EFFECT OF LUPEOL, A PENTACYCLIC TRITERPENE, ON URINARY ENZYMES IN HYPEROXALURIC RATS

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SUMMARY: Investigations were undertaken to study the role of lupeol, a pentacyclic triterpene from Crataeva nurvala stem bark, in calcium oxalate experimental rat urolithiasis. A 2% solution of ammonium oxalate was administered by gastric intubation for inducing hyperoxaluric condition in adult male rats of Wistar strain. The duration of treatment was for 15 days. This resulted in increased urinary excretion of oxalate associated with reduction in citrate and glycosaminoglycans. The urinary marker enzymes which indicate renal tissue damage namely — lactate dehydrogenase, inorganic pyrophosphatase, alkaline phosphatase, gamma glutamyl transferase, β-glucuronidase and N-acetyl β-D glucosaminidase were found to be elevated. Lupeol administration (25 mg/kg body weight/day) reduced significantly the renal excretion of oxalate. It also reduced the extent of renal tubular damage as evidenced from the decreased levels of the above enzymes in urine. Such a reduction is likely to be beneficial in minimizing the deposition of stone-forming constituents in the kidney which provides antilithic effect.

INTRODUCTION

Urolithiasis occurs as a consequence of deposition of various stone-forming constituents like calcium and oxalate in the renal tissues. Such a deposition is preceded by tissue lesion which serves as a niche (1). Since various enzymes are found to be characteristically located in different regions of the kidney, they
serve as urinary markers in ascertaining the degree of lesions in the renal cellular components (2).

Baggio et al. (3) have reported higher than normal levels of renal enzymes in the urine of idiopathic calcium oxalate stone formers and in experimentally induced nephrolithiatic rats. According to Smith (4), the greater the extent of tubular injury, the higher the risk of calculi formation.

Urolithiasis is a disorder with increasing rate of incidence and recurrence due to economic and industrialization progress. The traditional systems of medicine are gaining importance in India and their scientific evaluation has become a necessity. Several herbal drugs are reported to have antilithic properties. One such is the bark decoction of the indigenous plant Crataeva nurvala Buch Ham (N. O. Capparidaceae). Recent studies in our laboratory has confirmed its antilithic role in experimental rat urolithiasis. A pentacyclic triterpene, lupeol was also isolated and it showed promising effect in rat models (5). The present work aims to investigate the effect of lupeol on urinary oxalate levels and urinary marker enzymes reflecting renal injury.

MATERIALS AND METHODS

Male albino rats of Wistar strain (150-180 g body weight) were acclimatized to laboratory conditions and were nourished with commercially available pelleted (Gold Mohur, Hindustan Lever Ltd., Bombay, India) rat feed. Water was given ad libitum. Experimental hyperoxaluric condition was induced by the gastric administration of 2% ammonium oxalate for 15 days. The dosage of lupeol was fixed at 25 mg/kg body weight/day/rat. It was arrived at from reports where 10-50 mg/kg body weight of lupeol was administered to implanted rat urolithiatic models (6).

Experimental set-up: The animals were divided into four groups of six rats each and treated as follows:

Group I: The animals served as controls and were maintained on normal diet for 15 days.

Group II: The animals were administered 2% ammonium oxalate by gastric intubation.

Group III: The animals were administered lupeol (25 mg/kg body wt/day) dissolved in olive oil for 15 days by gastric intubation.

Group IV: The animals received both 2% ammonium oxalate and lupeol as described above for 15 days.
At the end of the 14th day, the rats were housed in metabolic cages and their urine collected for 24 hr in beakers maintained at 0 C in an ice bath. The collected urine was centrifuged, a part of it was dialyzed at 4 C for 3 hr, and used for the assay of enzymes.

Oxalate was estimated in the undialyzed urine by the method of Hodgkinson and Williams (7). Creatinine was estimated by the method of Owen et al. (8), citric acid by the method of Rajagopal (9) and glycosaminoglycans by the modified method of Hwang et al (10).

The dialyzed urine was utilized for assaying various enzymes originating in the kidney. Alkaline phosphatase (ALP) was assayed by the method of King (11). Lactate dehydrogenase (LDH) was assayed by the method of King (12). The activities of inorganic pyrophosphatase and N-acetyl β-D glucosaminidase (NAG) were arrived at by the methods of Josse (13) and Marhun (14), respectively. Gamma-glutamyl transferase (γ-GT) was assayed by the method of Orlowski and Meister (15) and β-glucuronidase by the method of Kawai and Anno (16). The enzyme activities are expressed as units per mg creatinine per 24 hr urine at 37 C.

The values are expressed as mean ± standard deviation. Student's t test was used for the statistical evaluation of the data.

RESULTS

Table I represents the effect of lupeol on the urinary excretion of oxalate, creatinine, citric acid and glycosaminoglycans of control and hyperoxaluric rats. Urinary oxalate was found to be elevated in the ammonium oxalate administered (Group II) rats (p<0.001). Supplementation of lupeol brought about a reduction in the level of urinary oxalate on the 15th day in both controls (Group III) and hyperoxaluric rats (Group IV).

