7.1 Introduction

Kidney or renal stone is a common disease affecting 2% to 20% (average 10%) of population world-wide (Sayana et al., 2014, Gao et al., 2015). The disease is complex and multifactorial in nature. Calcium stones are found in 80% of cases and (72%) contain calcium oxalate, however (14.7%) contains phosphate oxalate or mixture of both. In calcium oxalate crystals, calcium oxalate monohydrate form is oxalate dependent, whereas dehydrate form is calcium dependent. Deposits of calcium can be located within urinary cavity and in urinary ducts. Although urine supersaturation is prerequisite for stone formation but it fails to explain the localization of crystals in urinary system. Different rodent models were developed to study pathophysiology of urolithiasis. Few models directly inject oxalate salts in to animals and others give products which after metabolism converts to oxalate. So these models are hyperoxaluria (high oxalate in blood) models which give rise to calcium oxalate monohydrate crystals in renal tubules (Daudon and Jungers, 2004, Bilbault and Haymann, 2016).

Ethylene glycol and ammonium chloride induced urolithiasis

Ethylene glycol (EG) is well known chemical for urolithiasis. EG on administration, metabolizes to glycolate, glyoxylate, and oxalate. Each metabolite plays an important role in formation of calcium oxalate monohydrate (COM) crystal in urine and kidney. Rats receiving EG (0.75 % v/v) in drinking water develop hyperoxalurea and hypercalciurea. Intra tubular crystal deposition can be observed from next day of the administration. Induction of macromolecules such as Osteopontine (OPN), Tamm-Horsfall (TH) protein can be detected by northen blot. Tamm-Horsfall protein is an inhibitor of crystal formation. OPN’s role is still debatable in urolithiasis (Bilbault and Haymann, 2016). In vitro, it inhibits crystal aggregation, however in vivo it may facilitate crystal binding to kidney epithelial cells (Kleinman et al., 2004). On the other hand glycolate and glyoxylate deforms the epithelial cells to crystal binding epithelial cells. This will facilitate crystal binding site, where in crystal can grow in size (Bilbault and Haymann, 2016). Glycolate (glycolic acid) also causes severe systemic acidity and oxalate (oxalic acid) precipitate as a calcium oxalate in the kidneys (Albayrak et al., 2013). Ammonium chloride given orally causes metabolic acidosis and promot calcium oxalate crystal deposition in animal kidneys (Fan et al., 1999).
7.2 Methods

7.2.1 Experimental animals

Six to seven week-old male Wistar albino rats weighing 180 - 230 g were obtained from the animal house of Manipal central animal facility, Manipal, India. The animals were housed individually in stainless steel wire meshed plastic cages in a temperature (23±2) °C and humidity (55% - 60%) controlled room with a 12 h light-dark cycle. The animals were supplied with standard rat pellet diet and drinking water *ad libitum* during the entire period of the study. The study protocol was approved by the Institutional Animal Ethical Committee (No: IAEC/KMC/18/2013). Experiment was carried out in accordance with accepted standard guidelines for safe use of animals in scientific research.

7.2.2 Acute toxicity study

Acute oral toxicity was performed in male rats as per Organization for Economic Cooperation and Development (OECD) guideline 425 and maximum tolerable dose (MTD) was determined. LPEA was found to be safe up to 2000 mg/kg without any signs of toxicity. Animals were observed for any toxicity signs (behavioral, neurological and morphological profiles) for the first 4 h continuously and thereafter daily for 14 days. No mortality or toxicities were observed with any of the treatments. 1/10th and 1/20th of safe dose i.e. (200 mg/kg and 100 mg/kg) were selected for the efficacy study. Quercetin was given at 50 mg/kg (Zhu et al., 2014, Hou et al., 2014) and gallic acid was given at 40 mg/kg (Akomolafe et al., 2014). Cystone, a herbal drug formulation from Himalaya drug company was used as standard at 750 mg/kg body weight (Patel et al., 2012).

