






# Toxicity (acute and subacute) assessment and *in-vivo* antiurolithiatic activity of ethanolic extract of *Caesalpinia bonducella* seed in albino Wistar rat

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## ABSTRACT

The objective of the present work is to find supportive evidence for the use of *Caesalpinia bonducella* in nephrolithiasis. For safety assessment of ethanolic extract of seed of *C. bonducella* (ECB), acute and subacute oral toxicity studies was performed. In the acute oral toxicity study at a dose of 2,000 mg/kg and subacute oral toxicity study at dose of 250, 500, and 1,000 mg/kg, no changes were seen in the behavior, haematological parameters, biochemical parameters, histopathology of major organ, and body weight of rats. The efficacy of ECB at dose of 200 and 400 mg/kg was studied in ethylene glycol, glycolic acid, and sodium oxalate induced urolithiasis in rats and marketed cystone tablet at a dose of 750 mg/kg was used as the standard drug. The mean body weight difference, urinary parameters (urine pH, urine volume, levels of calcium, magnesium, and phosphorus), and serum biochemical parameters [urea, Blood urea nitrogen (BUN), creatinine, uric acid, and alkaline phosphatase] were measured. Urolithiasis caused a significant decrease in mean body weight, pH, and volume of urine and increased in both serum biochemical parameters and urinary electrolyte concentration as compared with the normal control group. Histopathology of kidneys were showed increased glomerular hypercellularity, hydropic tubular degeneration, and cast in the induced control group. Ethanolic Extract of *Caesalpinia bonducella* (ECB) was found to be effective to normalise the above all changes. It was concluded that the ECB was effective in the prevention and elimination of calcium oxalate crystal deposition into kidneys.

## INTRODUCTION

Urolithiasis also termed kidney stone or nephrolithiasis, is a common worldwide disease with a high prevalence and recurrence rate. Chronic diseases, including diabetes mellitus, obesity, kidney disease, metabolic disorder, and hypertension, have a high tendency to form stones compared with healthy people (Besiroglu and Ozbek, 2019). It causes pyelonephritis or acute renal failure and severe acute back pain (Namburu *et al.*,

2017; Yasui *et al.*, 2017). Urolithiasis typically occurs between the age of 20 and 60 years and is more occurrences in hot climates. It affects nearly 10% of men and 6% of women over their lifetime. The recurrence rate is 50% within 5–10 years and 75% within 20 years of initial treatment (Dawson and Tomson, 2012; Yasui *et al.*, 2017). The overall recurrence rate is high in male (70%–80%) as compared with female (47%–60%) (Ahmed *et al.*, 2018; Wang *et al.*, 2019). Approximately, 80% of stones are calcium-containing stones which are found as pure calcium oxalate (CaOx) (50%), pure calcium phosphate (1%), or a mixture of both (45%), and other stones are struvite (10%), uric acid (9%), and cystine (1%) (Bashir and Gilani, 2009; Namburu *et al.*, 2017). CaOx (CaC<sub>2</sub>O<sub>4</sub>) stones are found as CaOx monohydrate or CaOx dehydrate form. Struvite stones are also called triple phosphate or infection stones or magnesium ammonium phosphate and develop

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when the urease-producing bacteria such as proteus cause the urine pH higher than 7.2 by ammonia (urea convert in ammonia in presence of urease enzyme) (Ahmed *et al.*, 2018).

*Caesalpinia bonducella* Roxb. (Caesalpinaceae) is extensively used in the traditional medicine (Bawari *et al.*, 2020; Bashir and Gilani, 2009; Datta *et al.*, 1998; Iheagwam *et al.*, 2019; Liu *et al.*, 2020). The various parts of *C. bonducella* have been reported for anti-cancer and cytotoxic activities (Iheagwam *et al.*, 2019); contractile activity of uterine smooth muscle (Datta *et al.*, 1998); inhibit nuclear factor-kappa B and type-4 phosphodiesterase (Liu *et al.*, 2020); antimicrobial activities (Arif *et al.*, 2009). The objective of this study is to perform toxicity study (acute and subacute oral toxicity) and to evaluate antiurolithiatic activity of ECB in experimentally induced CaOx stone model.

## MATERIALS AND METHODS

### Material

Chemicals of analytical grade were utilized in the studies. Cystone was procured from Varanasi's local market. Albumin, total protein, calcium, phosphorous, magnesium, uric acid, urea, creatinine, uric acid estimation kits were procured from Coral Clinical Systems, a division of Tulip Diagnostics (P) Ltd., Uttarakhand, India. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) were procured from Autospan Liquid Gold, Arkray Healthcare Pvt. Ltd., Maharashtra, India. UV-Visible spectrophotometer (Systronics Double Beam UV-VIS Spectrophotometer: 2202) was used for analysis.

### Plant material and extraction

The plant seeds samples were collected from the local market in Varanasi, India. A voucher specimen was deposited (number 201901) and authenticated by Dr. Ashwani K. Kushwaha, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi. The coarsely powdered seeds of *C. bonducella* (100 g) were soaked in 1 l of 95% ethanol for a week with occasional shaking. The crude extract was obtained by filtration through filter paper. The filtrate was evaporated by rotatory evaporator to give a viscous brownish dark mass and the extract was preserved in a refrigerator at 2°C–8°C for use in the experiments (Bashir and Gilani, 2009). The yield value of the extract was 6% (w/w). The extract was suspended in 0.5% carboxy methyl cellulose (CMC) for pharmacological screening.

### Animals

Adult albino Wistar rats, 12 weeks old, 130–150 g bodyweight, were used for the study. They were placed under standard conditions (22°C ± 3°C) and allowed free access to standard food pellets and water. After 1 week of acclimatization, rats were used for the experiment. Rats were handled according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and procedures were approved by the ethical committee of the Institute of Medical

Sciences, BHU, Varanasi, India (2211, 07.11.2020) for the care and use of Laboratory Animals.

### Toxicity study

#### Acute oral toxicity

The acute toxicity was performed according to the Organisation for Economic Co-operation and Development (OECD) (423, December 17, 2001) test guidelines. Rats were divided into two groups ( $n = 3$ ). Group I (control) received p.o. vehicle (0.5% CMC), while Group II was treated with ECB at a single dose of 2,000 mg/kg p.o.

#### Subacute oral toxicity

The subacute toxicity was performed according to the OECD (407, October 3, 2008) guidelines with slight modification. Rats were divided into four groups ( $n = 5$ ). Group I (control) received vehicle orally (0.5% CMC), while Group II, III, and IV received orally 250, 500, and 1,000 mg/kg per day dose of ECB, respectively, for 28 consecutive days (Ekanayake *et al.*, 2019; Hasan *et al.*, 2020; Kharchoufa *et al.*, 2020; Loha *et al.*, 2019; Meguellati *et al.*, 2019; Porwal *et al.*, 2017; Silva *et al.*, 2011).

#### Behavioral changes and mortality

Animals were observed for food intake, water intake, bodyweight, behavioral changes, seizure, hair loss, allergic reaction, hazardous symptoms, and number of deaths during the entire experiment.

