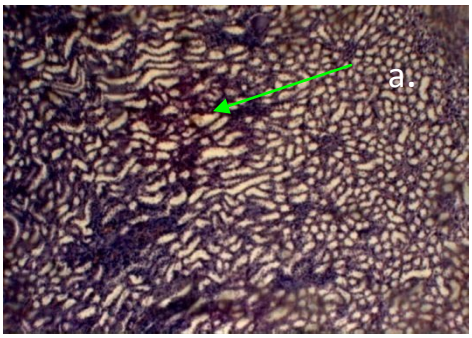
When observed under microscope, many crystalline deposits in the histological preparations were seen in tubules of all regions of kidneys: cortex, medulla and papilla, of all the animals in the nephrolithiatic induced group (Fig25 b). Control rats showed normal glomerular and tubular histology (Fig25a) whereas ethylene glycol group was found to cause glomerular, peritubular and blood vessel congestion and result in the presence of inflammatory cells in kidney sections (Fig25 b). Concurrent treatment with the *Bryophyllum pinnatum* leaf extract was found to reduce such changes in kidneys histology induced by ethylene glycol which have been shown in fig.25 and Table-23.

Table9: Histopathological features of the kidneys of rats of different treatment groups

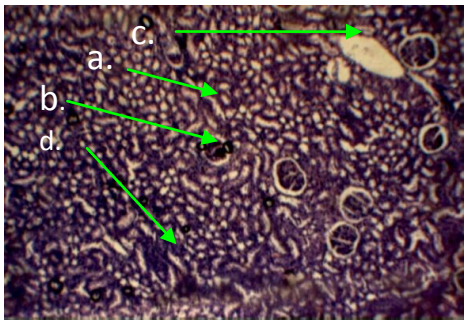
Histopathological Feature	Group I Normal Control	Group II EG treated	Group III EG treated and *B.Pinnatum* treated (30mg/kg i.p.)	Group IV EG treated and *B.Pinnatum* treated (50mg/kg i.p.)	Group IV treatedwith (Std)
Glomerular congestion	–	+++	++	+	+
Peritubular congestion	–	+++	++	+	+
Epithelial desquamations	–	+	–	–	–
Blood vessel congestion	–	+++	++	–	–
Interstitial edema	–	+	–	–	–
Inflammatory cells	–	+++	++	–	–
Necrosis	–	+	–	–	–
Tubular casts	–	+	–	–	–

+++ = Highly ++ = moderately, + = mild

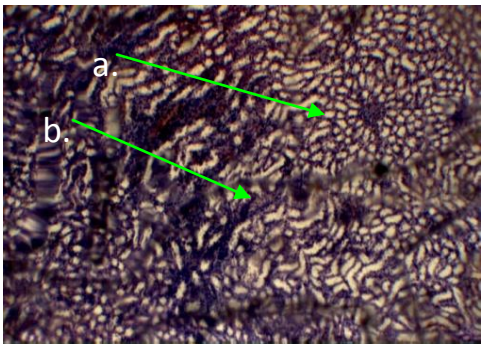
Fig 10: Histopathological examination of Kidney tissue of different groups



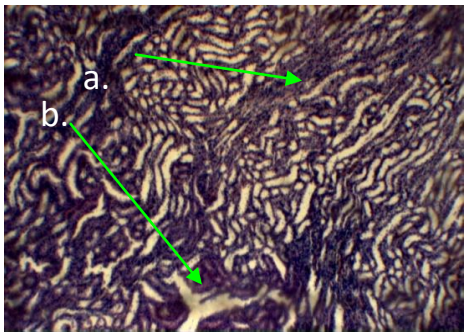
1. Control



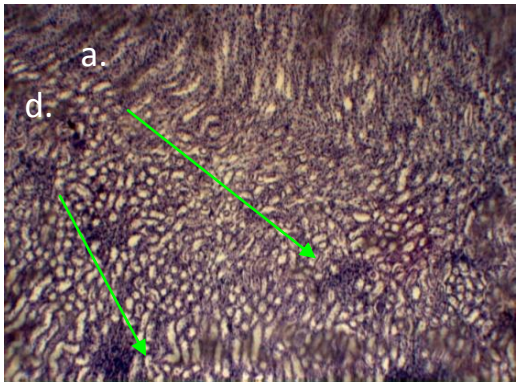
2. untreated(EG 0.75% v/v)



3. Test (BPE30mg /kg)



4. Test (BPE 50 mg/kg)



a. Glomerular & peritubular congestion.

b. Inflammatory cell infiltration.

c. Necrosis.

d. Blood vessel congestion.

5. Standard (Cystone treated)

Results for Implantation of Zinc disc in urinary bladder

General Observations:

The results of body weights, water intake and urine volume, and urinary pH have been recorded before the commencement of treatment and were found to have insignificant difference amongst the groups. The parameters recorded from the groups of animals at the end of 28 days of treatment period have been given in Table 24. Weight of untreated group decreased significantly ($p<0.01$) in comparison to normal and treated groups. However the treatment group show little change in body weight which has been shown in Fig.26. Water intake significantly increased ($p<0.01$) in all groups compared to control group.

