

Evaluation of Antiuro lithiatic Potential of *Piper cubeba* Dried Fruits on Sodium Oxalate Induced Urolithiasis in Rats

Sura Suman^{1,*}, Solleti Venkata Suresh Kumar²

¹Research Scholar, Department of Pharmacy, JNTUA, Ananthapuramu, Andhra Pradesh, INDIA.

²Department of Pharmacognosy and Phytochemistry, Creative Educational Society's College of Pharmacy, Kurnool, Andhra Pradesh, INDIA.

ABSTRACT

Objectives: The main endeavor of the work is to assess the antiuro lithiatic potentials of ethanolic extract of *Piper cubeba* dried fruits (EEPC). **Methods:** EEPC was prepared using soxhlation, subjected to phytochemical screening and determined total phenolic and flavonoid content using Folin-Ciocalteu reagent and aluminium chloride colorimetric assay methods. *In vitro* antioxidant activity was evaluated using methods like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation assays using ascorbic acid as standard. Sodium oxalate (70 mg/kg, i.p) was given to rats for 5 days to promote urolithiasis. Cystone (750mg/kg, p. o), EEPC (100, 200 and 400 mg/kg, p.o) was administered to the respective group of rats from 6th to 15th day. On the 15th day, serum and urine were collected from individual animals and biochemical parameters like BUN, creatinine and uric acid in serum and sodium, chloride, potassium, calcium, phosphate, oxalate in urine and calcium, phosphate and oxalate in kidney homogenate have been measured. The kidney sections have been prepared and histopathologically tested to check the stones. **Results:** Preliminary phytochemical analysis discloses the existence of phenolics, tannins, steroids, terpenoids

and flavonoids. The EEPC enriched with phenols and flavonoids which correlates with its antioxidant potentials. Following treatment with cystone and EEPC, concentrations of BUN, creatinine, uric acid in serum and sodium, chloride, potassium, calcium, phosphate, oxalate in urine and calcium, phosphate, oxalate in kidney homogenate ($P < 0.001$ vs. control) were significantly reduced in urolithiasis caused by sodium oxalate in a dose-dependent way. **Conclusion:** The promising results suggest that EEPC would act as a potential agent in the management of urolithiasis along with its antioxidant properties.

Key words: Antioxidant, Calcium oxalate, Flavonoid content, Sodium oxalate, Total phenolic content, Urolithiasis.

Correspondence

Mr. Sura Suman

Research Scholar, JNTUA, Ananthapuramu-515002, Andhra Pradesh, INDIA.

Phone no: +91-9949521100

Email: kalyan.suman1985@gmail.com

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INTRODUCTION

Urolithiasis implies to the compacted non-metallic aggregates in the urinary tract. Calcium oxalate is the most common of many types of kidney stones. The formation of such calculi involves several physicochemical incidents, starting with the nucleation and aggregation of crystals and ending with urinary tract retention.¹ Several mechanisms concerned with urolithiasis etiology are among the most significant reasons for the failure to expand antiuro lithiatic drugs. Reactive oxygen species (ROS) are recognized to disrupt the oxidant-antioxidant balance in the kidney cells resulting in cellular damage.² Renal epithelial cells induce oxidative stress when exposed to different crystals such as CaOx, calcium phosphate and uric acid.³ Hence, the imbalance between lithogenesis promoters and inhibitors contributes to the formation of renal calculi. Therefore, drugs with various targets, such as antioxidant, anti-spasmodic and anti-inflammatory features, are an obvious choice for the development of antiuro lithiatic drugs.

Piper cubeba L. or tailed pepper belongs to the family of Piperaceae. This plant is a folk herb and has been used as a spice in many countries, including Indonesia, India, Europe (Middle Ages) and Morocco. As per the classical Unani system of medicine, *Piper cubeba* (Kabab chini) are reported to be useful in the treatment of a wide range of ailments including a diuretic, lithotriptic, cathartic for kidney and bladder, demulcent, astringent, antiseptic, de-obstructive, hepatonic, anti-inflammatory, mouth Refreshner, stimulant, anti-asthmatic, carminative, sedative, gastrotonic.⁴⁻⁶

However, no scientific data are available to develop the antiuro lithiatic activity of *P. cubeba* dried fruits ethanolic extract. In the present research, an attempt was made to establish the scientific validity of the antiuro lithiatic activity of *Piper cubeba* dried fruits ethanolic extract against urolithiasis induced by sodium oxalate in rats.