#### Hematological and biochemical analysis

Animals were anesthetized with chloroform once the toxicity investigation was completed, and blood was taken via retro-orbital puncture with Ethylenediamine tetraacetic acid (EDTA) coated capillary tube. Hematological parameters were determined by Auto-Haematology analyser (Arkray Autocell Plus). The serum parameters were determined by using standard diagnostic kits.

#### Histopathological studies

After completion of toxicity study duration, all animals were anesthetized with chloroform and sacrificed. The organs, like liver, heart, lungs, and kidney, were collected and preserved in 10% formalin solution. The preserved organs were fixed in melted paraffin wax, then allowed to solidify. The tissue sections were prepared using a microtome (thickness of 4 µm). After staining with hematoxylin and eosin, the pathological changes of the tissue sections were examined and identified under microscope [Olympus Magnus Inverted (INVI) inverted microscope] in comparison with the control group (Ekanayake *et al.*, 2016).

### In vivo urolithiasis

#### Ethylene glycol (EG) induced urolithiasis

The antiurolithiasis activity of ECB was evaluated by EG induced urolithiasis model (Bawari *et al.*, 2020; Lonkala, 2020; Moriyama *et al.*, 2009; Patel and Acharya, 2020; Tzou *et al.*, 2016) with slight modification. Animals were divided into five groups ( $n = 5$ ). Group I (normal control) received p.o. vehicle (0.5% CMC) for 28 days. Group II, III, IV, and V received p.o.

0.75% v/v EG and 1% w/v ammonium chloride for 28 days. Group II (induced control) received vehicle 0.5% CMC p.o. once daily from 15th day to 28th day. Group III, IV, and V received p.o. 200, 400 mg/kg dose of ECB and 750 mg/kg dose of cystone tablet (a standard polyherbal formulation), respectively, once daily from 15th day to 28th day.

#### Sodium oxalate (SO) induced urolithiasis

The antiurolithiasis activity of ECB was evaluated by SO induced urolithiasis model (Araujo *et al.*, 2020; Elhan *et al.*, 2014; Tzou *et al.*, 2016) with slight modification. Rats were divided into five groups ( $n = 5$ ). Group I (normal control) received vehicle 0.5% CMC p.o. once daily for 21 days. Group II, III, IV, and V received SO (70 mg/kg per day, i.p.) for 7 days. Group II (induced control group) received vehicle 0.5% CMC p.o. once daily for next 14 days. Group III, IV, and V received p.o. 200, 400 mg/kg dose of ECB and 750 mg/kg dose of cystone, respectively, once daily for next 14 days.

#### Glycolic acid (GA) induced urolithiasis

The antiurolithiasis activity of ECB was evaluated by GA induced urolithiasis model (Mitra *et al.*, 1998; Sathya and Kokilavani, 2012; Shehzad *et al.*, 2021; Tzou *et al.*, 2016) with slight modification. The rats were divided into five groups ( $n = 5$ ). Group I (normal control) received vehicle 0.5% CMC p.o. once daily for 21 days. Group II, III, IV, and V received 3% v/v GA per day, p.o. for 7 days. Group II (induced control) received vehicle 0.5% CMC p.o. once daily from for next 14 days. Group III, IV, and V received p.o. 200, 400 mg/kg dose of ECB and 750 mg/kg dose of cystone, respectively, once daily for next 14 days.

#### Body weight

In EG induced urolithiatic model, changes in bodyweight of individual rats were measured on the 1st, 14th, and 28th days while, in the GA and SO induced urolithiatic model, the body weight was measured on 1st, 7th and 21st days.

#### Urine analysis

At the end of experiment, all animals were kept in metabolic case for 24 hours urine collection. A drop of conc. Hydrochloric acid (HCl) was mixed in urine to prevent microbial growth (Patel and Acharya, 2020). Urine samples were used to measurement of urine volume and then quantitative estimation of calcium, phosphorus, and magnesium. Urine pH was analyzed by pH meter (Eutech Instruments CyberScan pH 510)

#### Biochemical analysis

Animals were anesthetized with chloroform once the toxicity investigation was completed, and blood was taken via retro-orbital puncture with capillary. After centrifugation at 5,000 g for 15 minutes, serum was collected and stored in a deep freezer (Patel and Acharya, 2020). The serum parameters were determined by using diagnostic kits.

#### Histopathological studies

At the end of experiment, all rats were anesthetized with chloroform and sacrificed and the kidneys were collected and preserved in 10% formalin. Kidney sections were prepared as the

above-described procedure. The pathological changes in kidney sections were identified by comparison with control group and photographs were taken by microscope (Olympus Magnus INVI inverted microscope).

#### Statistical analysis

The data were expressed as mean  $\pm$  SEM and analyzed by using software GraphPad Prism 8.0.2. ordinary one-way and two-way analysis of variance with Dunnett's multiple comparisons test was applied.

## RESULTS AND DISCUSSION

In order to investigate the effect of medicinal plants on urolithiasis, we chosen *C. bonducella* that have been claimed to have diverse therapeutic characteristics. Extraction of *C. bonducella* seed were performed in ethanol (95%) based on the literature studies stating that ethanol (95%) can offer superior extraction results for phenolic like chemicals because it dissolves the most polar and non-polar compounds. Extraction was done using the cold percolation process, and the operation was repeated three times with new solvents for a greater yield. Semisolid extracts were obtained and kept in the freezer for scientific investigations. In this investigation, ethanolic solvent yielded higher extract yields for *C. bonducella* (6%).

The signs and symptoms of toxicity, including morbidity or mortality, were not observed in both acute and subacute oral toxicity. LD<sub>50</sub> of ECB could be greater than 2,000 mg/kg because

**Table 1.** Effects of *C. bonducella* extract on haematological parameters in acute oral toxicity study.

Parameter	Control	2,000 mg/kg
Hb (g/dl)	14.63 $\pm$ 0.43	14.57 $\pm$ 0.38*
PCV (%)	46.23 $\pm$ 0.87	46.09 $\pm$ 1.24*
RBC count (mill/mm <sup>3</sup> )	9.16 $\pm$ 0.22	8.55 $\pm$ 0.38*
MCV (fl)	50.51 $\pm$ 0.75	54.01 $\pm$ 0.97*
MCH (pg)	15.98 $\pm$ 0.15	17.15 $\pm$ 1.21*
MCHC (g/dl)	31.64 $\pm$ 0.46	31.69 $\pm$ 1.66*
RDW (%)	22.21 $\pm$ 0.98	23.02 $\pm$ 0.95*
TLC (thou/mm <sup>3</sup> )	11.04 $\pm$ 0.71	9.82 $\pm$ 0.51*
Segmented neutrophils (%)	15.13 $\pm$ 0.10	13.72 $\pm$ 1.37*
Lymphocytes (%)	79.28 $\pm$ 0.61	81.56 $\pm$ 1.02*
Monocytes (%)	2.65 $\pm$ 0.25	2.00 $\pm$ 0.55*
Eosinophils (%)	2.62 $\pm$ 0.74	2.27 $\pm$ 0.62*
Basophils (%)	0.32 $\pm$ 0.11	0.46 $\pm$ 0.22*
Neutrophils (thou/mm <sup>3</sup> )	1.67 $\pm$ 0.10	1.36 $\pm$ 0.20*
Lymphocytes (thou/mm <sup>3</sup> )	8.76 $\pm$ 0.61	8.01 $\pm$ 0.41*
Monocytes (thou/mm <sup>3</sup> )	0.30 $\pm$ 0.05	0.19 $\pm$ 0.05*
Eosinophils (thou/mm <sup>3</sup> )	0.28 $\pm$ 0.07	0.22 $\pm$ 0.05*
Basophils (thou/mm <sup>3</sup> )	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02*
Platelets (thou/mm <sup>3</sup> )	858.33 $\pm$ 11.05	861.67 $\pm$ 16.50*
Mean platelet volume(fl)	8.13 $\pm$ 0.33	7.97 $\pm$ 0.28*