7.2.3 Ethanyle glycol and ammonium chloride induced urolithiasis in rats

Animals were randomized based on body weight in nine groups containing six to seven animals in each group (n = 6 - 7) to study the effect of LPEA, quercetin and gallic acid in ethylene glycol and ammonium chloride induced urolithiasis in rats (Fan et al., 1999). Grouping of the animals is given in table 7.1.

**Normal control:** Animals received normal drinking water from day 1 to day 28 along with 0.25 % w/v carboxy methyl cellulose (CMC) orally.
**Disease control:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 orally.

**Standard:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with cystone 750 mg/kg in 0.25% CMC orally.

**LPEA 100:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with LPEA 100 mg/kg in 0.25% CMC orally as preventive regimen.

**LPEA 200:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with LPEA 200 mg/kg in 0.25% CMC orally as preventive regimen.

**Quercetin:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with quercetin 50 mg/kg in 0.25% CMC orally as preventive regimen.

**Gallic acid:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with gallic acid 40 mg/kg in 0.25% CMC orally as preventive regimen.

**LPEA 100C:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with LPEA 100 mg/kg in 0.25% CMC from day 15 to 28, orally as curative regimen.

**LPEA 200C:** Animal received ethylene glycol (0.75 % v/v) and ammonium chloride (0.5% w/v) in drinking water from day 1 to day 28 along with LPEA 200 mg/kg in 0.25% CMC from day 15 to 28, orally as curative regimen.
Table 7.1 Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration (In days)</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control (0.25% w/v CMC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Disease Control</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; to 28&lt;sup&gt;th&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cystone 750 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LPEA 100 mg/kg p.o.</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; to 28&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Preventive</td>
</tr>
<tr>
<td>5</td>
<td>LPEA 200 mg/kg p.o.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Quercetin 50 mg/kg p.o.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gallic acid 40 mg/kg p.o.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>LPEA 100 mg/kg p.o.</td>
<td>15&lt;sup&gt;th&lt;/sup&gt; to 28&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Curative</td>
</tr>
<tr>
<td>9</td>
<td>LPEA 200 mg/kg p.o.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Induction of urolithiasis was confirmed by collecting urine and blood on day 14, for the analysis of urine parameters like pH, calcium, creatinine, uric acid and serum parameters like calcium, creatinine and uric acid.

7.2.3.1 Collection and analysis of urine

Metabolic cages were used to collect urine of individual animal for 24 h on 29<sup>th</sup> day. The volume of urine and pH were determined. Urine was acidified with one drop of concentrated HCl and stored at -20 °C to analyze oxalate, uric acid, calcium, magnesium, and creatinine using commercially available kits (Aspen laboratories Pvt Ltd, India). Oxalate was estimated using colorimetric reported method. Urine was acidified with HCl (1 mL Conc HCl per 100 mL urine) to ensure solubility of calcium oxalate. Exactly 0.5 mL of urine was taken and diluted with 1.5 mL water. Urine was neutralized with diluted NaOH. About 2 mL of saturated aqueous calcium sulphate and 14 mL of ethanol was added. Allowed the mixture to stand for 4 h. Mixture was centrifuged at 2000 rpm for 10
min. After decanting, residue was dissolved in 2 mL 2 N H$_2$SO$_4$. Few pieces of fresh zinc was added and mixture was heated on boiling water bath for 30 min or till volume reduces to 0.5 mL. About 0.5 mL of 1% chromotropic acid (in water) and 5 mL of Conc H$_2$SO$_4$ was added. Mixture was heated for 30 min in boiling water bath. After cooling, sample was diluted to 20 mL with 10 N H$_2$SO$_4$. Absorbance was recorded at 570 nm. Calibration curve was prepared using standard oxalic acid anhydrous at concentration of (0, 100, 200, 500, 1000, 2000, 5000) µg/mL. Sample absorbance was plotted and final concentration was mentioned as µg/mL (Hodgkinson and Williams, 1972).