MATERIALS AND METHODS

Collection of plant material

The collection of dried fruits of *Piper cubeba* was done from Sirigiri Venkappa ayurvedic stores, Kurnool, Andhra Pradesh, India in July 2017 for the present study and conformed by Raw material herbarium and museum, NISCAIR, Delhi, India with a reference number (NISCAIR/RHMD/Consult/2017/3091-40). The dried fruits have been pulverized to get the coarse powder used for extraction.

Preparation of extract

The coarse powder of *Piper cubeba* dried fruits crammed in a thimble and was subjected to extraction by using the Soxhlet apparatus, ethanol as a solvent. The extract was filtered and the residue obtained after concentration in the water bath was further evaporated at laboratory temperature and stored in desiccator till use.

Phytochemical screening

The ethanolic extract of *Piper cubeba* dried fruits (EEPC) was subjected to preliminary phytochemical screening for alkaloids, glycosides,

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tannins, phenols, steroids, flavonoids and terpenoids following standard procedures.⁷

Estimation of Phenolic content

The total phenolic content of the ethanolic extract of *Piper cubeba* dried fruits (*EEPC*) was measured by the using Folin ciocalteu process.⁸ 10 ml stock solution of the extract was prepared in the respective solvent with a concentration of 2mg/ml. 1ml of the extract solution was taken into a 25ml volumetric flask from the stock solution to this 10ml of water and 1.5ml Folin ciocalteu reagent added. The mixture was placed aside for 5 min and then 4ml of 20% sodium carbonate solution was added and volume was made up to 25ml with double distilled water. The mixture was placed aside for 30min and the absorbance of blue color formed was quantified at 765nm. The solutions of standard gallic acid have been prepared in a concentration range of 50 to 250 µg / ml for calibration curve preparation. The standard calibration curve for gallic acid was attained by plotting absorbance on the y-axis and their concentration on the x-axis respectively.

Estimation of total flavonoid content

The total flavonoid content of the ethanolic extract of *Piper cubeba* dried fruits (*EEPC*) was estimated by earlier reported methods.⁹

Aluminium chloride method

The aluminium chloride colorimetric method was carried out using the earlier reported procedure. Quercetin was used to make the calibration curve. From the stock solution of standard, 0.1, 0.2, 0.3, 0.4 and 0.5 ml were taken which gave 10, 20, 30, 40 and 50 µg concentrations respectively. 0.5 ml of diluted standard solutions were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415nm, after incubation at room temperature for 30min. the amount of 10% aluminium chloride was substituted by same amount of distilled water in blank. Similarly, 0.5 ml of ethanolic extract of *Piper cubeba* dried fruits (*EEPC*) was reacted with aluminium chloride for determination of total flavonoid content as described above.

2,4-dinitro phenyl hydrazine method

The method reported by chang *et al.* was used for this estimation. Naringenin was used as a reference sample for the preparation of the calibration curve. 20 mg of naringenin was dispersed in methanol and then diluted to provide concentrations of 250, 500, 1000, 1500 and 2000 µg/ml. 1 ml of each of the diluted standard solutions was independently mixed with 2 ml of 1% 2,4-dinitro phenyl hydrazine reagent and 2 ml of methanol at 50°C for 50 min, after cooling to room temperature the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2min, then 1 ml of the mixture was taken and mixed with 5 ml of methanol and centrifuged at 1000 rpm to remove the precipitate formed. The supernatant was filtered and adjusted to 25 ml, the absorbance of the supernatant was measured at 459 nm. Similarly, 5 ml of each concentration of ethanolic extract of *Piper cubeba* dried fruits (*EEPC*) were treated with 2,4-dinitro phenyl hydrazine reagent for determination of flavonoid content as described above. For the blank, the amount of 2,4-dinitro phenyl hydrazine reagent was replaced by methanol.