Hb, Haemoglobin; PVC, Packed cell volume; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; TLC, Total leucocytes count; RDW, Red cell distribution width; WBC, White blood cell; MPV, Mean platelet volume.

Values represent the mean  $\pm$  SEM ( $n = 3$ ).

\*  $p > 0.05$ .

**Table 2.** Effects of *C. bonducella* extract on biochemical parameters in acute oral toxicity study.

Parameter	Control	2,000 mg/kg
Urea (mg/dl)	24.20 ± 1.58	25.79 ± 1.09*
Uric acid (mg/dl)	2.50 ± 0.75	2.46 ± 0.76*
Creatinine (mg/dl)	0.56 ± 0.14	0.60 ± 0.10*
ALP (U/l)	134.05 ± 1.57	134.33 ± 2.12*
AST (U/l)	74.87 ± 1.14	74.33 ± 1.24*
ALT (U/l)	45.74 ± 1.53	45.72 ± 1.54*
Albumin (mg/dl)	3.90 ± 0.13	3.73 ± 0.31*
Globulin (mg/dl)	3.44 ± 0.06	3.45 ± 0.22*
Total protein	7.34 ± 0.19	7.18 ± 0.53*
Albumin/globulin ratio	1.13 ± 0.02	1.08 ± 0.03*
Initial wt (g)	143.50 ± 1.95	141.67 ± 2.06*
1 week (g)	148.33 ± 1.74	146.83 ± 1.58*
2 weeks (g)	154.00 ± 1.67	152.33 ± 1.65*
BWG (g)	10.50 ± 0.56	10.67 ± 0.88*
Food intake (g/day)	23.50 ± 0.99	23.83 ± 1.05*
Water intake (ml/day)	30.17 ± 1.28	27.67 ± 0.99*

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; BWG, Bodyweight gain.

Values represent the mean ± SEM ( $n = 3$ ).

\*  $p > 0.05$ .

there was no death recorded in rats during treatment. In acute and subacute toxicity study, the bodyweight of both the treated and normal control group were gradually increased and no significant change in mean body weight gain (Table 2 and 4). The results of hematological and biochemical parameters of treated groups were showed non-significant ( $p > 0.05$ ), similar and within range as compared with normal control group in both acute (Table 1 and 2) and subacute oral toxicity study (Table 3 and 4). The results of hematological and biochemical parameters of all groups were found to be normal and nearly similar to previously reported studies (Han *et al.*, 2015; Porwal *et al.*, 2017; Silva *et al.*, 2011). The histopathology of the liver showed no inflammation, normal hepatocytes cords, no acute or chronic damage in both acute (Fig. 1D), and subacute oral toxicity (Fig. 4) study. The histopathology of the kidney showed normal glomeruli, tubules, vessels with no features of damage or inflammation in both acute (Fig. 1A), and subacute oral toxicity (Fig. 2). The section of lungs showed normal patent alveoli lined by pneumocytes and there is no collapse, exudate, inflammatory infiltrate, or necrosis in the lung parenchyma in both acute (Fig. 1B) and subacute oral toxicity (Fig. 3). The section of the heart showed cardiomyocytes arranged in pseudosyncytium with normal thickness and there is no myonecrosis or inflammation in both acute (Fig. 1C) and subacute oral toxicity (Fig. 5).

In EG, GA, and SO-induced urolithiasis model, the bodyweight of both *C. bonducella* and cystone-treated groups was increased significantly ( $p < 0.01$  to  $p < 0.0001$ ) as compared

**Table 3.** Effects of *C. bonducella* extract on haematological parameters in subacute oral toxicity study.

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Hb (g/dL)	14.57 ± 0.20	14.37 ± 0.33*	13.82 ± 0.45*	14.17 ± 0.38*
PCV (%)	47.30 ± 0.23	45.53 ± 1.80*	45.80 ± 0.97*	46.64 ± 0.90*
RBC count (mill/mm <sup>3</sup> )	9.28 ± 0.16	8.53 ± 0.31*	8.88 ± 0.18*	9.13 ± 0.19*
MCV (fL)	51.02 ± 0.82	53.64 ± 3.60*	51.57 ± 0.25*	51.07 ± 0.35*
MCH (pg)	15.72 ± 0.45	16.88 ± 0.47*	15.56 ± 0.46*	15.52 ± 0.33*
MCHC (g/dl)	30.80 ± 0.39	31.72 ± 2.03*	30.17 ± 0.76*	30.38 ± 0.45*
RDW (%)	22.23 ± 0.55	23.49 ± 0.33*	22.52 ± 0.47*	22.45 ± 0.44*
TLC (thou/mm <sup>3</sup> )	11.10 ± 0.63	9.44 ± 0.27*	11.11 ± 0.59*	10.70 ± 0.26*
Segmented neutrophils (%)	15.57 ± 0.80	13.43 ± 1.85*	15.81 ± 0.28*	14.99 ± 2.72*
Lymphocytes (%)	79.79 ± 1.55	81.61 ± 1.61*	79.14 ± 0.30*	80.01 ± 3.58*
Monocytes (%)	2.33 ± 0.52	2.34 ± 0.62*	2.50 ± 0.29*	2.21 ± 0.68*
Eosinophils (%)	2.06 ± 0.47	2.15 ± 0.33*	2.23 ± 0.30*	2.34 ± 0.59*
Basophils (%)	0.24 ± 0.11	0.46 ± 0.10*	0.32 ± 0.12*	0.44 ± 0.18*
Neutrophils (thou/mm <sup>3</sup> )	1.72 ± 0.09	1.26 ± 0.15*	1.75 ± 0.07*	1.59 ± 0.26*
Lymphocytes (thou/mm <sup>3</sup> )	8.86 ± 0.54	7.71 ± 0.38*	8.79 ± 0.43*	8.58 ± 0.58*
Monocytes (thou/mm <sup>3</sup> )	0.26 ± 0.06	0.22 ± 0.06*	0.28 ± 0.04*	0.23 ± 0.07*
Eosinophils (thou/mm <sup>3</sup> )	0.23 ± 0.06	0.20 ± 0.03*	0.25 ± 0.05*	0.25 ± 0.06*
Basophils (thou/mm <sup>3</sup> )	0.03 ± 0.01	0.04 ± 0.01*	0.04 ± 0.01*	0.05 ± 0.02*
Platelets (thou/mm <sup>3</sup> )	854.00 ± 3.06	860.67 ± 12.02*	870.00 ± 8.19*	835.00 ± 20.82*
Mean platelet volume (fl)	8.00 ± 0.23	7.83 ± 0.18*	8.27 ± 0.27*	8.10 ± 0.32*

Hb, Haemoglobin; PVC, Packed cell volume; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; TLC, Total leucocytes count; RDW, Red cell distribution width; WBC, White blood cell; MPV, Mean platelet volume.