Microscopic Urinalysis:

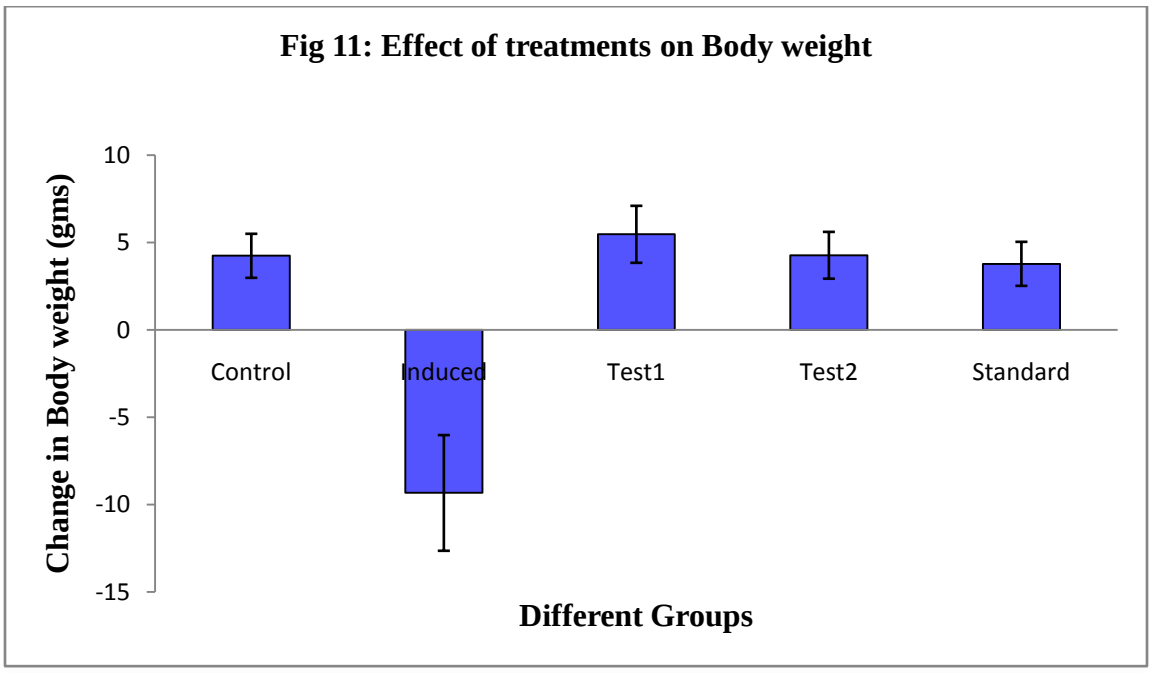
The Change in body weight, Daily water intake, & Crystalluria (No. of Crystals/mm3) levels in normal control, nephrolithiatic induced control, Cystone (standard drug) treated and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups have been tabulated in Table-24. Only a few CaOx crystals were seen in the 24 h morning urine of the vehicle control animals, whereas, the hyperoxaluric treatment induced group increased significantly ($p< 0.01$) CaOx crystalluria. At 30 and 50 mg/kg body weight i.p. *Bryophyllum pinnatum* leaf extract visibly reduced the crystal size with significantly decreased ($p<0.05$) number of crystals but at 50 mg/kg body weight i.p. there was a more significantly (0.01) decrease in urine crystal count as well as crystal size (Fig.27 & 33).

Table 10 : Effect of treatments on Body weight, water-intake & Crystalluria

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Change in Body weight (gms)	4.24 ±1.26	-9.33 ±3.31[b]	5.47 ±1.63[d]	4.27 ±1.34[d]	3.78 ±1.26[d]
Daily Water Intake (ml/24hr)	10.50 ±0.33	18.53 ±0.38[b]	22.22 ±0.37[b]	28.62 ±1.38[b, d]	35.58 ±1.20[b, d]
Crystalluria (No of Crystals/mm3)	3.43 ±1.25	244.320 ±6.50[b]	45.02 ±1.61[b, d]	24.32 ±1.35[b, d]	12.20 ±1.16[b, d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a p<0.05 compared with control group; b p<0.01 compared with control group;
c p<0.05 compared with Induced group; d p<0.01 compared with Induced group.

Fig. 11: The effect of treatment on body weight. (Weight of Induced group decreased significantly (P< 0.01) in comparison to normal and treated group. The treatment groups show little change in body weight)



Urine Analysis:

The results of urine analysis in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table 25. Lithogenic treatment with zinc implantation in urinary bladder significantly reduced (P<0.01) the pH of urine in the induced group as compared to that of the control group. A co-treatment with *Bryophyllum pinnatum* leaf extract at both the doses (30 mg/kg body weight i.p. and 50 mg/kg body weight i.p.) increased urine volume in a dose-dependent manner, although these parameters remained higher than those of the induced animals even at 30 mg/kg body weight i.p. ($p < 0.05$). In parallel with crystalluria, there was an increased calcium and oxalate concentrations of the urine collected from the induced animals. *Bryophyllum pinnatum* leaf extract at 30 & 50 mg/kg body weight i.p. prevented the change in urinary calcium and oxalate ($p < 0.05$) and ($p < 0.01$) respectively as compared to induced group (Table-25 & Fig. 28).

