Antiuroliothiatic activity of *Phaseolus vulgaris* seeds against ethylene glycol-induced renal calculi in Wistar rats

Sree Lakshmi Namburu, Sujatha Dodoala, Bharathi Koganti, K. V. S. R. G. Prasad

*Department of Pharmacology, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women’s University), Tirupati, Andhra Pradesh, India*

**Abstract**

**Objective:** The present study was aimed to evaluate the antiurolithiatic potential of the ethanolic extract of the seed of *Phaseolus vulgaris* (EPV).

**Materials and Methods:** Calcium oxalate urolithiasis in male Wistar rats was induced by ethylene glycol (EG) (0.75% v/v) and ammonium chloride (1% w/v) administration in drinking water. Cystone (750 mg/kg, p.o.) was used as a standard drug, and EPV was administered at doses of 200 and 400 mg/kg, p.o. Both preventive and curative effects of EPV were evaluated. Urinary biochemical parameters such as calcium, oxalate, phosphate, uric acid and creatinine; deposition of calcium and oxalate in the kidney; and serum uric acid, creatinine, and blood urea nitrogen (BUN) were assessed. Creatinine clearance was calculated. Oxalate associated oxidative stress in the kidney was assessed by estimating *in vivo* antioxidant parameters such as lipid peroxidation, superoxide dismutase, catalase, and reduced glutathione. Histopathological studies of the kidney were carried out.

**Results:** In the preventive and curative disease-control groups, urinary excretion of calcium, oxalate, and their deposition in the kidney were significantly increased. Elevated levels of phosphate and uric acid in urine and uric acid, creatinine, and BUN in serum were observed in both the control groups. Creatinine clearance was reduced in the control groups. On treatment with cystone and EPV, all the urinary, serum biochemical, and oxidative stress parameters were reversed to almost normal values. Cystone and EPV significantly restored the *in vivo* antioxidant enzymes by decreasing the lipid peroxidation in the kidney. This study demonstrated the antiurolithiatic activity of the ethanolic extract of *P. vulgaris* seeds against EG-induced renal calculi in Wistar rats.

**Key words:** Ammonium chloride, antiurolithiatic activity, ethylene glycol, *Phaseolus vulgaris*

**INTRODUCTION**

Urinary calculus (kidney stones) is one of the painful disorders of the urinary tract. It is estimated that 10% of the population in the industrialized areas of the world are affected by urinary tract stone disease with an incidence of 0.5–1.9%. In India, upper and lower urinary tract stones occur frequently, but the incidence depends on regional, climatic, and socioeconomic conditions. Approximately 80% are of calcium-containing stones are in the form of pure calcium oxalate (CaOx) (50%) or calcium phosphate (1%) and a mixture of both (45%), and other stones are Struvite (10%), uric acid (9%), and cystine (1%). Although the mechanisms involved in the formation of calcific stones are not fully understood, it is generally agreed that urinary lithiasis is a multifaceted process involving a series of events such as supersaturation of urine, crystal nucleation, aggregation, and growth of insoluble particles that are finally retained in the urinary tract. Studies have also shown that tubular cell injury facilitates CaOx crystal formation and deposition in the renal tubules.

In the management of urolithiasis, combinations of medical and surgical techniques are commonly employed. Endoscopic stone removal and extracorporeal shock wave lithotripsy are commonly used methods in stone removal. These procedures are relatively costly and painful and cause undesirable side effects.

**Address for correspondence:**
K. V. S. R. G. Prasad, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, (Women’s University), Tirupati - 517 502, Andhra Pradesh, India. E-mail: kvsrgprasad@gmail.com

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effects such as hemorrhage, hypertension, tubular necrosis, and subsequent fibrosis of the kidney leading to cell injury, and also, recurrence of stone is quite common. Thiourea diuretics and alkali-citrate are being used to prevent the recurrence of hypercalciuria and hyperoxaluria-induced calculi, but scientific evidence for their efficacy is less reliable. Hence, there is a need for the search of new drug therapy, which will be cost-effective, target multiple etiological risk factors in urolithiasis and reduce the rate of recurrence.

In India, medicinal plants in the traditional medicine have played a prominent role in the treatment of various diseases since ancient. The plants provide a cheap source of drugs for majority of the world’s population. Data from in vitro, in vivo, and clinical trials reveal that phytotherapeutic agents could be used as either alternative or an adjunctive therapy in the management of urolithiasis.