Values represent the mean ± SEM ( $n = 5$ ).

\*  $p > 0.05$ .

**Table 4.** Effects of *C. bonducella* extract on biochemical parameters in subacute oral toxicity study.

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Urea (mg/dl)	23.01 ± 1.79	24.39 ± 2.11*	24.99 ± 2.08*	22.64 ± 1.34*
Uric acid (mg/dl)	2.77 ± 0.66	2.49 ± 0.60*	2.55 ± 0.70*	2.92 ± 0.50*
Creatinine (mg/dl)	0.74 ± 0.14	0.54 ± 0.10*	0.49 ± 0.15*	0.62 ± 0.11*
ALP (U/l)	135.16 ± 0.55	135.47 ± 2.90*	133.66 ± 2.28*	135.42 ± 1.01*
AST (U/l)	75.03 ± 0.74	75.14 ± 1.27*	75.82 ± 2.15*	75.73 ± 1.01*
ALT (U/l)	45.85 ± 0.85	47.21 ± 1.57*	47.56 ± 2.35*	47.23 ± 1.48*
Albumin (mg/dl)	3.63 ± 0.14	3.59 ± 0.20*	3.75 ± 0.05*	3.70 ± 0.12*
Globulin (mg/dl)	3.44 ± 0.15	3.39 ± 0.07*	3.42 ± 0.06*	3.36 ± 0.09*
Total protien	7.07 ± 0.29	6.97 ± 0.27*	7.17 ± 0.09*	7.06 ± 0.20*
Albumin/globulin ratio	1.06 ± 0.02	1.08 ± 0.04*	1.11 ± 0.03*	1.12 ± 0.03*
Initial wt (g)	143.50 ± 2.17	143.33 ± 1.78*	141.17 ± 2.21*	140.33 ± 1.36*
1 week (g)	148.00 ± 2.14	147.67 ± 1.91*	146.67 ± 2.35*	145.67 ± 1.15*
2 weeks (g)	153.33 ± 1.80	151.83 ± 1.74*	151.00 ± 2.18*	150.17 ± 1.17*
3 weeks (g)	157.67 ± 1.74	157.33 ± 1.86*	156.00 ± 2.18*	154.83 ± 1.25*
Final weight (g)	163.00 ± 1.48	163.33 ± 1.76*	161.67 ± 1.98*	161.50 ± 1.09*
BWG (g)	19.50 ± 0.89	20.00 ± 1.15*	20.50 ± 0.72*	21.17 ± 1.01*
Food intake (g/day)	23.67 ± 1.33	24.67 ± 1.12*	22.33 ± 0.99*	22.00 ± 0.93*
Water intake (ml/day)	29.83 ± 1.40	27.33 ± 1.20*	25.67 ± 1.54*	28.17 ± 0.87*

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; BWG, Mean bodyweight gain.

Values represent the mean ± SEM ( $n = 5$ ).

\*  $p > 0.05$ .

**Table 5.** Body weight change during treatment.

		Body weight (g)				
		Group I	Group II	Group III	Group IV	Group V
EG-induced urolithiasis model	Initial	144.4 ± 2.29	143.4 ± 1.33	143.6 ± 1.72*	142.8 ± 1.88 *	142.4 ± 1.72 *
	14 days	158.2 ± 2.08	139.6 ± 1.21	138.6 ± 1.40 *	137.8 ± 2.01 *	136.8 ± 1.83 *
	28 days	167.2 ± 1.12	128.6 ± 1.12	138 ± 2.17**	147.4 ± 1.96****	149.2 ± 1.36****
SO-induced urolithiasis model	Initial	145.8 ± 2.48	140.8 ± 1.43	142.4 ± 2.44*	142.8 ± 3.09 *	141.6 ± 2.23 *
	7 days	151.4 ± 3.12	137.4 ± 2.34	139.2 ± 1.66 *	137.4 ± 2.69 *	138.6 ± 2.11 *
	21 days	163.6 ± 2.18	129.2 ± 2.18	145.2 ± 3.04***	148.6 ± 1.81****	149.2 ± 4.10****
GA-induced urolithiasis model	Initial	143.2 ± 3.06	141.2 ± 2.50	144.4 ± 2.29 *	142.8 ± 2.92 *	140.8 ± 2.87 *
	7 days	150.8 ± 1.71	137 ± 2.05	140.8 ± 1.36 *	138.2 ± 2.96 *	137.2 ± 1.85 *
	21 days	162 ± 1.72	129.6 ± 1.72	143.4 ± 3.46****	148.2 ± 2.56****	150 ± 2.74****

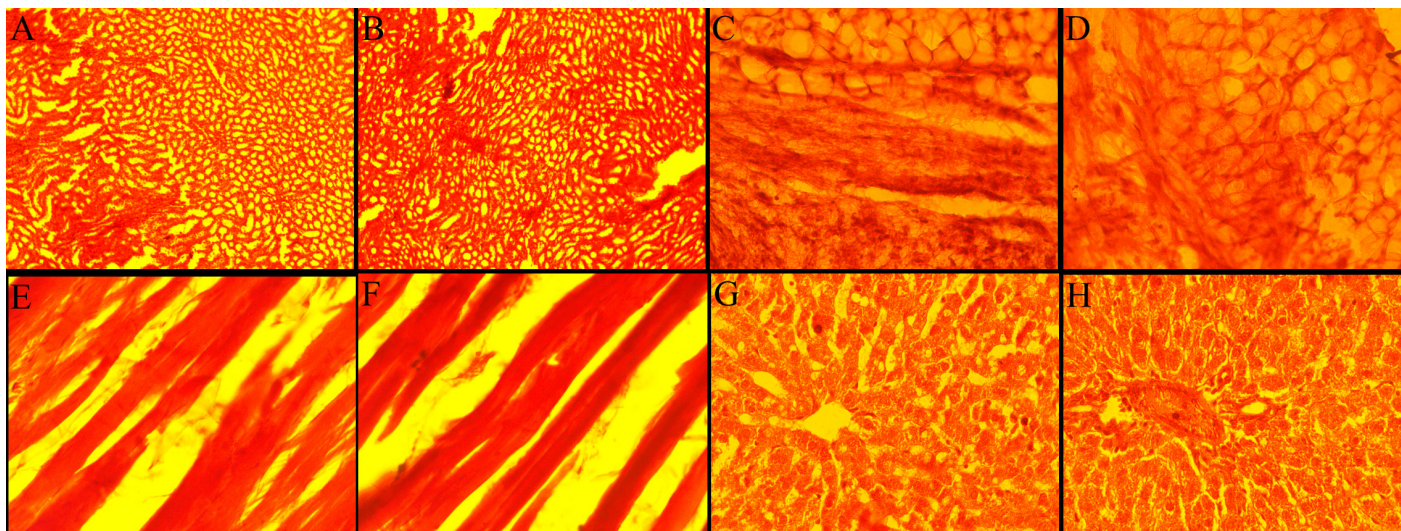
Data are expressed as mean ± SEM ( $n = 5$ ).