Table 10: Effect of treatments on twenty four Hours Urine Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Volume (ml/24hr)	12.47 ±0.23	06.25 ±0.37 [b]	10.07 ±0.32 [b,d]	16.06 ±1.23 [b,d]	18.34 ±1.33 [b,d]
pH	7.4 ±0.03	6.1 ±0.08.	7.3 ±0.07	7.4 ±0.07	7.4 ±0.14
Calcium (mg/dl)	1.662 ±0.021	3.743 ±0.195 [b]	2.031 ±0.079 [b, d]	1.906 ±0.052 [b, d]	1.842 ±0.061 [a, d]
Oxalate (mg)	2.087 ±0.041	3.172 ±0.064 [b]	2.055 ±0.069 [a,d]	1.960 ±0.062 [b, d]	1.852 ±0.098 [b, d]
Phosphorus (mg/dl)	134.921 ±2.059	184.622 ±2.508 [a]	147.270 ±3.160 [a, c]	143.660 ±2.857 [b, c]	127.630 ±6.148 [a, d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Fig 12: Effect of treatments on crystalluria

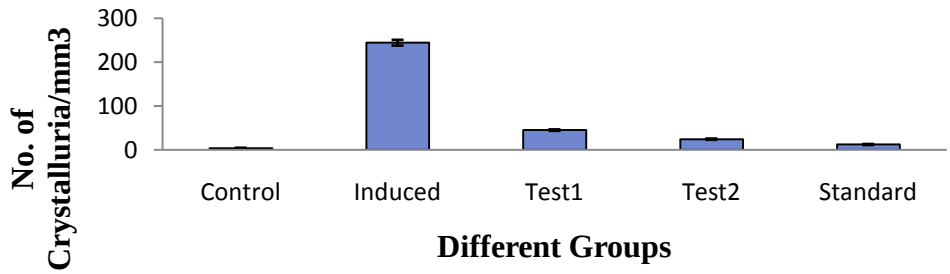
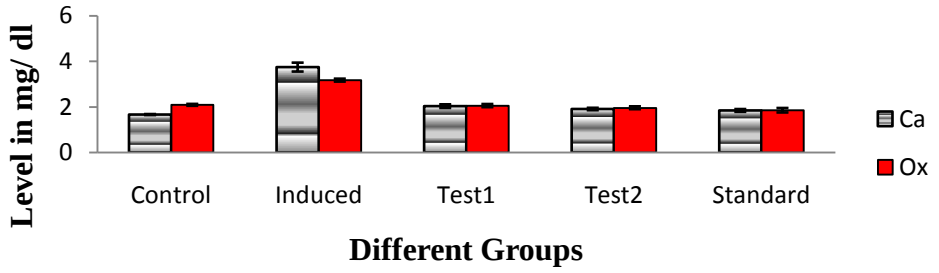


Fig 13 : Effect of treatment on oxalate & calcium excretion in 24hr urine



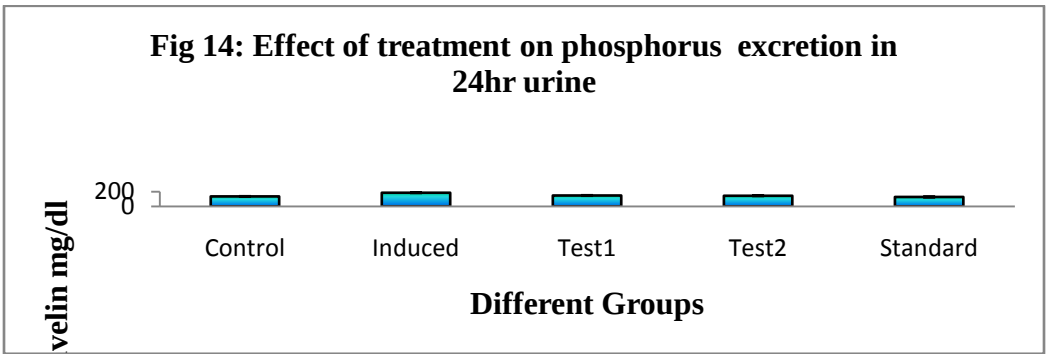


Fig 14: Effect of treatment on phosphorus excretion in 24hr urine

Serum Analysis:

The results of serum analysis for the measurement of renal function in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table-26. The raised BUN (Blood Urea Nitrogen) levels and serum Creatinine leves have been shown in Fig-30 & Fig-31 and significantly reduced creatinine clearance ($p < 0.01$), which were dose-dependently prevented in the animals receiving a simultaneous treatment with *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. Oxalate serum level were also significantly elevated ($p<0.01$) in induced animals due to lithogenic treatment (Fig-32).

Table 11 : Effect of Treatments on Serum Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Blood Urea Nitrogen(BUN)	64.510 ±1.520	95.355 ±1.954 [b]	68.203 ±1.396 [d]	46.918 ±0.904 [c,d]	45.787 ±2.660 [d]
Creatinine (mg/dl)	0.714 ±0.143	1.571 ±0.238 [b]	0.761 ±0.145 [d]	0.348 ±0.084 [d]	0.602 ±0.063 [d]
Phosphate (mg/dl)	23.647 ±1.231	31.004 ±1.250 [a]	26.789 ±0.180 [d]	26.679 ±0.667 [c]	25.266 ±0.298 [d]
Oxalate (mg/dl)	0.247 ±0.022	1.711 ±0.270 [b]	1.125 ±0.051 [d]	0.765 ±0.025 [b, d]	0.645 ±0.054 [d]
Calcium (mg/dl)	1.884 ±0.101	2.820 ±0.087 [b]	2.186 ±0.045 [d]	1,943 ±0.143 [d]	1.941 ±0.026 [d]

All values are expressed as mean ± S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; **b** $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; **d** $p<0.01$ compared with Induced group.