**Phaseolus vulgaris**, also known as kidney beans, called Raajmah in India, is a common Indian dish. The seeds of *P. vulgaris* are gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals such as proteins, amino acids, complex carbohydrates, dietary fibers, oligosaccharides, phenols, saponins, flavonoids, alkaloids, and tannins, with potential health benefits. The seeds were claimed to possess diuretic activity and were commonly used in water retention treatment in pregnant women. Studies indicate that seeds of *P. vulgaris* were found to have activities such as enhancement of the bifidogenic, antioxidant, antimutagenic, anticarcinogenic, and antihyperglycemic effects.

Our previous study demonstrated in vitro antiurolithiatic activity of ethanolic seed extract of *P. vulgaris* on CaOx crystallization. No studies were carried out on the in vivo antiurolithiatic activity. Hence, in the present study, in vivo antiurolithiatic activity of the ethanolic extract of the seed of *P. vulgaris* (EPV) was investigated in Wistar rats against CaOx urolithiasis induced by ethylene glycol (EG) and ammonium chloride (AC) in drinking water.

**MATERIALS AND METHODS**

**Chemicals**

In the present study, analytical grade chemicals (Merck India Ltd, Hi-media and Sigma Aldrich) were used. Cystone, a polyherbal formulation from the Himalaya Drug Co., Bengaluru, India, was used as a standard drug.

**Plant Material**

Seeds of *P. vulgaris* were purchased from the local market, authenticated by the Dr. B. Sitaram, Professor, Department of Dravyaguna, S.V. Ayurvedic Medical College, Tirupati. Voucher specimens were kept in the Sri Padmavati Mahila Visvavidyalayam (Women’s University), Tirupati. The seeds were coarsely powdered and used for extraction.

**Preparation of Ethanolic Seed Extract of *P. vulgaris***

The seed powder (200 g) was macerated with 95% ethanol (1 L) for 24 h at room temperature followed by Soxhlet extraction for 6 h. The extract was concentrated under reduced pressure, and the obtained semisolid mass (11 g) was stored in an airtight container in a refrigerator.

**Preliminary Phytochemical Screening**

Preliminary phytochemical screening of EPV was carried out for the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins, sterols, and tannins using standard procedures. Quantification of total polyphenolic content in the EPV was determined spectrometrically and expressed as µg of gallic acid per gram of dry extract. Flavonoids content was determined by aluminum chloride colorimetric assay and expressed as µg of quercetin per gram of dry extract.

**Experimental Animals**

Healthy male Wistar albino rats weighing between 150 and 200 g were procured from Sri Venkateswara Enterprises, Bengaluru. Animals were acclimatized to standard laboratory conditions. They were provided with standard rat food pellets and drinking water ad libitum. The animal care and experimental protocols were in accordance with the CPCSEA. The study was approved by the Institutional Animal Ethical Committee (IAEC) with the approval No. 1677/PO/a/12/IAEC-Feb-14/07.

**Acute Toxicity Studies**

Oral acute toxicity studies were carried out in accordance with the OECD guidelines 423.

**Experimental Design**

Rats were divided into nine groups of six animals each (*n* = 6). CaOx stones were induced in rats by administering 0.75% v/v of EG and 1% w/v of AC in drinking water for 15 days. The treatment schedule was planned as follows:

- **Group I:** Normal (untreated)
- **Group II:** Preventive control (EG + AC + vehicle from day 1 to 15)
- **Group III:** Preventive standard (EG + AC + cystone 750 mg/kg, orally from day 1 to 15)
- **Group IV:** Preventive low dose (EG + AC + EPV 200 mg/kg, orally from day 1 to 15)
- **Group V:** Preventive high dose (EG + AC + EPV 400 mg/kg, orally from day 1 to 15)
- **Group VI:** Curative control (EG + AC from day 1 to 15, vehicle from day 16 to 30)
- **Group VII:** Curative standard (EG + AC from day 1 to 15, cystone 750 mg/kg, orally from day 16 to 30)
Group VIII: Curative low dose (EG + AC from day 1 to 15, EPV 200 mg/kg, orally from day 16 to 30)
Group IX: Curative high dose (EG + AC from day 1 to 15, EPV 400 mg/kg, orally from day 16 to 30)