\*  $p > 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  compared with induce control group II.

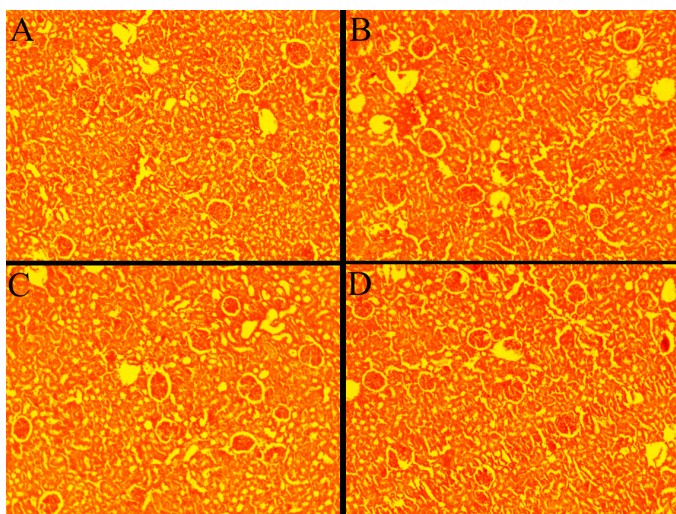
with the induced control group (Table 5). Administration of EG, GA, and SO caused induction of renal stone formation and led to a significant decrease in body weight in the induced control group when compared with the normal control group. EG, GA, and SO caused body weight loss. After treatment with the 200 mg/kg of *C. bonducella*, slight body weight gain was observed; and treatment with 400 mg/kg of *C. bonducella*, maximum body

weight gain was observed which was similar to cystone-treated group. Ahmed *et al.* (2013); Bawari *et al.* (2020); Bayir *et al.* (2011); Shehzad *et al.* (2021); Kaushik *et al.*, (2019) reported that lithogenic treatment causes a decrease in body weight.

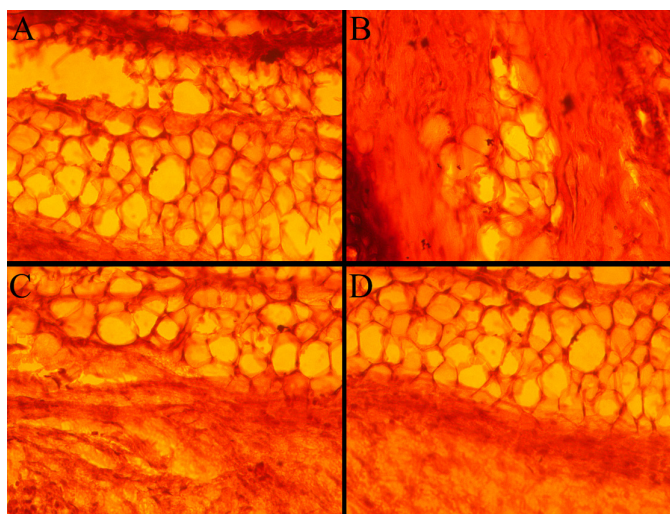
EG, GA, and SO administration cause significant ( $p < 0.05$  to  $p < 0.0001$ ) increase in the urinary calcium, phosphorus level and significant ( $p < 0.05$  to  $p < 0.0001$ ) decrease in the urine



**Figure 1.** Histology of albino Wistar rat's kidney (A), lung (C), heart (E), liver (G), of the control group and kidney (B), lung (D), heart (F), liver (H) of the group treated with 2,000 mg/kg dose in acute oral toxicity.



**Figure 2.** Histology of albino Wistar rat's kidney (A) control group (A) 250 mg/kg (B), 500 mg/kg (C), and 1,000 mg/kg (D) treated group in subacute oral toxicity study.

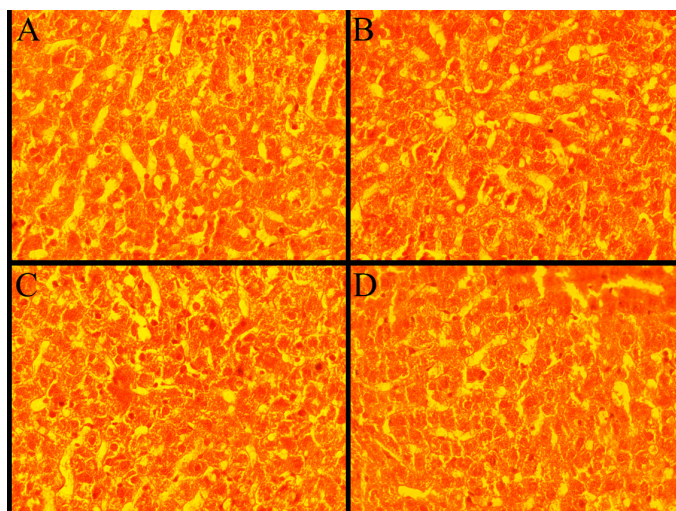


**Figure 3.** Histology of albino Wistar rat's lung (A) control group (A) 250 mg/kg (B), 500 mg/kg (C), and 1,000 mg/kg (D) treated group in subacute oral toxicity study.

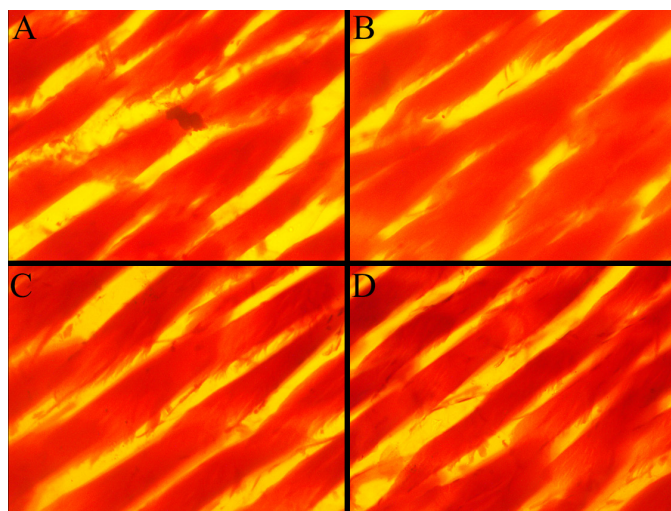
pH, urine volume, urinary magnesium level in the induced control group as compared with the normal control group. Treatment with 200 and 400 mg/kg of *C. bonducella* extract causes a significant ( $p < 0.05$  to  $p < 0.0001$ ) decrease in the levels of urinary calcium and phosphorus along with an increase in magnesium urinary level as compared with the induced control group (Table 6). The lithogenic substance decreases the urinary pH by its metabolism in the acidic compound which causes urinary acidosis. The decrease in urinary pH favors the CaOx precipitation by decreasing CaOx solubility and saturation level in urine which promotes stone formation. Low urinary output also plays a major role by decreasing the volume of urine which got easily supersaturated with oxalates and calcium ions (Patel and Acharya, 2020). The urinary pH and volume of the induced control group were decreased significantly and urine gets supersaturated with CaOxs, initiating stone formation. Volume

and pH of urine in *C. bonducella* extract (200 and 400 mg/kg) and cystone-treated group was found significantly increased, which indicates that CaOx solubility and saturation level were increased in urine. Maximum recovery in urinary parameters were seen with highest dose (i.e., 400 mg/kg) which is similar with cystone-treated group.