Evaluation of *in vitro* antioxidant activity of *Piper cubeba* dried fruits ethanolic extract

2,2 diphenyl 1 picrylhydrazyl (DPPH) assay

The *Piper cubeba* dried fruits ethanolic extract effect on DPPH radical was estimated using the method described by Sharifi-Rad.¹⁰ A 0.135mM

DPPH methanol solution was formulated as well as its 1.0ml amount was combined with 1.0ml amount of *Piper cubeba* dried fruits ethanolic extract having concentrations (25-3200µg/ml). The mixture of the reaction was carefully vortexed as well as placed in the shade at 37°C room-temperature for 30 min. Spectrophotometrically, at 517nm solution's absorbance, is measured. For instance, Ascorbic acid was used. The following equation calculated the scavenge DPPH radical ability "as: DPPH radical scavenging activity (%) = [(Abs_{control} - Abs_{sample})] / (Abs_{control}) × 100

Where Abs_{control} represents DPPH radical + methanol absorbance;

Abs_{sample} represents the DPPH radical + sample extract /standard absorbance.

Lipid peroxidation assay method

Lipid peroxidation inhibition of *EEPC* was determined using an earlier reported method.¹¹ Further, homogenization of 10gm of rat liver tissue was done in phosphate buffer solution along with a polytron homogenizer (Remi) having 7.4pH which produced homogenate of 25%w/v. Next, for 10 min centrifugation of homogenation was done at 4000 rpm. Supernatant's 0.1 ml was combined with 0.1ml of different concentrations of ethanolic extract of *Piper cubeba* dried fruits (25-3200 µg/ml) and 0.1 ml KCl (30 mM), 0.1ml "ascorbic acid" (0.06mM), 0.1ml "ammonium ferrous sulphate" was added as well as at 37°C it was incubated for 1hr. After that, 1.5ml TBA (0.8%), 1.5ml of 20% glacial acetic acid, as well as sodium dodecyl sulphate 0.2ml (8%) were used to treat the reaction mixture. Distilled water was used so that the total mixture volume is attained was 4ml and then for 1hr at 100°C, it was placed in an oil bath. After the mixture was cooled down, 5ml 15:1v/v butanol-pyridine mixture, as well as 1ml distilled water, is added to it. Furthermore, for 10 min tubes were centrifuged at 4000rpm after vigorous shaking. At 532nm, the organic layer's absorbance is measured that contains TBARS (Thiobarbituric Acid Reactive Substance). Instead of a test compound, 0.1ml respective vehicle was used for preparing the control sample. It resulted in the 50% inhibition concentration (IC₅₀) and percentage inhibition.

Evaluation of pharmacological studies

Healthy mice weighing about 20-30 g and rats weighing about 150-180 g are procured from animal house, CES College of pharmacy, Kurnool. They were placed in polypropylene cages and kept under a temperature of 27 ± 2°C, relative humidity 65 ± 10% and 12 h light/dark cycles. The animals were given a standard diet manufactured by Nutrivet Life Sciences, Pune, India. The protocol was reviewed and approved by the Committee on Institutional Animal Ethics (Ref. No.: IAEC / CESCOP/2017-10) created following CPCSEA India guidelines.

Acute toxicity studies

OECD-423 procedures were used to carry out the acute oral toxicity study.¹² For the effective dosage non-median lethal dose's (LD₅₀) 1/10th part is used.¹³

Antiuro lithiatic activity

Sodium oxalate induced urolithiasis in rats

Antiuro lithiatic activity of ethanolic extract of *Piper cubeba* dried fruits in albino rats was evaluated by using an earlier reported method.¹⁴ Thirty-six rats have been divided into the following six groups (Normal Control, Disease Control, Standard and 3 Extract Doses) each of which consisted of six rats. 70 mg/kg of sodium oxalate (in physiologic saline) was given by intraperitoneal injection (i.p) for 5 days to provoke urolithiasis. After induction of urolithiasis, standard drug cystone (750mg/kg, p.o) and three distinct doses of *EEPC* (100mg/kg, 200mg/kg and 400mg/kg, p.o) were administered to animals by suspending in 1 % carboxymethyl

cellulose (CMC) for 10 days. Only a regular diet and potable water were given to normal group rats. On the 15th day, approximately 1.5 to 2ml of blood was collected through retro-orbital plexus under mild anaesthesia using diethyl ether,¹⁵ serum was separated by centrifugation at $10,000 \times g$ for 10 min and blood urea nitrogen (BUN), creatinine and uric acid were analysed and a 24-hr urine sample had also been collected by putting rats in metabolic cages and drinking water was provided to rats and analysed for sodium, chloride, potassium, calcium, phosphate and oxalate. After blood and urine samples collection, the rats were euthanized using a CO₂ chamber and the abdomen was opened to remove both kidneys from each animal. Isolated kidneys have been washed out of extraneous tissue, flushing out of ice-cold physiological saline and the right and left kidneys have been used for homogeneous renal analysis and histopathological analysis, respectively.