Urine and blood samples were collected from all the groups of animals after completion of their respective treatment schedules. Rats were orally hydrated with 5 ml of drinking water, placed in individual metabolic cages and 24 h urine was collected. Urinary volume was noted, and urinary pH was determined. Urine was centrifuged at 2,500 rpm at 30 ± 2°C for 5 min, and the supernatant was used to estimate calcium, phosphate, uric acid, creatinine using commercially available diagnostic kits (ERBA Diagnostic Mannheim and Span Diagnostics Ltd., India) according to manufacturer’s instructions. Oxalate was estimated by the method of Hodgkinson and Williams, 1972. Blood samples were collected from the retro-orbital venous plexus and serum was separated by centrifuging at 1500 rpm for 15 min and used for the estimation of uric acid, creatinine, and Blood urea nitrogen (BUN) using ERBA diagnostic kits according to the manufacturer’s instructions. Fully automated autoanalyzer (Erba EM-200, Transasia Bio-medicals Ltd, Mumbai) was used for the estimations. Creatinine clearance was considered as kidney function parameter and was calculated using the formula:\[24]

\[
\text{Creatinine clearance (ml / min)} = \frac{(\text{mg creatinine / dl urine} \times (24 \text{ h urine output (ml)}))}{(\text{mg creatinine / dl serum}) \times 1440}
\]

After collecting urine and blood, rats were sacrificed by cervical decapitation; kidneys were perfused with ice-cold saline (0.9% w/v sodium chloride) and carefully isolated. One kidney from each animal was used for the estimation of calcium and oxalate as well as \textit{in vivo} antioxidant parameters such as lipid peroxidation expressed as malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Other kidney was fixed in 10% solution of buffered formalin (pH 7.4) and processed to paraffin wax. The sections were made using microtome and stained with hematoxylin and eosin and were examined under light microscope for renal tubular necrosis.

Statistical Analysis

The values were expressed as mean ± standard error of mean. Data obtained were analyzed by one-way ANOVA followed by \textit{post hoc} Bonferroni multiple comparisons test using GraphPad Prism (version 5.0).

RESULTS

Preliminary phytochemical analysis revealed the presence of flavonoids, polyphenols, saponins, and tannins in the ethanolic extract of \textit{Phaseolus vulgaris} seeds. Total phenolic content of EPV was found to be 418.78 μg/g gallic acid equivalents and total flavonoid content of EPV was 181.47 μg/g quercetin equivalents.

In the oral acute toxicity studies, EPV was found to be safe as it did not cause any mortality up to 2000 mg/kg. Hence, 200 and 400 mg/kg doses were selected for the present study.

Deposition of Calcium and Oxalate in the Kidney

On administration of EG and AC in drinking water for 15 days, a significant increase in the deposition of calcium and oxalate levels in the kidney were observed in both the preventive-control (II) and curative-control (VI) groups when compared to the normal (I). In the preventive regimen, animals treated with cystone (III) and EPV at doses 200 and 400 mg/kg (IV and V), a significant decrease in calcium and oxalate deposition in the kidney was observed when compared to the preventive-control group (II). In the curative regimen, animals treated with cystone (VII) and EPV (VIII and IX) also showed a significant decrease in calcium and oxalate deposition in the kidney when compared to curative-control group (VI). These effects were found to be dose-dependent [Table 1].

Urinary Biochemical Parameters

A significant increase in the urinary excretion of calcium, oxalate, phosphate, and uric acid was observed in both the preventive and curative-control (II and VI) group animals when compared to the normal group (I). On treatment with cystone and EPV, there was a significant decrease in the excretion of calcium, oxalate, phosphate and uric acid in the preventive-treated (III, IV and V) and curative-treated groups (VII, VIII, and IX) compared to their respective control groups (II and VI) [Table 2].

The urinary pH in normal animals was between 6.0 and 7.0. On induction of CaOx stones, the pH reduced to 5.0-6.0 in both the preventive and curative-control (II and VI) groups whereas in preventive (III, IV, and V) and curative (VII, VIII, and IX) group animals treated with cystone, EPV low and high doses showed an increase in the urinary pH (6.5–7.5) when compared to their respective control groups.

Serum Biochemical Parameters

Administration of EG and AC in drinking water for 15 days in both the control groups (II and VI), increased serum levels of uric acid, creatinine and BUN when compared to the normal group (I). These levels were significantly decreased in both preventive and curative regimens on treatment with cystone and EPV. A significant decrease in creatinine clearance was observed in the control groups (II and VI) and this was improved in groups treated with cystone and EPV in both the preventive and curative regimens [Table 3].
In Vivo Antioxidant Parameters

A significant increase in MDA levels was observed in both the control groups (II and VI) on administration of EG and AC, when compared to the normal group (I), indicating increased lipid peroxidation. These values were significantly decreased in groups treated with cystone and EPV [Table 4]. In vivo, antioxidant parameters such as SOD, CAT, and GSH were significantly decreased in both preventive and curative controls, and these levels were significantly improved in groups treated with cystone and EPV [Table 4].