**7.2.3.2 Collection and analysis of serum**

The total body weight of the animals was recorded every week throughout the duration of the study. Blood was collected from retro-orbital puncture under light ether anesthesia on 29$^{th}$ day. Serum was separated by centrifugation at 10000 rpm for 12 min and analyzed for calcium, creatinine, uric acid, blood urea nitrogen (BUN), AST, ALP, and ALT. At the end of the study, animals were overdosed with anesthesia for scarification and kidneys were dissected out and weighed.

**7.2.3.3 Estimation of biochemical and oxidative-stress related biomarkers**

Right kidney was excised from the animals and blood was removed by washing with ice-cold PBS, blotted and weighed on 29$^{th}$ day. 10% w/v homogenate of the tissues was prepared in cold 1.15% w/v potassium chloride solution. This homogenate was centrifuged at 10000 rpm for 10 min at 4 °C to obtain a clear supernatant which was used for the estimation of anti-oxidant and biochemical parameters.

**7.2.3.3.1 Lipid peroxidation**

To 200 µL of 10% w/v tissue homogenate, 200 µL of sodium dodecyl sulphate (8.1%), 1.5 mL of 20% acetic acid solution (pH 3.5, adjusted with sodium hydroxide) and 1.5 mL of aqueous solution of thiobarbituric acid (0.8 %) were added. The total volume was made up to 4 mL with distilled water and heated at 95 °C in a water bath for 1 h. The mixture was then cooled, and 1 mL of distilled water and 5 ml of mixture of n-butanol and pyridine (15:1) were added. The mixture was shaken vigorously and centrifuged at 5000 rpm for 5 min. The upper layer was removed and absorbance was measured at 532 nm using UV/Visible spectrophotometer (Ohkawa et al., 1979).
7.2.3.3.2 Estimation of catalase

50 μL of tissue homogenate was added to 3 mL of freshly prepared 0.036% w/w of hydrogen peroxide solution (prepared in phosphate buffer using 30% w/w H₂O₂) and the absorbance was recorded at 240 nm for 0 to 30s (Cohen et al., 1970).

7.2.3.3.3 Estimation of SOD

The SOD estimation assay was based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome. 50 μL of tissue homogenate was added to the reaction mixture consisting of 3 × 10⁻⁴ M adrenaline and 0.05 M carbonate buffer (pH 10.2). The decrease in absorbance of adrenochrome was recorded at 480 nm. 1 unit of SOD activity is equal to the amount of enzyme, needed to inhibit 50% auto oxidation of adrenaline (Misra and Fridovich, 1972).

7.2.3.3.4 Estimation of glutathione (GSH)

To the tissue homogenate (in 0.1 M phosphate buffer pH 7.4), equal volume of 20% TCA containing 1 mM EDTA was added to precipitate the proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 5000 rpm. The supernatant was added to 1.8 mL of Ellman's reagent (DTNB), 0.1 mM), prepared in 0.3 M phosphate buffer with 1% sodium citrate solution. The volume was made up to 2 mL in all the tubes and absorbance recorded at 412 nm against blank. The GSH concentration was calculated from the standard curve for reduced glutathione (concentration range 0–0.1 mM) and results were expressed as nmol/mg protein (Ellman, 1959).

7.2.3.3.5 Total protein

Total protein was estimated in kidney homogenate colorimetrically using Total Protein Kit, Micro (Sigma; TP0100) (Bradford, 1976, Sedmak and Grossberg, 1977).

7.2.3.4 Estimation of serum biomarkers/elemental analysis

Aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, and blood urea nitrogen (BUN) were determined using appropriate kits in an autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Calcium was
estimated colorimetrically (Aspen colorimetric assay kit) (Patel et al., 2012, Atici et al., 2005).