Kidney homogenate analysis

The left kidney was finely chopped and 20% homogenate has been prepared in Tris-HCl buffer (0.02 mmol/l, pH 7.4). Kidney homogenate was used for assaying tissue calcium, phosphate and oxalate.

Histopathological analysis

The left kidney was fixed in 10% neutral formalin buffer, sectioned at 5µm thickness and stained with hematoxylin-eosin dye and mounted with Canada balsam. The histopathological examination of slides was executed under a plain and polarized light microscope (40X) and photographed by an Olympus Digital Camera.

Statistical analysis

All the values are articulated as mean \pm SEM. The data was statistically analysed by using one-way ANOVA followed Dunnett's *t*-test in GraphPad Prism 5.03 version software.

RESULTS

Preliminary phytochemical screening of ethanolic extract *Piper cubeba* dried fruits

Phytochemical screening discloses the presence of phenolics, tannins, steroids, terpenoids and flavonoids in the ethanolic extract of *Piper cubeba* dried fruits.

Phenolic content estimation

Folin ciocalteu process is used for measuring the *Piper cubeba* dried fruits ethanolic extract's total phenolic content through a standard gallic acid. The phenolic content was attained to be 185.65 µg/ml.

Estimation of total flavonoid content

Piper cubeba dried fruits ethanolic extract's total flavonoid content was determined to be 65.83 µg/ml from the quercetin and naringenin calibration curve.

In vitro antioxidant activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The effect of ascorbic acid and ethanolic extract of *Piper cubeba* dried fruits was showed significant DPPH radical scavenging activity. It was thought that the effect of antioxidants on DPPH radical scavenging is due to their capacity to donate hydrogen. The antioxidant activity was obtained to be in a dose-dependent way (Figure 1) and they showed an IC₅₀ value of 185.68µg/ml and 338.50 µg/ml respectively.

Lipid peroxidation inhibition assay

The effect of ascorbic acid and ethanolic extract of *Piper cubeba* dried fruits inhibit the lipid peroxidation induced by Fe²⁺ -ascorbate system in the homogenate of rat liver. Malondialdehyde (MDA) generation and related substances which interact with thiobarbituric acid were identified to be impeded by the ascorbic acid and ethanolic extract of *Piper cubeba* dried fruit (Figure 2). IC₅₀ value of the ascorbic acid and ethanolic extract of *Piper cubeba* dried fruits was identified to be 210.45 µg/ml and 495.68 µg/ml respectively.

Acute toxicity studies

The limit test was performed with a dose of *EEPC* (2000 mg/kg, b.w) given by oral route to a group of mice using an oral feed needle (22 gauge). Upon treatment, mice were examined for 14 days and no changes in normal behavior were detected, as a result of which it was concluded that the *EEPC* was practically non-toxic in normal mice and that the non-medium lethal dose of 1/20th (100 mg/kg b.w), 1/10th of the dose (200 mg/kg b.w), 1/5th of the dose (400 mg/kg b.w) was considered to be smaller, medium and high doses for further pharmacological studies.

Sodium oxalate induced urolithiasis in rats

Serum analysis

After treatment with sodium oxalate (70mg/kg b.w, i.p) results in a significant increase ($***p < 0.001$) in the serum levels of blood urea nitrogen (BUN), creatinine and uric acid when compared to the normal group. These levels were restored significantly ($***p < 0.001$) after treatment with standard drug cystone (750 mg/kg, b.w, p.o). However, treatment with *EEPC* (100 mg/kg, 200mg/kg, 400 mg/kg, p.o) pointedly ($***p < 0.001$) restored the elevated levels of blood urea nitrogen, creatinine and uric acid respectively in a dose-dependent manner (Table 1).

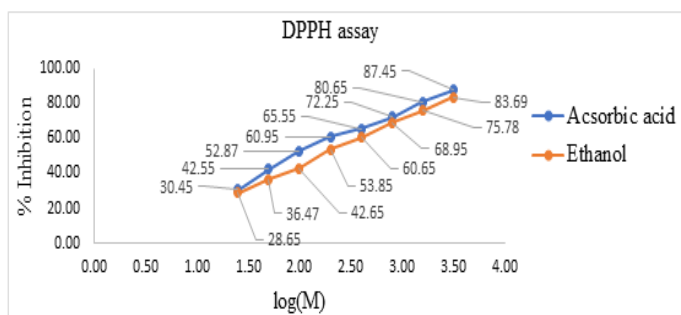


Figure 1: Effect of different concentrations of ascorbic acid and ethanolic extract of *Piper cubeba* dried fruits on DPPH* radicals.