The levels of creatinine and citric acid were found to be decreased in hyperoxaluric rats (Group II) and were normalized to a great extent on lupeol supplementation. Glycosaminoglycans (GAGs) being calcium-complexing agents were found to be lowered in the urine of lithogenic rats. On administration of lupeol, their excretion was further reduced (Group IV).

The urinary excretion of various enzymes originating in the kidney has been depicted in Table II. LDH, the major oxalate-synthesizing enzyme, was elevated in the urine of hyperoxaluric rats (p<0.001), as was the level of inorganic pyrophosphatase (p<0.001). Lupeol administration lowered their release (Group IV) into urine considerably when compared to hyperoxaluric (Group II) rats.
Table I. Effect of lupeol on certain urinary constituents (Mean ± S.D. for six rats in each group)

<table>
<thead>
<tr>
<th>No.</th>
<th>Constituents</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oxalate (mg/24 hr urine at 37C)</td>
<td>2.83 ± 0.08</td>
<td>13.63 ± 0.12a*</td>
<td>2.34 ± 0.01b*</td>
<td>8.15 ± 0.08c<em>d</em>e*</td>
</tr>
<tr>
<td>2.</td>
<td>Creatinine (mg/24 hr urine at 37C)</td>
<td>13.20 ± 0.71</td>
<td>6.75 ± 0.81a*</td>
<td>14.85 ± 1.41b@</td>
<td>10.36 ± 0.59c<em>d</em>e*</td>
</tr>
<tr>
<td>3.</td>
<td>Citric acid (mg/24 hr urine at 37C)</td>
<td>2.83 ± 0.44</td>
<td>1.60 ± 0.15a*</td>
<td>2.77 ± 0.54</td>
<td>1.99 ± 0.19c<em>d</em>e#</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosaminoglycans (mg/24 hr urine at 37C)</td>
<td>4.23 ± 0.71</td>
<td>3.67 ± 0.19a*</td>
<td>4.58 ± 0.07b*</td>
<td>3.39 ± 0.10c<em>d</em>e*</td>
</tr>
</tbody>
</table>

A. ox - ammonium oxalate.
Group I: Control rats fed on normal pelleted feed; Group II: Rats administered with 2% A. ox; Group III: Control rats administered with lupeol (25 mg/kg body weight/day); Group IV: Rats administered with 2% A. ox and lupeol (25 mg/kg body weight/day).

Comparisons were made between: (a) Group I and Group II, (b) Group I and Group III, (c) Group I and Group IV, (d) Group II and Group IV, (e) Group III and Group IV.

Statistical significance is expressed as: @p<0.05, #p<0.01, *p<0.001.
Table II. Effect of lupeol on urinary marker enzymes (Mean ± S.D. for six rats in each group)

<table>
<thead>
<tr>
<th>No.</th>
<th>Enzymes</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lactate dehydrogenase (micro moles of pyruvate released/mg creatinine/24 hr at 37 C)</td>
<td>0.35±0.16</td>
<td>0.73±0.19a#</td>
<td>0.27±0.04</td>
<td>0.39±0.08c<em>d#e</em></td>
</tr>
<tr>
<td>2.</td>
<td>Inorganic pyrophosphatase (micro moles of phosphorus released/mg creatinine/24 hr at 37 C)</td>
<td>8.95±0.65</td>
<td>13.68±2.14a*</td>
<td>8.08±0.41b#</td>
<td>10.17±0.71c@d#e*</td>
</tr>
<tr>
<td>3.</td>
<td>Alkaline phosphatase (micro moles of phenol released/mg creatinine/24 hr at 37 C)</td>
<td>0.58±0.05</td>
<td>2.86±0.37a*</td>
<td>0.52±0.05</td>
<td>1.15±0.13c<em>d</em>e*</td>
</tr>
<tr>
<td>4.</td>
<td>γ-glutamyl transferase (micro moles of p.nitroaniline released/mg creatinine/24 hr at 37 C)</td>
<td>2.77±0.31</td>
<td>8.33±1.99a*</td>
<td>2.41±0.14b#</td>
<td>5.07±0.98c<em>d#e</em></td>
</tr>
<tr>
<td>5.</td>
<td>β-glucuronidase (nanomoles of p.nitrophenol released/mg creatinine/24 hr at 37 C)</td>
<td>0.19±0.03</td>
<td>0.55±0.07a*</td>
<td>0.16±0.03</td>
<td>0.26±0.04c#d<em>e</em></td>
</tr>
<tr>
<td>6.</td>
<td>N-acetyl β-D glucosaminidase (nanomoles of p.nitrophenol released/mg creatinine/24 hr at 37 C)</td>
<td>0.35±0.05</td>
<td>1.39±0.36a*</td>
<td>0.32±0.06</td>
<td>0.74±0.16c<em>d#e</em></td>
</tr>
</tbody>
</table>