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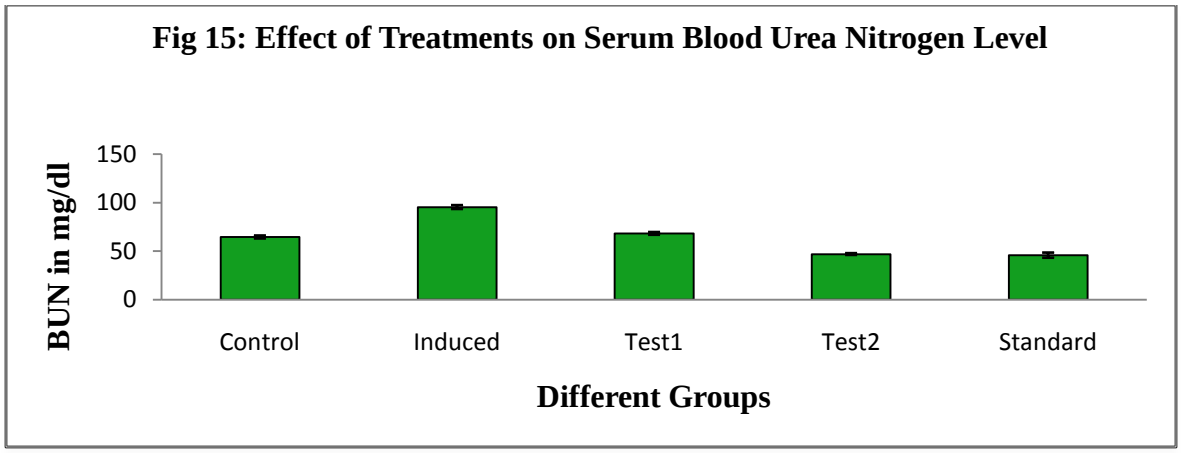

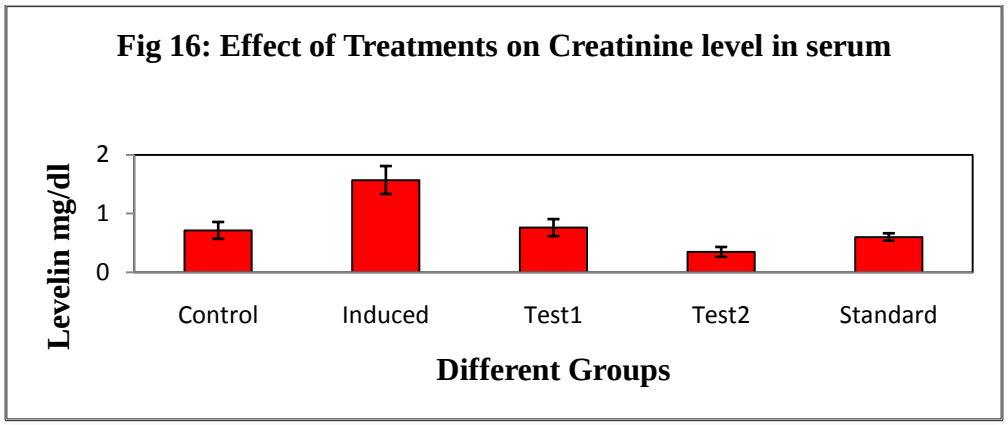

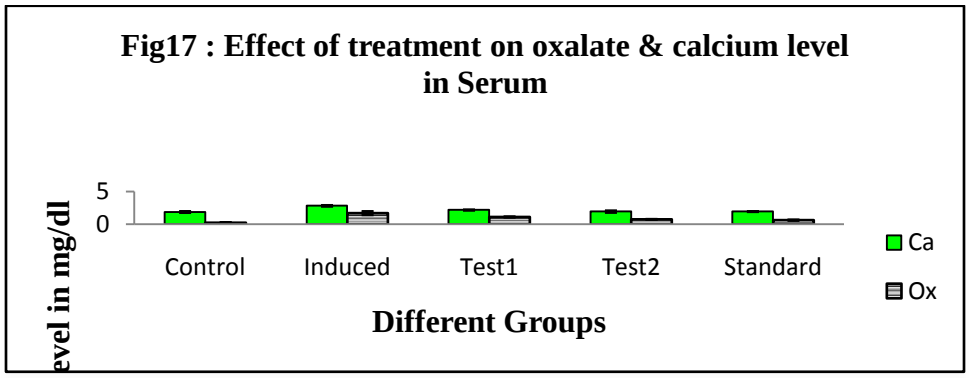

The effect of treatment on bladder parameters in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaf extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table-27. A significant increase ($P<0.01$) in bladder weight and stone weight was observed in nephrolithiatic induced

animals as compared to normal animals. The bladder weight was found near to normal in animals treated with *Bryophyllum pinnatum* leaf extract at 50 mg/ kg body weight i.p. and a significant decreased ($p< 0.01$) stone weight in animals treated with *Bryophyllum pinnatum* leaf extract at 50 mg/ kg body weight i.p.

Table 12: Effect of treatments on Bladder Parameters

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Bladder wt (gms)	0.365 ±0.055	0.870 ±0.050[b]	0.536 ±0.060 [b, d]	0.386 ±0.018[d]	0.435 ±0.015 [d]
Stone wt (gms)	0.001 ±0.001	0.258 ±0.027 [b]	0.138 ±0.013 [b, d]	0.016 ±0.003[d]	0.013 ±0.002 [d]

All values are expressed as mean **±** S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; b $p<0.01$ compared with control group;
c $p<0.05$ compared with Induced group; d $p<0.01$ compared with Induced group.

Weight of stone

The difference between the weight of the implanted zinc disc at the time of Implantation & final weight of the dried calculi taken out from the bladder with stone at the end of 4 weeks period indicated the amount of deposited stone (Table 27).

Gross pathology of the kidney:

At the end of 28 days, the kidneys of the induced rats were enlarged. The renal pelvis was dilated and contained a purulent exudate. There was presence of necrotic and ulcerative papillitis & complete or partial disappearance of the tip of the papilla which is further depicted by the histopathological findings.