Urinary magnesium works as an inhibitor in CaOx stone formation. Magnesium preferentially binds with oxalate and forms a stable complex that is highly water-soluble (Bano *et al.*, 2018; Kaushik *et al.*, 2019). A low level of magnesium is seen in the induced control group and after treatment with the *C. bonducella* extract, a nearly normal Mg level was resumed. Increased levels of calcium and phosphorus in the urine favor the growth of CaOx crystals. Phosphorous as phosphates was deposited on CaOx stone (Kaushik *et al.*, 2019). The level of



**Figure 4.** Histology of albino Wistar rat's liver (A) control group (A) 250 mg/kg (B), 500 mg/kg (C), and 1,000 mg/kg (D) treated group in subacute oral toxicity study.



**Figure 5.** Histology of albino Wistar rat's heart (A) control group (A) 250 mg/kg (B), 500 mg/kg (C), and 1,000 mg/kg (D) treated group in subacute oral toxicity study.

**Table 6.** Effect of *C. bonducella* on urine parameter in urolithiasis.

Model	Urinary parameters (per 24 hours)	Group I	Group II	Group III	Group IV	Group V
EG-induced urolithiasis model	Calcium (mg)	3.86 ± 0.09	9.55 ± 0.16	5.25 ± 0.13****	4.29 ± 0.18****	4.05 ± 0.07****
	Phosphorous (mg)	4.08 ± 0.17	7.36 ± 0.15	5.89 ± 0.05****	4.87 ± 0.07****	4.69 ± 0.08****
	Magnesium (mg)	6.52 ± 0.22	1.03 ± 0.08	1.72 ± 0.19*	5.01 ± 0.12****	5.55 ± 0.19****
	Urine volume ml	13.65 ± 0.59	6.25 ± 0.38	13.80 ± 0.59****	14.55 ± 0.25****	12.25 ± 0.22****
	Urine pH	6.74 ± 0.06	5.30 ± 0.10	6.22 ± 0.18****	6.76 ± 0.11****	6.71 ± 0.09****
SO-induced urolithiasis model	Calcium (mg)	3.02 ± 0.29	9.04 ± 0.98	5.55 ± 0.29***	3.80 ± 0.40****	4.32 ± 0.28****
	Phosphorous (mg)	3.88 ± 0.29	7.78 ± 0.45	5.69 ± 0.20***	4.97 ± 0.15****	4.55 ± 0.18****
	Magnesium (mg)	6.77 ± 0.34	0.96 ± 0.13	2.82 ± 0.24***	5.43 ± 0.30****	5.96 ± 0.36****
	Urine volume ml	11.65 ± 0.71	7.35 ± 0.33	12.70 ± 0.59****	13.20 ± 0.46****	11.95 ± 0.32****
GA-induced urolithiasis model	Urine pH	6.70 ± 0.10	5.34 ± 0.13	6.36 ± 0.15****	6.62 ± 0.08****	6.74 ± 0.09****
	Calcium (mg)	3.30 ± 0.39	10.15 ± 0.59	4.84 ± 0.31****	3.19 ± 0.60****	3.35 ± 0.40****
	Phosphorous (mg)	3.63 ± 0.42	8.09 ± 0.48	6.01 ± 0.09**	4.08 ± 0.53****	4.96 ± 0.26****
	Magnesium (mg)	7.60 ± 0.68	0.96 ± 0.13	3.42 ± 0.37*	5.55 ± 0.52****	7.04 ± 0.84****
	Urine volume ml	12.85 ± 0.35	6.70 ± 0.22	12.80 ± 0.50****	13.10 ± 0.75****	12.15 ± 0.22****
	Urine pH	6.73 ± 0.10	5.42 ± 0.12	6.40 ± 0.08****	6.60 ± 0.14****	6.71 ± 0.11****

Data are expressed as mean ± SEM ( $n = 5$ ).

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$  compared with induced control group II.

calcium and phosphorous was decreased in the *C. bonducella* extract-treated group.

The serum biochemical parameters like uric acid, urea, creatinine, BUN, ALP level were significantly ( $p < 0.05$  to  $<0.0001$ ) increased in the induced control group as compared to the normal group. However, these biochemical parameters were

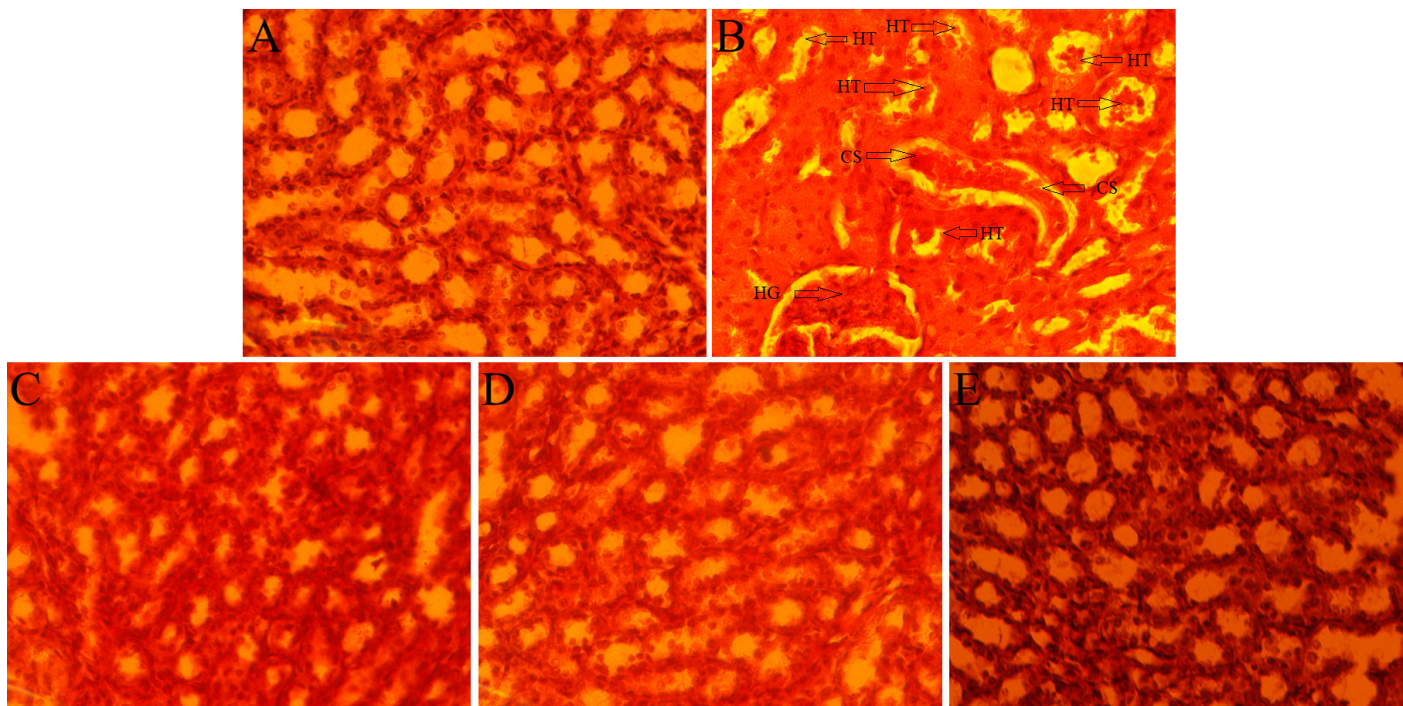
found to be significantly ( $p < 0.05$  to  $<0.0001$ ) decreased in both *C. bonducella* extract and cystone-treated groups as compared to the induced control group (Table 7). Maximum recovery in biochemical parameters were seen with highest dose (i.e., 400 mg/kg) which is similar with cystone-treated group. Stone deposition in tubules and glomerulus obstruct the outflow of urine and decreases