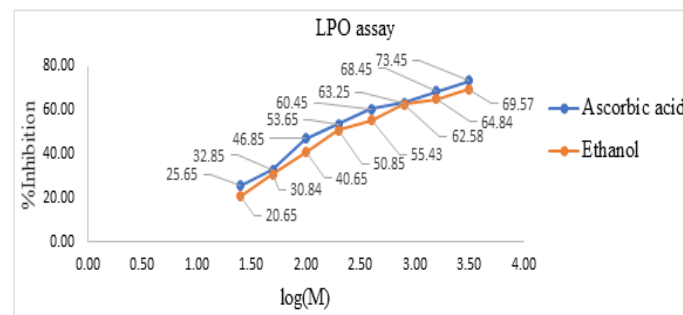


Figure 2: Effect of different concentrations of ascorbic acid and ethanolic extract of *Piper cubeba* dried fruits on LPO inhibition.

Urine analysis

The urinary levels of sodium oxalate (70mg/kg b.w, i.p) administered rats resulted in a significant ($***p<0.001$) increase in excretion of sodium, chloride, potassium, calcium, phosphate and oxalate respectively as compared to normal rats. However, Cystone group rats significantly ($***p<0.001$) and *EEPC* (100 mg/kg, 200mg/kg, 400 mg/kg, p.o) treated rats pointedly reduced the urinary excretion levels of sodium, chloride, potassium, calcium, phosphate and oxalate respectively as compared to disease control rats (Table 2).

Kidney homogenate analysis

In the renal tissue, the levels of calcium, phosphate and oxalate were significantly ($***p<0.001$) increased in the sodium oxalate induced rats as compared to normal rats, while in rats treated with cystone (750mg/kg, p.o) pointedly reduced the levels of calcium, phosphate and oxalate were significantly ($***p<0.001$) compared to disease control rats. However, rats treated with *EEPC* (100 mg/kg, 200mg/kg, 400 mg/kg, p.o) significantly ($***p<0.001$) lowered the levels of calcium, phosphate and oxalate respectively than disease control rats (Table 3).

Kidney Histopathology

Histopathological studies of kidney tissue revealed that there were no changes in renal tubules and glomerulus, no deposition of CaOx in the

kidney tissues (Figure 3A). The disease group showed marked calcium oxalate crystals deposition, significant tubular dilatation and infiltration (Figure 3B). There was no hemorrhage and no necrosis in the standard drug-treated group (Figure 3C). However, at a dose of *EEPC* 100 mg - the treated group exhibited a reduced amount of calcium oxalate crystal deposition, moderate infiltration but no hemorrhage (Figure 3D), at a dose of 200 mg of *EEPC* treated group also exhibited mild infiltration but no hemorrhage (Figure E) and *EEPC* 400 mg treated group exhibited reduced tubular dilatation and infiltration (Figure 3F). There was a predominant recovery of CaOx crystals in renal tissues at all three doses (100, 200 and 400 mg) of *EEPC*.

DISCUSSION

A variety of animal models using rats were used to induce calcium oxalate urolithiasis.¹⁶ Among these methods, the sodium oxalate simulated hyperoxaluria rat model triggers the rapid development of calcium oxalate stones in experimental renal tubules and is therefore commonly used for the rapid screening of antiuro lithiatic drugs.¹⁷

Table 1: Effect of *EEPC* on serum parameters.

S.no	Groups	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal	26.29±1.057	0.50±0.08	1.16±0.39
2	Disease Control	44.51±1.61 ^{***}	4.46±0.36 ^{***}	4.60±0.33 ^{***}
3	Standard (Cystone750mg/kg, p.o)	29.61±0.69 ^{***}	1.74±0.32 ^{***}	1.98±0.39 ^{***}
4	<i>EEPC</i> (100 mg/kg, p.o)	35.69±1.46 ^{***}	2.26±0.44 ^{***}	2.33±0.20 ^{***}
5	<i>EEPC</i> (200 mg/kg, p.o)	31.18±1.03 ^{***}	1.96±0.30 ^{***}	2.03±0.39 ^{***}
6	<i>EEPC</i> (400 mg/kg, p.o)	30.46±1.28 ^{***}	1.70±0.40 ^{***}	2.01±0.23 ^{***}

All values are expressed as mean ±S.E.M (n=6). Comparisons made between $***p<0.001$, $**p<0.01$, $*p<0.05$; Normal Vs Disease control, $***p<0.001$, $**p<0.01$, $*p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's -t test.