The effect of treatment on kidneys parameters in normal control, nephrolithiatic induced control, Cystone (Standard) treated, and *Bryophyllum pinnatum* leaves extract treated at 30 mg/kg body weight i.p. and 50 mg/kg body weight i.p. groups has been tabulated in Table-28. A significant increase ($p<0.01$) in kidney weight and enlarged kidney size was observed in nephrolithiatic induced animals as compared to normal control animals. The kidney weight and kidney size were found near to normal in animals treated with *Bryophyllum pinnatum* (Lam.) leaf extract at 50 mg/ kg body weight i.p. and treated with standard drug Cystone (Himalaya health care Pvt. Ltd) at 5 ml/kg body weight p.o. as compared to nephrolithiatic induced groups.

TABLE 13: EFFECT OF TREATMENTS ON KIDNEY PARAMETERS

Observational Parameter	Group 1 Control	Group 2 Induced	Group 3 Test 1	Group 4 Test 2	Group 5 Standard
Kidney wt (gms)	0.751 ±0.043	1.885 ±0.037 [b]	0.821 ±0.047[d]	0.733 ±0.021[d]	0.691 ±0.021 [a, d]
Kidney (Size)	Nornal	Enlarged	Normal	Normal	Nomal

All values are expressed as mean **±** S.E.M. for six animals in each group.
a $p<0.05$ compared with control group; **b** $p<0.01$ compared with control group;

c $p<0.05$ compared with untreated group; **d** $p<0.01$ compared with untreated group.

Histopathology:
The kidneys and urinary bladders of animals that had zinc disc implanted in their bladders were examined microscopically. Out of the 24 rats harboring the disc impregnated, 18 developed pyelonephritis as well as urinary bladder infections. Four of the remaining six animals developed lesions of the urinary bladder (Table29), whereas two of them exhibited no lesions whatsoever. Histological Examination of the 18 rats that were assessed as demonstrating renal involvement revealed important and severe changes in the renal pelvis and medulla. The tip of the renal papilla was necrotic, and calcareous deposits were randomly distributed in necrotic and adjacent tissues.

A zone of polymorphonuclear leukocytes and intense collateral hyperemia divided necrotic areas from living tissues of the pyramid. Tubules in the medulla adjacent to necrotic tissue had undergone granular degeneration, were sometimes dilated, and occasionally contained casts. There were dense concentrations of leukocytes in and around these tubules. As the lesion progressed, immature granulation tissue was observed in the medulla. The inflammatory process extended into the cortex in some kidneys. The renal lesion was characterized as an acute necrotizing pyelonephritis. The control rats that had only sterile disc implanted in the urinary bladder had no remarkable lesions in their kidneys with the exception of an occasional randomly distributed calcareous deposit.

The microscopic features of urinary bladders from rats infected disc and erosion of transitional epithelium of the mucosa. Inflammatory cells, principally polymorphonuclear leukocytes with lesser numbers of lymphocytes, extended into the submucosa. Squamous metaplasia of transitional epithelium was observed in some areas adjacent to mucosal ulceration. Calcareous deposits were scattered throughout necrotic tissue, and occasional bacteria were observed. This pathological process was diagnosed as an acute necrotizing cystitis. The urinary bladders of rats into which sterile zinc disks had been implanted were characterized by focal mucosal ulceration and focal necrosis. Inflammatory cells were often present in the mucosa and submucosa, and/or foreign body granulomas were induced as a result of the trauma of disc implantation.

Finally none of the normal control rats developed bladder wall abnormalities of any recognisable nature. The kidneys of induceded group had urothelial lesions and bladder stones were present and treated group rats had little urothelial lesions and bladder stone formation. This represented a significant differrence between the development of bladder stones and the occurrence of urothelial lesions in treated group rats. Urothelial lesions, therefore, can be reproduced in untreated group rats. Their occurrence appears independent of bladder stone formation (Table 14).

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Table 14: Histopathological features of the Urinary bladder of different groups

Histopathological features	Group1 Normal Control	Group2 Induced with Zinc implanted	Group3 Test1	Group4 Test2	Group5 Standard (Cystone)
Urothelial lesions	_	+++	++	+	+
Inflammatory cells	_	+++	++	+	+
Mucosal ulceration	_	+++	++	+	+
Focal necrosis.	_	+++	+	_	_
Calcareous deposits	_	+++	++	+	+
Squamous metaplasia of transitional epithelium	_	++	+	_	_

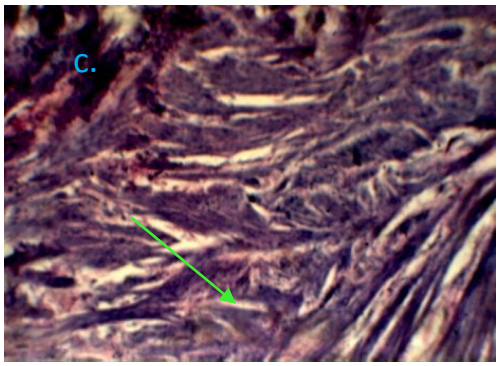
+++ = Highly ++ = moderately, + = mild

Table 15: Bacterial infection in urine of different groups

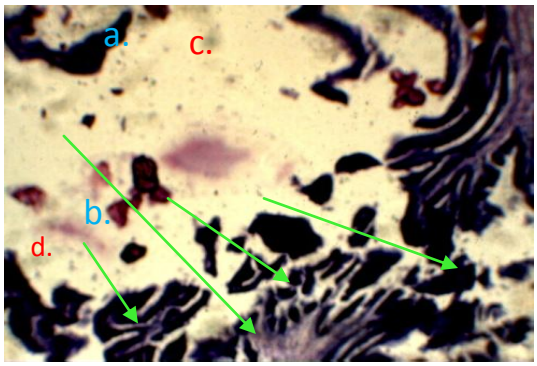
Bacterial Examination	Group1 Normal	Group2 Induced	Group3 Test1	Group4 Test2	Group5 (Std)
Gram +ve	_	+++	++	+	_
Gram – ve	_	++	+	+	+