**Table 7.** Effect of *C. bonducella* on serum biochemical parameters in urolithiasis

Model	Serum parameters	Group I	Group II	Group III	Group IV	Group V
EG-induced urolithiasis model	Urea (mg/dl)	27.72 ± 2.52	40.68 ± 2.52	33.92 ± 1.57*	28.04 ± 1.28****	31.46 ± 0.82***
	Uric acid (mg/dl)	2.618 ± 0.18	8.024 ± 0.20	3.22 ± 0.20*****	2.932 ± 0.07*****	2.878 ± 0.26*****
	Creatinine (mg/dl)	0.544 ± 0.05	1.824 ± 0.12	0.912 ± 0.04*****	0.696 ± 0.05*****	0.65 ± 0.04*****
	BUN	12.95 ± 0.64	19.00 ± 1.18	15.84 ± 0.73**	13.09 ± 0.60*****	14.69 ± 0.38***
	ALP (U/l)	139.04 ± 2.94	183.46 ± 4.19	172.20 ± 2.54**	141.70 ± 2.95*****	142.84 ± 0.92*****
SO-induced urolithiasis model	Urea (mg/dl)	23.40 ± 4.14	44.39 ± 4.14	32.01 ± 2.32**	25.48 ± 2.18***	22.76 ± 2.59***
	Uric acid (mg/dl)	2.232 ± 0.22	8.67 ± 0.38	2.904 ± 0.30*****	2.616 ± 0.19*****	2.772 ± 0.27*****
	Creatinine (mg/dl)	0.642 ± 0.13	2.478 ± 0.27	1.072 ± 0.13*****	0.756 ± 0.08*****	0.58 ± 0.10*****
	BUN	10.93 ± 0.99	20.73 ± 1.93	14.95 ± 1.08**	11.90 ± 1.02****	10.63 ± 1.21*****
	ALP (U/l)	135.82 ± 4.60	211.06 ± 12.74	179.34 ± 6.89**	139.28 ± 4.58*****	133.68 ± 2.91*****
GA-induced urolithiasis model	Urea (mg/dl)	24.80 ± 3.18	41.01 ± 3.18	30.08 ± 2.81**	26.33 ± 2.10***	27.04 ± 2.26***
	Uric acid (mg/dl)	2.464 ± 0.45	8.74 ± 0.80	3.60 ± 0.47*****	2.564 ± 0.34*****	2.286 ± 0.48*****
	Creatinine (mg/dl)	0.46 ± 0.09	2.25 ± 0.24	1.12 ± 0.21****	0.50 ± 0.13*****	0.38 ± 0.14*****
	BUN	11.58 ± 0.78	19.15 ± 1.49	14.05 ± 1.31**	12.30 ± 0.98***	12.63 ± 1.05***
	ALP (U/l)	130.42 ± 5.63	196.35 ± 7.18	162.50 ± 8.00***	138.06 ± 4.94*****	145.98 ± 3.65*****

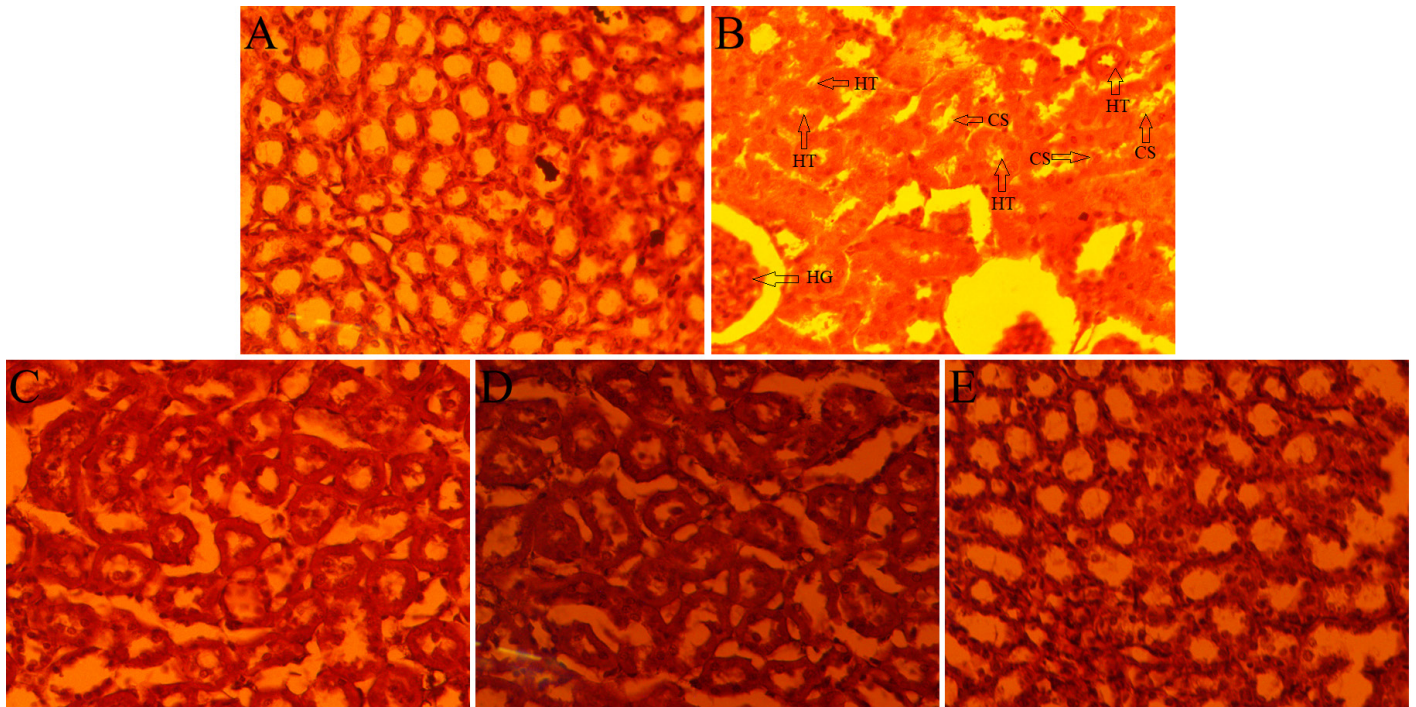
Data are expressed as mean ± SEM (n = 5).