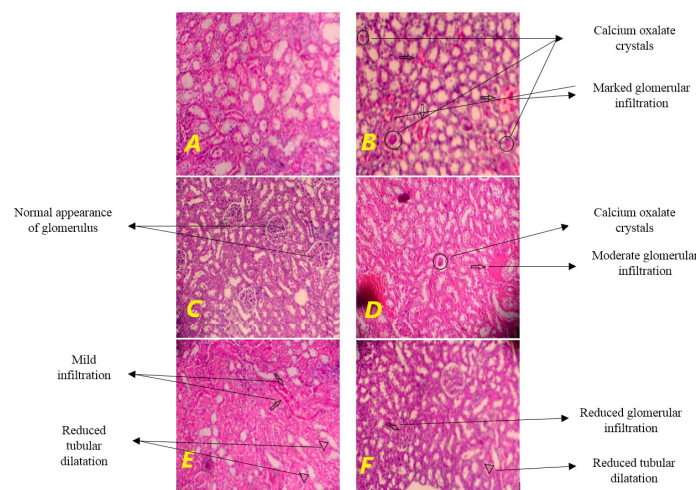


Figure 3: Histopathological appearance of the experimental group rat kidneys stained with hematoxylin and eosin (HE). Sections were viewed using a polarized light microscope (40X) and photographed by an Olympus Digital Camera.

- A-Normal group
- B-Disease control (sodium oxalate 70mg/kg b.w, i.p)
- C- Standard (Cystone 750 mg/kg, p.o)
- D- *EEPC* (100 mg/kg, p.o)
- E- *EEPC* (200 mg/kg, p.o)
- F- *EEPC* (400 mg/kg, p.o)

Table 2: Effect of *EEPC* on urinary parameters.

S. No	Groups	Sodium (mEq/L)	Chloride (mEq/L)	Potassium (mEq/L)	Calcium (mg/dl)	Phosphate (mg/dl)	Oxalate (mg/dl)
1	Normal	132.6±2.10	92.28±1.74	83.12±2.64	3.06±0.34	2.66±0.22	1.84±0.18
2	Disease Control	173.5±2.88 ^{***}	155.20±2.95 ^{***}	129.0±3.92 ^{***}	7.31±0.40 ^{***}	5.18±0.17 ^{***}	5.85±0.54 ^{***}
3	Standard (Cystone750mg/kg, p.o)	134.6±2.64 ^{***}	113.2±2.68 ^{***}	96.61±3.83 ^{***}	3.88±0.25 ^{***}	2.47±0.28 ^{***}	2.89±0.32 ^{***}
4	<i>EEPC</i> (100 mg/kg, p.o)	143.9±3.03 ^{***}	126.8±3.26 ^{***}	111.5±2.29 ^{***}	4.53±0.39 ^{***}	2.99±0.26 ^{***}	3.52±0.31 ^{***}
5	<i>EEPC</i> (200 mg/kg, p.o)	141.7±2.72 ^{***}	122.9±3.48 ^{***}	107.2±1.45 ^{***}	4.38±0.23 ^{***}	2.76±0.32 ^{***}	3.23±0.23 ^{***}
6	<i>EEPC</i> (400 mg/kg, p.o)	137.2±1.91 ^{***}	121.5±2.43 ^{***}	103.9±2.33 ^{***}	4.06±0.21 ^{***}	2.69±0.37 ^{***}	3.06±0.19 ^{***}

All values are articulated as mean ±S.E.M (n=6). Comparisons made between $***p<0.001$, $**p<0.01$, $*p<0.05$; Normal Vs Disease control, $***p<0.001$, $**p<0.01$, $*p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's -t test.

Table 3: Effect of EEPC on renal parameters.