+++ = Highly ++ = moderately, + = mild

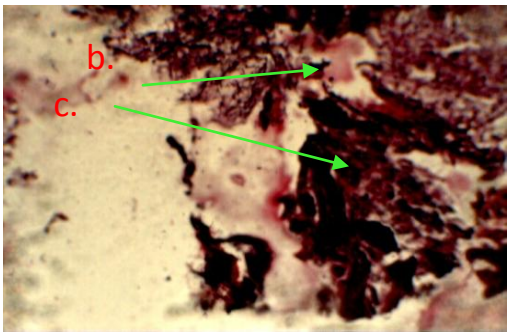
Fig18 : Histopathological examination of Urinary bladder tissue of diff. groups



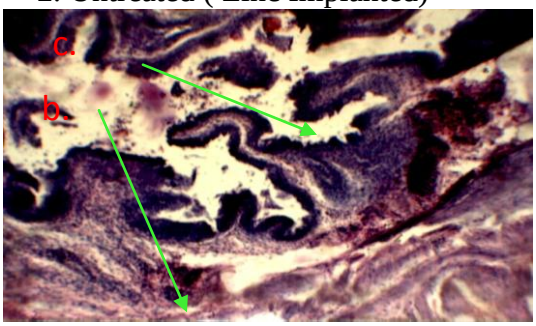
1.Control



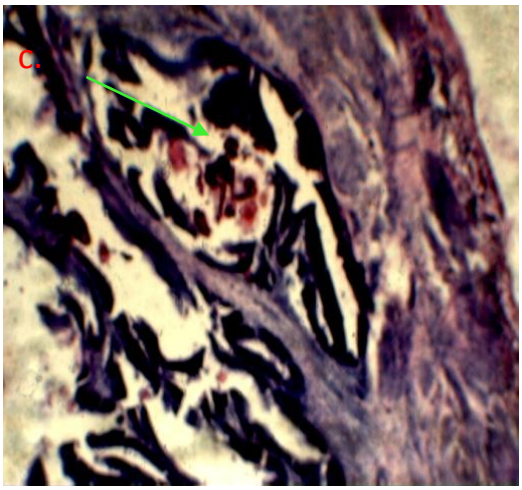
2. Untreated (Zinc Implanted)



3.Treated with BPE 30 mg/kg



4. Treated with BPE 50 mg/kg



a.Focal necrosis
b. Urothelial lesions
c. Mucosal Ulceration
d. Calcareous deposits

Histopathological examination of Kidneys:

When observed under microscope, many crystalline deposits in the histological preparations were seen in tubules of all regions of kidneys: cortex, medulla and papilla, of all the animals in the induced group (Fig34 b). Control rats showed normal glomerular and tubular histology whereas zinc implanted group was found to cause glomerular, peritubular and blood vessel congestion and result in the presence of inflammatory cells in kidney sections. Concurrent treatment with the *Bryophyllum pinnatum* leaf extract was found to reduce such changes in kidney histology induced by zinc implantion in urinary bladder. (Figure19 and Table. 15).

Table: 15 Histopathological features of the kidneys of Rats of different treatment groups

Histopathological Feature	Group I Normal Control	Group II EG treated	Group III EG treated and BPE treated at (30mg/kg i.p.)	Group IV EG treated and BPE treated at (50mg/kg, i.p.)	Group IV EG & Std
Glomerular congestion	–	++	+	–	–
Peritubular congestion	–	++	+	+	+
Epithelial desquamations	–	+	–	–	–
Blood vessel congestion	–	++	+	–	–
Interstitial edema	–	+	–	–	–
Inflammatory cells	–	++	+	–	–
Tubular casts	–	+	–	–	–