A. ox - ammonium oxalate.
Group I: Control rats fed on normal pelleted feed; Group II: Rats administered with 2% A. ox; Group III: Control rats administered with lupeol (25 mg/kg body weight/day); Group IV: Rats administered with 2% A. ox and lupeol (25 mg/kg body weight/day).
Comparisons were made between: (a) Group I and Group II, (b) Group I and Group III, (c) Group I and Group IV, (d) Group II and Group IV, (e) Group III and Group IV.
Statistical significance is expressed as: @p<0.05, #p<0.01, *p<0.001.
ALP and γ-GT, the enzymes specific for the renal proximal tubules, were increased in the lithogenic group (Group II). The lysosomal marker enzymes namely, β-glucuronidase and NAG also followed the same pattern of excretion. When lupeol was supplemented to these hyperoxaluric rats, a dramatic decrease in the levels of these enzymes was observed in the urine.

**DISCUSSION**

Urinary oxalate is an important determinant of urinary calcium oxalate supersaturation than is calcium (17). Ammonium oxalate (2%) administration to rats for 15 days resulted in a multifold increase in urinary oxalate excretion as already reported by Khan et al (1). Elevated oxalate in the kidney exerts marked effect on the physicochemical properties of the urine and may also damage the tubular epithelium.

Reduced urinary creatinine was indicative of abnormal renal functioning of hyperoxaluric rats, which was normalized by lupeol. Calcium oxalate crystal growth and aggregation in the kidney were inhibited by urinary macromolecules like GAGs (18) and citric acid (19) by combining of calcium ions. Their lowered excretion is observed during urolithiatic affliction (20). Urinary GAGs are filtered from serum as end products of connective tissue metabolism (21) and may possibly enter the urine from other sites along the genitourinary tract (22). On lupeol administration, the lowered citric acid level was found to be considerably elevated, thus ascertaining diminished calcium oxalate crystallization. Though significant lowering of total GAGs was found to be associated with stone formers as compared with controls (23,24), the clinical role of GAGs on renal calculi formation remains controversial. With lupeol supplementation, the level of GAGs was found to be further lowered (Group IV), which may be attributed to the decreased entry of GAGs from the sites along the urinary tract due to amelioration of tubular injury.

Most of the urinary enzymes originate specifically from various portions and cellular components of the nephron (25). Increased oxalate content in the tissues cause damage to proximal tubular epithelium which is generally associated with shedding of the brush border membrane, thereby facilitating crystal retention (26). Hence, the measure of various urinary enzymes could be directly related to
the extent of tissue derangement caused by hyperoxaluria and reversal of this condition would reduce the risk of calculi formation.

LDH, a renal cytoplasmic enzyme, is one of the marker enzymes for various diseases of the urinary tract. Under lithogenic condition, its activity has been reported to increase in urine (27). In the present investigation, LDH being an oxalate-synthesizing enzyme is found to be inhibited by the external milieu of oxalate reaching the kidney, leading to its increased excretion. Supplementation of lupeol reduces its excretion (Group IV, Table II), thereby relieving the kidney of the high oxalate content.

Inorganic pyrophosphate is an inhibitor of calcium oxalate crystallization (28) and its non-availability as a substrate to inorganic pyrophosphatase might be the reason for its increased level in the urine of hyperoxaluric rats (Group II). Increased oxalate content has also been reported to inhibit pyrophosphatase activity (29). Lupeol administration lowered the enzyme excretion to a remarkable extent (Group IV).

ALP and ϒ-GT are specific for the brush border membrane of renal proximal tubules (30). In the present investigation, the calculogenic rats (Group II) manifested a massive rise in their urinary levels analogous to the observations made by Padmaja and Varalakshmi (31) in urolithiatic rats.

The hyperoxaluric rats exhibited significant elevation in the urinary levels of β-glucuronidase and NAG. These are lysosomal enzymes with their maximal activity in the straight part of the proximal tubule (32). Subha and Varalakshmi (33) have observed increased activities of these enzymes in the urine of stone forming rats, which correlates with our observation.

Hyperoxaluric condition makes the proximal tubular epithelium more susceptible to oxalate-induced injury because of its major role involved in the handling of oxalate (34). This epithelial tissue damage is reflected by the increased release of the tubular marker enzymes in the urine of hyperoxaluric rats (Group II).

Lupeol, an active constituent from the stem bark of C. nurvala, is effective in bringing down the levels of various urinary enzymes. Such a protective effect of lupeol may be attributed to its diuretic activity which facilitates regular excretion of oxalate during the course of the treatment. This reduces saturation of oxalate in the renal tissues thereby minimizing cellular injury. This concept is supported by various reports establishing the diuretic activity of related triterpenoids (35,36).
From the above results and considering various long-term harmful side effects caused by the different less invasive techniques, like percutaneous nephrolithotomy (PCN) and extracorporeal shockwave lithotripsy (ESWL), lupeol may be useful as an antilithic drug and further work is in progress on these lines.

REFERENCES