S. No	Groups	Calcium (mg/dl)	Phosphate (mg/dl)	Oxalate (mg/dl)
1	Normal	0.39±0.06	1.15±0.10	2.02±0.21
2	Disease Control	1.55±0.22 ^{***}	3.92±0.57 ^{***}	4.84±0.67 ^{***}
3	Standard (Cystone 750mg/kg, p.o)	0.55±0.11 ^{***}	1.16±0.14 ^{***}	2.31±0.13 ^{***}
4	EEPC (100 mg/kg, p.o)	0.71±0.22 ^{**}	1.95±0.12 ^{***}	2.76±0.26 ^{***}
5	EEPC (200 mg/kg, p.o)	0.65±0.13 ^{**}	1.87±0.21 ^{***}	2.64±0.27 ^{***}
6	EEPC (400 mg/kg, p.o)	0.58±0.04 ^{***}	1.68±0.40 ^{**}	2.45±0.24 ^{***}

All values are articulated as mean ±S.E.M ($n=6$). Comparisons made between ^{***} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Normal Vs Disease control, ^{***} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's - t test.

Intraperitoneal administration of sodium oxalate triggers hyperoxaluria in rats. Biochemical pathways for sodium oxalate-induced lithiasis are associated with a rise in the urinary concentration of oxalate.^{18,19} This triggers precipitation of oxalate in the urine as calcium oxalate due to low solubility. High oxalate levels cause several changes in epithelial cells of renal tissue, such as increased production of free radical and decreased antioxidant status, followed by cell damage and cell death. Such changes are important predisposing factors for promoting adherence and retention of crystals.²⁰ Oxalate mediated damage and free radical development are attenuated by antioxidants *in vivo*²¹ and *in vitro*.²² Calculi stimulation with sodium oxalate results in hyperoxaluria. Persistent hyperoxaluria is a more important risk factor for renal stone pathogenesis.^{23,24} Hyperoxaluria can also contribute to the aggregation of calcium oxalate in multiple organs.

In the present study, the administration of EEPC results in a significant decrease in the serum levels of blood urea nitrogen, creatinine and uric acid in a concentration-dependent manner, similar to standard (cystone) drug which was elevated by the renal damage induced by sodium oxalate administration.

In the current research, elevated urinary levels of sodium, potassium, chloride, calcium, oxalate and phosphate were identified in lithiatic rats in line with previously published studies.²⁵ However, supplementation with ethanolic extract of *P. cubeba* in different doses leads to a significant decrease in the urinary levels of sodium, potassium, chloride, calcium, oxalate and phosphate respectively as that of standard (cystone) drug compared to lithiatic group.

In line with previous studies, the amount of calcium, oxalate and phosphate in the renal tissue homogenate in the disease control rats was significantly increased compared to normal rats. However, this elevated renal calcium, oxalate and phosphate levels were significantly decreased by treatment with EEPC at dose-dependently as that of standard (cystone) drug compared with the lithiatic group.

Sodium oxalate administration resulted in evident histological changes such as a marked accumulation of calcium oxalate crystals, major tubular dilation, swelling and inflammation of the kidneys, leading to a rise in the index of kidney injury. Treatment with EEPC reversed these histological changes, resulting in a substantial decrease in the damage index. This indicates that the EEPC reduces damage to the renal tubules which might be the partly attributable antioxidant property of the plant.²⁶

Overall, this significant recovery in serum parameters, urinary parameters and renal parameters by EEPC in sodium urolithiasis-

induced urolithiasis models themselves indicates an extensive range of activity of these extracts covering all phases of pathogenesis. The extent of the action of plant material is primarily determined by the prominent nature of phytochemicals and their contact with each other.

CONCLUSION

This study demonstrated that the ethanolic extract of *Piper cubeba* possesses a significant antioxidant and antiuro lithiatic activity which might be due to the presence of phytoconstituents like flavonoids, terpenoids and saponins. The different doses of EEPC possess significant activity among in this 400mg/kg dose produces potent activity. The mechanism involved in this finding is arbitrated collectively through antioxidant, nephroprotective properties and by reducing the concentration of urinary stone-forming constituents.

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CONFLICT OF INTEREST

The authors express that there is no conflict of interest.

ABBREVIATIONS

CaOx: Calcium oxalate; **CMC:** Carboxymethyl cellulose; **DPPH-2,2:** diphenyl-1-picrylhydrazyl; **EEPC:** Ethanolic extract of *Piper cubeba* dried fruits.

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