Histopathological Studies

Histological examination revealed marked congestion, dilation of Bowman’s capsules, scattered mononuclear inflammatory infiltrations and necrotic changes in both preventive and curative groups. EG induced necrotic changes were decreased in the animals treated with cystone and EPV [Figure 1a-i].

DISCUSSION

Formation of kidney stones is a complex process and involves a series of biological events that are most likely triggered by genetic susceptibility together with dietary factors and lifestyle changes.[20] Several in vivo animal models have been developed to investigate the mechanisms involved in the formation of urinary stones. However, rat model has been widely used for the study of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans.[21] In this study, male rats were selected to induce urolithiasis as the amount of stone deposition in...
Table 3: Effect of EPV on serum biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>2.40±0.12</td>
<td>0.74±0.12</td>
<td>18.71±0.59</td>
<td>0.088±0.003</td>
</tr>
<tr>
<td></td>
<td>On day 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>On day 30</td>
<td>2.54±0.27</td>
<td>0.66±0.19</td>
<td>18.10±0.77</td>
<td>0.082±0.005</td>
</tr>
<tr>
<td>II</td>
<td>Preventive control</td>
<td>6.54±0.27</td>
<td>1.74±0.07</td>
<td>37.12±1.41</td>
<td>0.015±0.003</td>
</tr>
<tr>
<td>III</td>
<td>Preventive standard</td>
<td>2.50±0.19</td>
<td>0.79±0.19</td>
<td>24.12±0.87</td>
<td>0.060±0.002</td>
</tr>
<tr>
<td>IV</td>
<td>Preventive low dose</td>
<td>5.48±0.29</td>
<td>1.01±0.07</td>
<td>31.11±1.47</td>
<td>0.025±0.003</td>
</tr>
<tr>
<td>V</td>
<td>Preventive high dose</td>
<td>5.01±0.36</td>
<td>0.94±0.10</td>
<td>30.59±1.36</td>
<td>0.037±0.004</td>
</tr>
<tr>
<td>VI</td>
<td>Curative control</td>
<td>6.80±0.20</td>
<td>1.85±0.12</td>
<td>41.20±1.45</td>
<td>0.016±0.002</td>
</tr>
<tr>
<td>VII</td>
<td>Curative standard</td>
<td>2.71±0.40</td>
<td>0.81±0.09</td>
<td>26.70±1.48</td>
<td>0.064±0.002</td>
</tr>
<tr>
<td>VIII</td>
<td>Curative low dose</td>
<td>5.91±0.37</td>
<td>1.16±0.13</td>
<td>35.21±1.22</td>
<td>0.026±0.001</td>
</tr>
<tr>
<td>IX</td>
<td>Curative high dose</td>
<td>5.14±0.29</td>
<td>1.05±0.09</td>
<td>32.20±1.41</td>
<td>0.034±0.002</td>
</tr>
</tbody>
</table>

All values are expressed in mean±SEM; *P<0.001 when compared to Group I; †P<0.001, ‡P<0.01, §P<0.05 when compared to Group II; ¶P<0.001, ††P<0.01, ‡‡P<0.05 when compared to Group VI. EPV: Ethanolic extract of the seed of Phaseolus vulgaris, SEM: Standard error of the mean, BUN: Blood urea nitrogen.