7.2.3.5 DNA Fragmentation in tissue samples
Quantitation of DNA fragmentation was done by the colorimetric diphenylamine assay (Burton, 1956). The kidney homogenates were mixed with equal volume of buffer containing 20 mM Tris-HCl, 20 mM ethylene diamine tetracetate (EDTA), 0.5% Triton X-100, pH 7.5, and centrifuged at 15,000 rpm for 15 min at 4°C to separate intact DNA in the pellet from fragmented/damaged DNA in the supernatant fraction. Perchloric acid (final concentration 0.5 M) was added to the pellet and supernatant samples which were heated at 90°C for 15 min and then centrifuged to remove precipitated proteins. The resulting supernatants, whether containing whole or fragmented DNA, were treated with 58.7 mM diphenylamine for 16–20 h at room temperature in dark and the absorbance was recorded at 600 nm. DNA fragmentation was expressed as the percentage of fragmented DNA to total DNA.

7.2.3.6 Histopathology

Hematoxylin and eosin staining
Left kidney was fixed in 10% neutral buffered formalin (NBF), dehydrated with alcohol and cleared thoroughly using xylene. The tissues were impregnated with paraffin wax and 5µm sections were cut using a rotary microtome (RM2245, Leica Microsystems GmbH, Wetzlar, Germany), mounted on slides, de-waxed with xylene followed by rehydration through graded series of alcohol. The tissues were then stained with hematoxylin and eosin. The slides were observed under a microscope, analyzed by a pathologist who was blinded to the samples, and photographs taken. The slides were observed for the tissue changes (Patel et al., 2012).

Statistical analysis
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey’s post hoc test using Prism 6.05 Version (GraphPad Software Inc., La Jolla, California, USA). Results were expressed as Mean ± SEM and (p < 0.05) was considered significant.
7.3 Results and discussion

In vitro antiurolithiatic study showed ethyl acetate fraction of the L. prostrata (LPEA) was active in inhibiting calcium oxalate crystal formation. Fraction was also enriched with phenols and flavonoids. Phenols and flavonoids are good antioxidants. There is a positive relation between antioxidants and antiurolithiatic activity (Devkar et al., 2016). Therefore, LPEA was evaluated for efficacy study in ethylene glycol induced urolithiasis in rats.

7.3.1 In vivo induction of urolithiasis

Oral administration of ethylene glycol (0.75% v/v) and ammonium chloride (0.5% w/v) for 28 days caused significant induction of urolithiasis in experimental animals characterized by change in urine and serum biochemical parameters. There was a significant weight reduction in diseased control group observed during the progression of urolithiasis (data not shown). Induction of urolithiasis was confirmed by collecting urine and blood on day 14, for the analysis of urine parameters like pH, calcium, creatinine, uric acid and serum parameters like calcium, creatinine and uric acid (data not shown). Results for preventive and curative regimen are discussed below.

7.3.2 Urine parameters

Different urine parameters such as, volume, pH, calcium, magnesium, oxalate, creatinine, and uric acid were studied. Result for all the parameters shown in Figure 7.1. There is no significant difference observed in urine volume. The pH of the disease control group was significantly (p < 0.05) lower than normal control (Figure 7.1B). These findings are in accordance with the literature (Fan et al., 1999). Treatment with LPEA at 200 mg/kg in preventive and curative regimen and gallic acid was found to be significantly (p < 0.05) active in regulating pH (Figure 7.1B).
Figure 7.1. Effect of treatment on urine parameters; A: volume, B: pH, C: calcium, D: magnesium, E: creatinine, F: oxalate, G: uric acid.

$^p < 0.05$ compare to normal control

*p < 0.05* compare to disease control

All values are expressed as Mean ± SEM. Statistical analysis was carried out using One way ANOVA followed by post-hoc Dunnett’s test. (n=6-7)
A significant difference in urine calcium and magnesium was observed in control and normal group. Calcium was found to be higher and magnesium to be lower in diseased control, than normal control (Patel et al., 2012). However all the treatment groups were found significantly (p < 0.05) effective in lowering urine calcium (Figure 7.1C). No effect on magnesium was observed (Figure 7.1D).