+++ = Highly ++ = moderately, + = mild

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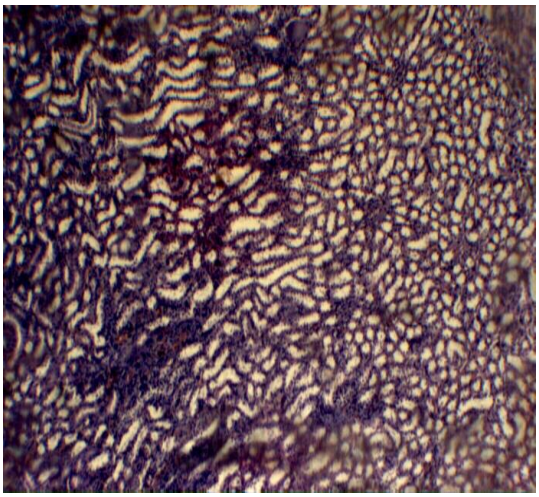
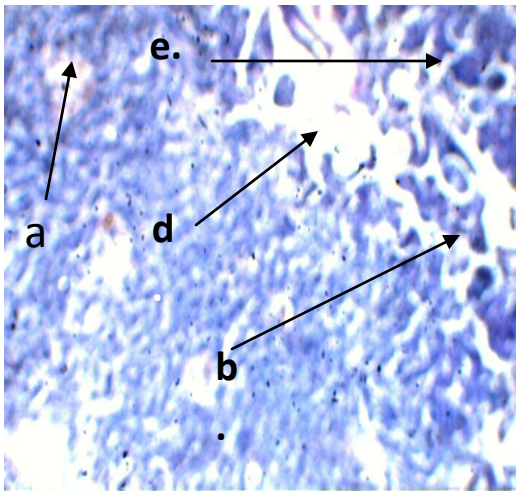
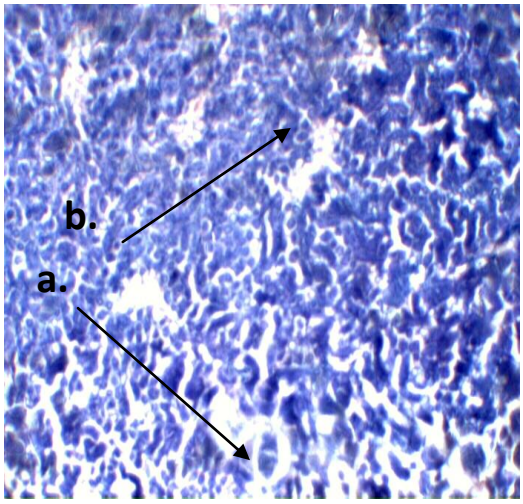
Fig19 : Histopathological examination of Kidney tissue of different groups

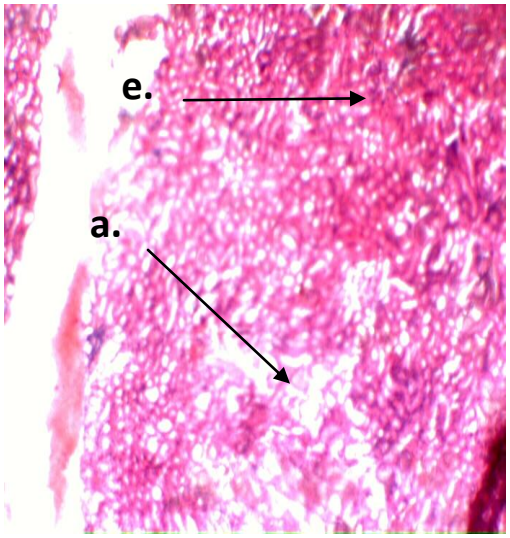


1.Normal

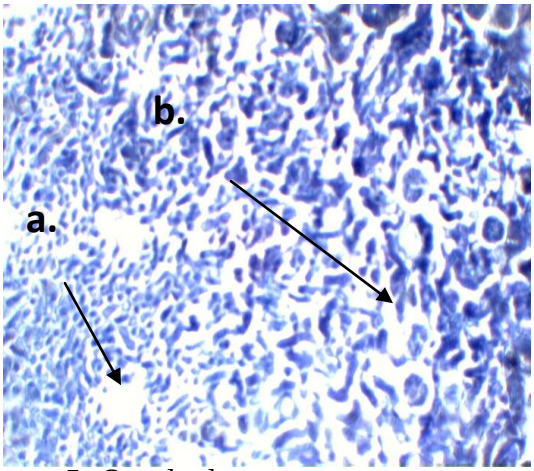


2. Induced





4. Test2



5. Standard

a. Glomerular and peritubular Congestion

b. Inflammatory cell infiltration

c. Epithelial desquamation

d. Necrosis

e. Blood vessel congestion

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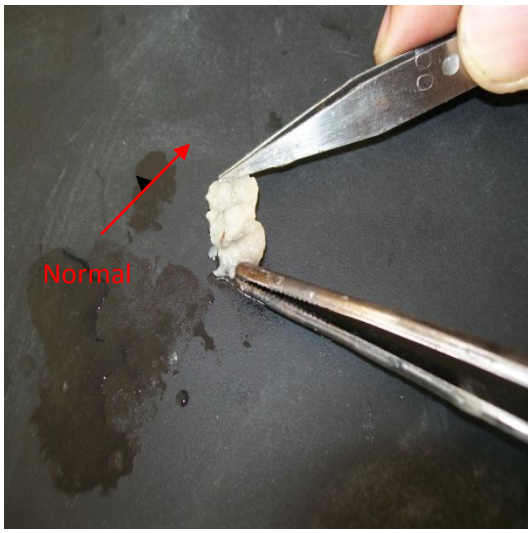
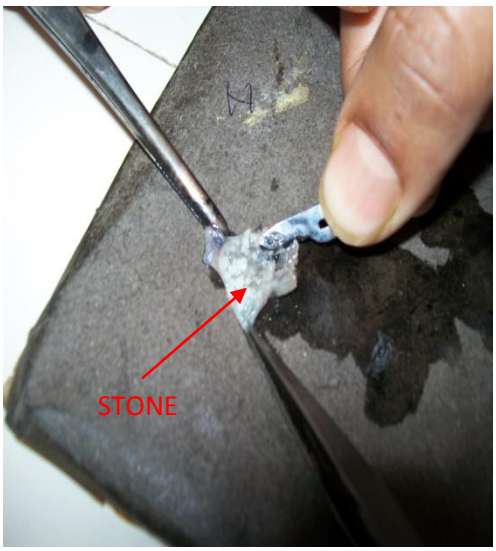
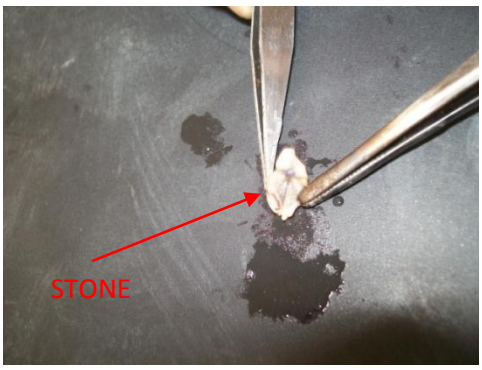
Fig20. Photographs of Urinary bladder with stones after Zinc implantation in different Groups.