Table 4: Effect of EPV on in vivo antioxidant parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>LPO (nMole of MDA/mg of protein)</th>
<th>SOD (units/mg of protein)</th>
<th>CAT (µM of H₂O₂ consumed/min/mg of protein)</th>
<th>GSH (nmole of GSH/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>0.30±0.17</td>
<td>6.11±0.33</td>
<td>0.059±0.001</td>
<td>1.97±0.09</td>
</tr>
<tr>
<td>II</td>
<td>Preventive control</td>
<td>5.01±0.53</td>
<td>2.18±0.49</td>
<td>0.025±0.002</td>
<td>0.53±0.14</td>
</tr>
<tr>
<td>III</td>
<td>Preventive standard</td>
<td>0.81±0.40</td>
<td>5.73±0.40</td>
<td>0.054±0.002</td>
<td>1.86±0.10</td>
</tr>
<tr>
<td>IV</td>
<td>Preventive low dose</td>
<td>3.10±0.31</td>
<td>3.68±0.71</td>
<td>0.036±0.001</td>
<td>0.79±0.21</td>
</tr>
<tr>
<td>V</td>
<td>Preventive high dose</td>
<td>1.98±0.89</td>
<td>5.12±0.67</td>
<td>0.040±0.003</td>
<td>1.20±0.19</td>
</tr>
<tr>
<td>VI</td>
<td>Curative control</td>
<td>5.65±0.51</td>
<td>2.39±0.33</td>
<td>0.018±0.001</td>
<td>0.53±0.28</td>
</tr>
<tr>
<td>VII</td>
<td>Curative standard</td>
<td>1.09±0.72</td>
<td>5.56±0.27</td>
<td>0.050±0.001</td>
<td>1.77±0.14</td>
</tr>
<tr>
<td>VIII</td>
<td>Curative low dose</td>
<td>2.80±0.39</td>
<td>4.10±0.38</td>
<td>0.027±0.002</td>
<td>1.24±0.19</td>
</tr>
<tr>
<td>IX</td>
<td>Curative high dose</td>
<td>2.15±0.71</td>
<td>5.11±0.55</td>
<td>0.041±0.001</td>
<td>1.50±0.17</td>
</tr>
</tbody>
</table>

All values are expressed in mean±SEM; *P<0.001 when compared to Group I; †P<0.001, ‡P<0.01, §P<0.05 when compared to Group II; ¶P<0.001, ††P<0.01, ‡‡P<0.05 when compared to Group VI. EPV: Ethanolic extract of the seed of Phaseolus vulgaris, SEM: Standard error of the mean, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced glutathione, LPO: Lipid peroxidation.

Female rats was significantly less.[30] Induction of CaOx type of urolithiasis by EG is a well-validated and clinically relevant model. EG is metabolized to form acidic metabolites such as oxalic acid, benzoic acid, formic acid, and hippuric acid, which causes metabolic acidosis. Subsequently, this acidic condition favors CaOx nucleation followed by growth, aggregation and crystallization, then finally retention at the renal tissue to cause renal mitochondrial toxicity similar to clinical CaOx renal calculi.[31,32]

Urinary supersaturation with respect to stone forming constituents is considered as one of the causative factors for calculogenesis. Previous studies indicated that administration of EG (1% v/v) for 14 days in young albino rats produced renal calculi composed mainly of CaOx associated clinical symptoms of hyperoxaluria, hypercalciuria, and hyperphosphaturia.[33] Moreover, hyperoxaluria is considered as a significant risk factor in the pathogenesis of kidney stones than hypercalciuria. AC was administered to rats along with EG, as it acidifies the urine and then accelerates the process of lithiasis.[34] Hence, EG and AC in drinking water were employed to induce CaOx urolithiasis in rats.

In this study, enhanced deposition of calcium and oxalate in the kidney and their urinary excretion was observed in the disease control group animals indicating that administration of EG and AC induced CaOx urolithiasis. These results were inconsistent with the earlier reports of Patel et al.[35] On administration of EPV, the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinary excretion in both the preventive and curative group animals reveals the potential of EPV in both preventing...
the formation and dissolving the preformed CaOx stones. Although the exact reason was not clear for these diminished levels of calcium and oxalate in EPV treated groups, it might be due to inhibition of the synthesis of oxalate or interrupting the oxalate metabolism. In addition to this, EPV might have increased the bioavailability of nitric oxide to sequester calcium through the cGMP (3', 5' cyclic guanosine monophosphate) pathway.[36]

An increase in urinary phosphate excretion was observed in both the disease control group animals. Elevated urinary phosphate excretion along with oxalate induced stress appears to provide a suitable environment for stone formation by forming calcium phosphate crystals, which epitaxially causes CaOx deposition.[37] Treatment with EPV lowered the excretion of phosphate and reduced the risk of stone formation.

On treatment with EG and AC, an increase in urinary uric acid excretion was observed in the disease control group animals. Uric acid interferes with CaOx solubility and also reduces the inhibitory activity of glycosaminoglycans on CaOx crystallization.[38] Groups treated with EPV, lowered the uric acid concentration in the urine and reduced the risk of stone formation. These results were inconsistent with the earlier reports of Selvam et al.[38]

In addition to urinary stone forming constituents, urinary pH and volume play a prominent role in the process of stone development. The type of stones formed in humans depends on the pH of urine. In general, CaOx stones occur at a pH 5.0–6.5.[39] In this study, in both the disease control groups, a decrease in urinary pH to 5.0–6.0 was observed compared to normal animals (pH of 6.0–7.0) indicating the induction of CaOx stones. Moreover, dissolution of calculi can be achieved by an alteration in urinary pH. Treatment with EPV reversed the acidic pH to normal. This increase in urinary pH might be responsible for dissolving the complexes of calcium and oxalate. Urine volume also played a major role in the CaOx stone formation. In this study, a decrease in urine output was observed in both disease controls, indicating an obstruction in the urinary flow due to the presence of the CaOx stones.