There was a significant (p < 0.05) increase in urine oxalate, creatinine, and uric acid in diseased control animals compare to normal control (Figure 7.1 E,F,G). LPEA at the dose of 200 mg/kg, quercetin and gallic acid in both preventive and curative group were found significantly (p < 0.05) effective in reducing urine creatinine (Figure 7.1E). A significant (p < 0.05) reduction in urinary oxalate was observed in all treatment groups except quercetin treated and LPEA at the dose of 100 mg/kg. LPEA at the dose of 200 mg/kg in preventive treatment was found to be most active in reducing urinary oxalate (Figure 7.1F). It was observed that a significant (p < 0.05) reduction in uric acid in all treatment group except LPEA in curative treatment groups (Figure 7.1G).

### 7.3.2 Serum parameters

Though calcium in the blood is critically regulated, a significant (p < 0.05) increase in serum calcium level was observed in disease control group compare to normal control (Figure 7.2). Serum creatinine, uric acid, and blood urea nitrogen (BUN) were found significantly (p < 0.05) high in disease control group than healthy rats (Figure 7.2). These findings were in accordance with earlier reports in ethylene glycol induced urolithiasis model (Shah et al., 2012, Patel et al., 2012, Albayrak et al., 2013).

Presence of higher calcium level in blood, facilitate precipitation as calcium oxalate in urine. There for regulating calcium in blood is one of the important treatment parameters. Studies also showed that calcium channel blocker can increase incidence of urolithiasis (Albayrak et al., 2013). Treatment with LPEA at 200 mg/kg in preventive group was found to be significantly (p < 0.05) active in reducing elevated serum calcium level (Figure 7.2A). Antioxidants quercetin and gallic acid were also found significantly (p < 0.05) effective in bringing elevated calcium level to the normal (Figure 7.2A).

Urolithiasis causes impairment in renal functions and tubular damage. This was evident by increased serum creatinine, uric acid and BUN compared to healthy rats (Patel et al., 2012). Cystone was found to be significantly (p < 0.05) effective in
controlling increased serum creatinine and BUN (Figure 7.2B, G). LPEA in preventive group and gallic acid were significantly effective in controlling creatinine and BUN (Figure 7.2B, G). LPEA at lower dose level was found to be most active in controlling BUN (Figure 7.2G).

Elevated serum uric acid levels were brought to normal significantly (p < 0.05) by LPEA preventive group and gallic acid. Other treatment groups were not found significantly (p < 0.05) effective in controlling serum uric acid (Figure 7.2C).

AST and ALT levels were significantly (p < 0.05) higher in diseased control group than normal control (Figure 7.2E, F). All the treatment groups except LPEA at lower dose in preventive group were found to be significantly (p < 0.05) effective in lowering elevated AST level (Figure 7.2E). LPEA showed dose dependent lowering in AST. Only Cystone and gallic acid were significantly (p < 0.05) effective in controlling elevated ALT levels (Figure 7.2F). No significant changes were observed in serum ALP levels (Figure 7.2D).
Chapter 7

In vivo study

Figure 7.2. Effect of treatment on serum parameters; A: calcium, B: creatinine, C: uric acid, D: ALP, E: AST, F: ALT, G: BUN.

All values are expressed as Mean ± SEM. Statistical analysis was carried out using One way ANOVA.

$p < 0.05$ compare to normal control

* $p < 0.05$ compare to disease control

\[ \text{Data were expressed as Mean ± SEM. Statistical analysis was carried out using One way ANOVA.} \]
7.3.3 Kidney parameters

Urolithiasis caused significant (p < 0.05) increase in MDA (Malondialdehyde) and total protein compared to normal control (Figure 7.3B, F). Level of glutathione and catalase were significantly (p < 0.05) reduced in diseased control compared to healthy rats (Figure 7.3A, C). No significant change was observed in SOD (Figure 7.3D).