\*  $p > 0.05$ ; \*\* $p < 0.05$ ; \*\*\* $p < 0.01$ ; \*\*\*\* $p < 0.001$ ; \*\*\*\*\* $p < 0.0001$  compared with induced control group II.

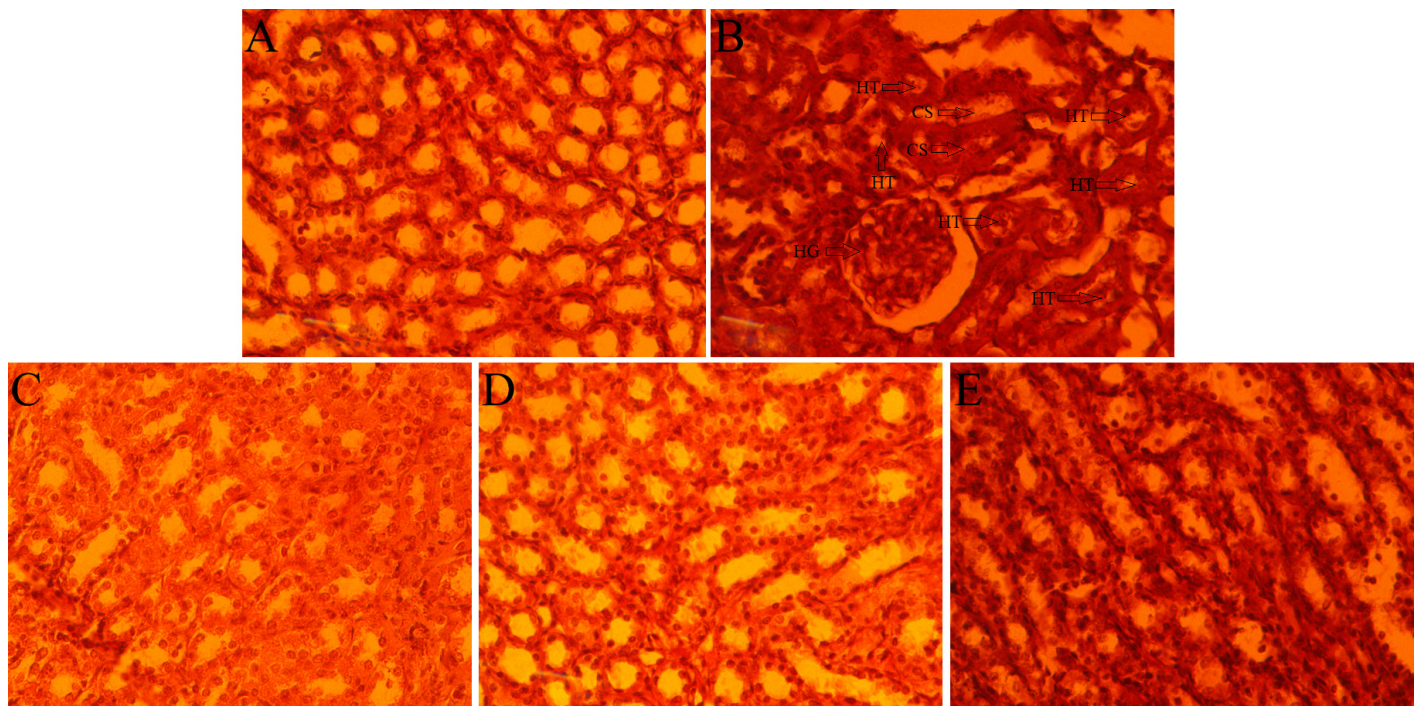


**Figure 6.** Histology of albino Wistar rat's kidney of the normal control group (A), induced control group (B), 200 mg/kg (C), 400 mg/kg (D), and cystone (E) treated group in EG-induced urolithiasis model.





**Figure 7.** Histology of albino Wistar rat's kidney of the normal control group (A), induced control group (B), 200 mg/kg (C), 400 mg/kg (D), and cystone (E) treated group in GA-induced urolithiasis model.



**Figure 8.** Histology of albino Wistar rat's kidney of the normal control group (A), induced control group (B), 200 mg/kg (C), 400 mg/kg (D), and cystone (E) treated group in SO-induced urolithiasis model.

the glomerular filtration rate which leads to the accumulation of nitrogenous waste products (urea, BUN, creatinine, and uric acid) in the blood (Bano *et al.*, 2018; Bayir *et al.*, 2011; Kaushik *et al.*, 2019). However, the accumulation of nitrogenous waste in blood was reduced on treatment with *C. bonducella* extract. ALP level is a marker for kidney damage. In urolithiasis, apical membranes of renal tubular epithelial are damaged because of CaOx crystals deposition. This membrane damage leads to a rise in ALP (Kaushik *et al.*, 2019).

The histopathological section of the kidney showed a mild increase in glomerular hypercellularity with severe tubular hydropic degeneration and casts in EG induced control group (Fig. 6B); moderate increased glomerular hypercellularity with moderate tubular hydropic degeneration and casts in GA induced control group (Fig. 7B); moderate increased glomerular hypercellularity, mild tubular hydropic degeneration and casts in SO-induced control group (Fig. 8B) as compared with the respective normal control group. In *C. bonducella* extract (200 and 400 mg/kg) treated rat's kidney section showed that glomerular hypercellularity, tubular hydropic degeneration, and casts were recovered in a concentration-dependent manner (Figs. 6C and D, 7C and D, 8C and D); and rats in the cysteine-treated group were also recovered (Figs. 6E, 7E and 8E) as compared with the respective induced control group.

## CONCLUSION

From the above results, it can be concluded that the ethanolic extract of *C. bonducella* has no toxic effect on albino Wistar rats in an acute and subacute oral study. 400 mg/kg of *C. bonducella* seed extract showed preventive effect in experimentally EG, GA, and SO-induced urolithiasis by normalizing imbalanced electrolytes in urine, nitrogenous substances in serum, and decreasing supersaturation of CaOx. Therefore, it is concluded that 400 mg/kg of *C. bonducella* seed extract could be used as a therapeutic agent to prevent urolithiasis and attenuate the problems associated with it.

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## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

All the experimental procedures were approved by the ethical committee of the Institute of Medical Sciences, BHU, Varanasi, India (2211, 07.11.2020) for the care and use of Laboratory Animals.

## DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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