Figure 1: Histopathological findings of kidney under a light microscope (×40). (a): Normal: Normal glomerular structure and renal tubules. (b): Preventive control: Oxalate renal stone and tubular dilation and renal tubular damage. (c): Preventive standard (cystone 750 mg/kg): Dilatation in the renal tubules and with normal glomerular structure. (d): Preventive low dose (EPV 200 mg/kg): Glomerular atrophy and tubular dilation. (e): Preventive high dose (EPV 400 mg/kg): Regenerated to normal glomerular structure with partial glomerular atrophy. (f) Curative control: Congested blood vessels and damage in the renal tubules. (g): Curative standard (cystone 750 mg/kg): Normal renal glomerular structure compared to normal. (h): Curative low dose (EPV 200 mg/kg): Renal tubular dilation and glomerular atrophy. (i) Curative high dose (EPV 400 mg/kg): Congested blood vessels and partial renal tubular dilation.
An increase in the urinary output was observed on treatment with EPV, indicating its diuretic action. Further, it dilutes the urinary electrolytes concentration and might decrease the chance of stone development.

In both the preventive and curative controls, elevated levels of serum uric acid, creatinine and BUN with a concurrent decrease in creatinine clearance indicates marked renal impairment. In both disease controls, the elevated level of nitrogenous waste (creatinine, uric acid, and BUN) in the serum was accompanied by the decreased glomerular filtration rate (GFR) due to obstruction of stones in the Bowman’s capsule.[40] Groups treated with cystone and EPV significantly reversed these levels through improved GFR.

In this study, EG and AC administration caused a significant increase in MDA levels through increased lipid peroxidation activity in the kidneys. This is further supported by earlier studies that exposure to high levels of oxalate and CaOx crystals produce cellular injury mediated by membrane lipid peroxidation through intracellular ROS (Reactive oxygen species) generation.[7,41] In this study, groups treated with EPV decreased the elevated MDA levels, indicating the effect of EPV in reducing oxalate associated lipid peroxidation through diminishing the oxalate levels and/or by scavenging the free radicals produced during the process of stone formation. In both the disease controls, a marked reduction in the levels of enzymatic antioxidants (SOD and CAT) and non-enzymatic antioxidant (GSH) levels were observed. These reductions in the levels, impaired antioxidant protection against oxidative stress, which might have further favored the accumulation and retention of oxalate and subsequent deposition of CaOx. Earlier studies suggested that treatment with antioxidants prevent CaOx deposition in the kidney.[42] In this study, groups treated with EPV improved these enzyme levels to normal, and this could be attributed due to the antioxidant principles/ phytoconstituents present in EPV. Preliminary phytochemical screening of EPV revealed the presence of flavonoids, polyphenols, tannins, and saponins. These phytochemicals may be responsible for the anticrystallization and antioxidant activities of EPV. Plant flavonoids are reported to possess antiurolithiatic activity through its antioxidant property.[43] Several reports suggest that saponins are having antiurolithiatic activity through its diuretic and disaggregating the suspension of mucoproteins.[34,44] An in vitro study suggests that phytic acid process anti-crystallization property by its chelating nature with the calcium.[45] P. vulgaris beans are rich in phytic acid, which combines with calcium to form calcium-phytate complex that reduces the availability of calcium for stone formation by reducing its intestinal absorption.[46]

Histopathological studies revealed congested blood vessels, tubular changes, irregular eosinophilic material, and scattered mononuclear inflammatory infiltration in both the diseased control groups. These remarkable histological changes might be due to oxalate induced lipid peroxidation and renal tissue damage. Treatment with EPV prevented these histological changes causing a significant decrease in the damage index of renal tissue.

Through several mechanisms are involved in the endogenous stone formation, in the present study, the antiurolithiatic activity of EPV may be due to its property of lowering stone forming constituents and by its antioxidant property. Further studies are needed to isolate the active principle/s responsible for the antiurolithiatic activity of P. vulgaris and to probe the mechanism/s of action.

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