LPEA preventive group and gallic acid were significantly (p < 0.05) effective in normalizing depleted glutathione levels (Figure 7.3A). Lipid peroxidation levels are an excellent indicator for the extent of oxidative damage in tissue (Janero, 1990). MDA (Malondialdehyde), the end product of lipid peroxidation, was estimated in the kidney tissues. The level of MDA was significantly (p < 0.05) increased in the disease control as compared to the normal control indicating oxidative stress in the kidney tissue by EG. Only gallic acid was found to be significantly (p < 0.05) effective in lowering elevated lipid peroxide; MDA level (Figure 7.3B). LPEA at both the dose levels in preventive and curative treatment group was found to be significantly effective (p < 0.05) in raising depleted catalase levels (Figure 7.3C). LPEA 200 mg/kg in preventive treatment and gallic acid were significantly (p < 0.05) effective in reducing total protein (Figure 7.3F).

Calcium content in kidney was determined. There was significant (p < 0.05) increase in kidney calcium was found in disease control compared to normal control. Treatment with Cystone, LPEA 200 mg/kg preventive, and gallic acid were found significantly (p < 0.05) effective in lowering elevated kidney calcium to the normal level (Figure 7.3E).
Figure 7.3. Effect of treatment on kidney parameters; A: glutatione, B: MDA, C: catalase, D: SOD, E: calcium, F: total protein.

\( \text{*p} \leq 0.05 \text{ compare to normal control}; \ \text{*p} \leq 0.05 \text{ compare to disease control} \)

All values are expressed as Mean ± SEM. Statistical analysis was carried out using One way ANOVA followed by post-hoc Dunnett’s test. (n=6-7)
7.3.4 DNA fragmentation assay

Oxidative stress causes production of reactive oxygen species (ROS) that leads to cell damage and consequently cell death; apoptosis is one of the mechanisms of cell death. In the study, an attempt was made to evaluate DNA damage during urolithiasis by apoptosis (Kizivat et al., 2017, Aggarwal et al., 2013). There was significant \( p < 0.05 \) increase in DNA fragmentation in diseased control animals as compared to normal control (Figure 7.4). Treatment with Cystone, LPEA 200 mg/kg in preventive group and gallic acid were found to significantly \( p < 0.05 \) reduce the DNA damage (Figure 7.4), thereby acting as a protective in nature. The antioxidant nature of the fraction (Devkar et al., 2016) could be responsible for reduction in DNA damage. Extract and gallic acid were found to be protective against DNA damage.

![Figure 7.4 DNA fragmentation assay by diphenylamine method](image)

7.3.5 Histopathology of kidney sample

Histopathology of kidney was performed. Slides were compared with normal control (Figure 7.5). In disease control animal, prominent tubular congestion, necrotic debris, calculi, tubular cell swelling, peritubular inflammation, interstitial edema, and peritubular fibrosis were observed.

In cystone treated group, calculi were absent. Peritubular fibrosis was reduced. Glomerular atrophy and interstitial edema were absent. LPEA in preventive group was found to be more effective even at lower dose of 100 mg/kg. Histopathology of LPEA treated group showed no evident calculi or peritubular inflammation. In curative group
there was no evidence of calculi present. Necrotic debris, peritubular fibrosis and interstitial edema were found absent. However glomerular congestion can be detected in curative regimen for LPEA (Plates 7.1).

A: Normal control, B: Disease control, C: Cystone, D: LPEA 100, E: LPEA 200, F: Quercetin, G: Gallic acid, H: LPEA 100C, I: LPEA 200C

**Plates 7.1 Histopathology (hematoxylin and eosin staining) of kidney (40X)**
Quercetin was also found effective in reducing calculi, however glomerular and tubular congestion was observed. Tubular swelling was reduced with no evidence of edema observed. Gallic acid was found effective in controlling calculi and tubular swelling. No peritubular inflammation or necrotic debris was observed in gallic acid treated animals.

To conclude, studies have shown that, *L. prostrata* possess marked antiurolithiatic activity with minimal signs of tissue toxicity. *L. prostrata* has greater antiurolithiatic potential as prophylactic rather than as a curative. Gallic acid is better candidate as an antiurolithiatic than quercetin.
7.4 References


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