3. Test 1



4. Test 2



5. Standard

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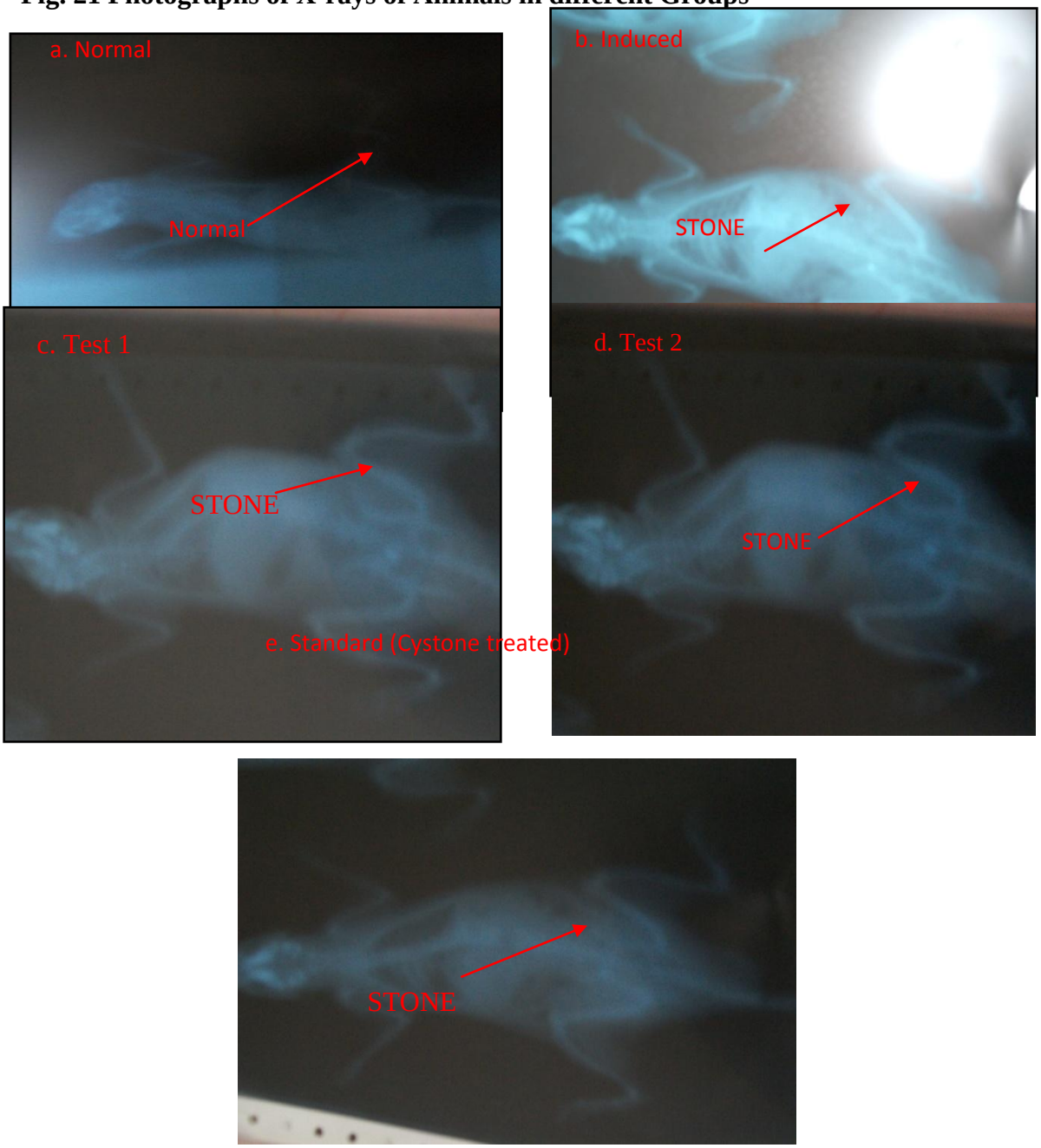
Radiographical Examination of stone

Radiographical examination was done before sacrificing the animals to confirm the formation of stone. The animal was kept under light ether anaesthesia in anteroposterior position to expose the pelvic region, on pleo dor 3 X-ray machine (Siemens India Ltd) film was kept 100 cm away from the tube.

Fig. 21 Photographs of X-rays of Animals in different Groups



CONCLUSION

The present investigation showed that the presence of antiurolithiatic effect in *Bryophyllum pinnatum* (Lam.) Oken hydroethanolic leaf extract against calcium oxalate stones. It inhibit the CaOx crystal growth and protect kidney from crystal induced oxidative stress and renal cell injury, possibly mediated through a combination of CaOx crystal inhibitory, diuretic, antioxidant activity. The studies rationalize its medicinal use for urinary stone disease. This confirms the utility of the plant in Folk medicine against urolithiasis. The finding were further substantiated by the histopathological studies which confirmed that *Bryophyllum pinnatum* leaf extract protected the renal cells from oxidative stress and injury induce by calcium oxalate crystals

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