HYPOLIPIDEMIC ACTIVITY OF AERVA LANATA ON ETHYLENE GLYCOL INDUCED CALCIUM OXALATE UROLITHIASIS IN RATS

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Summary

The hypolipidemic activity of Aerva lanata was assessed on ethylene glycol induced calcium oxalate urolithic rats. Total lipids, total cholesterol and triglycerides levels were significantly increased in serum, liver and kidney of calcium oxalate urolithic rats. Besides, phospholipids (PL), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) levels were altered in calcium oxalate urolithic rats. On supplementation of A. lanata aqueous suspension, the above changes were reverted to near normal. These results indicate that A. lanata aqueous suspension act as a hypolipidemic agent in calcium oxalate urolithiasis.

Key words: Aerva lanata; Calcium oxalate; Hypolipidemic; Urolithiasis

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Introduction

Urolithiasis is a disorder to occur 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females [1]. Calcium oxalate stones are most common, occurring in 70 to 80% of stone sufferers. Calcium oxalate stone formation was a result of a systemic abnormality in lipid metabolism. Light and electron microscopic studies in stone forming rats showed the accumulation of osmophilic lipid bodies along with oxalate crystals [2]. The serum lipid profile is generally considered to reflect the metabolism of lipid by liver and other tissues. Hyperlipidemia associated with the nephritic syndrome is a complex disorder involving abnormalities most likely induced by the glomerular barrier defect [3].

*Aerva lanata* is an erect or prostrate herbaceous weed, common throughout the hotter parts of India almost all over the plains up to an altitude of 3000m. A literature survey revealed that *A. lanata* is endowed with various chemical components such as flavonoids, alkaloids, triterpenes, steroids, polysaccharides, tannins, saponins, etc [4-6], which possibly contribute to its diverse uses in folklore medicine. In our previous study, *A. lanata* reduced the oxalate synthesizing enzymes, oxalate and urinary risk factors in ethylene glycol induced urolithiasis [7]. The present study was planned to evaluate the hypolipidemic activity of *A. lanata* aqueous suspension on lipids and lipoproteins alterations in ethylene glycol induced calcium oxalate urolithiasis in rats.

Methods

Plant material
*A. lanata* fresh aerial parts were collected during the months of September to December in Tamil University, Thanjavur, Tamilnadu, South India and authenticated. The aerial parts were dried thoroughly under shade and powdered finely. The powder was suspended in distilled water and used for the study.

Animals
Male albino rats of Wistar strain weighing approximately 140-150 gm were used. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee. Animals were housed in plastic cages with filter tops under controlled conditions of a 12 hour light/12 hour dark cycle, 50% humidity and 28°C. All the rats received standard pellet diet (Amrut rat feed, Pune) and water *ad libitum*.

Stone Induction
Experimental urolithiasis was induced by 0.75% ethylene glycol in drinking water for 28days *ad libitum*. After 28 days induction, the animals were used for the study.
Experimental design
In this experiment a total of 24 rats (12 urolithic rats, 12 normal rats) were used. The rats were divided into 4 groups of 6 rats each. Group I served as control. Group II served as drug control which are received \textit{A. lanata} aqueous suspension alone (2g/kg b.wt/dose/day/oral) for 28 days. Group III served as urolithic rats. Calcium oxalate urolithic rats were treated with \textit{A. lanata} aqueous suspension (2gm/kg b.wt/dose/day/oral) for 28 days (Group IV).

At the end of 28 days, the animals were anaesthetized with Pentobarbitol sodium (35mg/kg bodyweight, ip). Blood was drawn from the external jugular vein and serum was separated by centrifugation at 3000rpm. Liver and kidneys were excised and immersed in ice-cold physiological saline and blotted with filter paper. Known weight of tissues were homogenized in 0.1M tris-HCl buffer pH 7.4 containing 0.25M sucrose and used for biochemical estimations.

Estimations
The serum, liver and kidney homogenates were used to assay the contents of total lipids [8], total cholesterol [9], triglycerides [10] and phospholipids [11, 12]. In serum, HDL-cholesterol [9] was determined and the LDL cholesterol and VLDL were calculated using Friedwalds formula [13].

\begin{align*}
\text{VLDL-cholesterol} &= \frac{\text{Triglycerides}}{5} \\
\text{LDL-cholesterol} &= \text{Total cholesterol} - (\text{HDL+VLDL}) \\
\text{Atherogenic index} &= \frac{\text{LDL}}{\text{HDL}}.
\end{align*}

Statistical analysis
Values are mean±SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparison values of p<0.05 was considered to be significant. Statistical Package for Social Studies (SPSS) 7.5 version was used for this analysis.

Results
A significant (P<0.001) increase was absorbed in the levels of total lipids, total cholesterol and triglycerides in urolithic rats (Group III) (Table 1) where as the levels was minimized to near normal in \textit{A. lanata} post treated group (Group IV). \textit{A. lanata} treatment to normal rats (Group II) did not showed any alterations compared to control (Group I).

The phospholipids level was diminished in liver and kidney while elevated in serum of urolithic rats (Group III). The \textit{A. lanata} aqueous suspension was brought the levels in
liver and kidney and decreases the serum phospholipids levels (Group IV). Drug control rats did not showed significant changes (Group II) (Table 2).

Table 1. Effect of *A. lanata* on contents of total lipids, total cholesterol and triglycerides in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (mg/gm tissue)</th>
<th>Kidney (mg/gm tissue)</th>
<th>Serum (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>30.17 ± 1.80</td>
<td>20.14 ± 0.97</td>
<td>244.12 ± 7.12</td>
</tr>
<tr>
<td>II</td>
<td>30.12 ± 1.22</td>
<td>20.10 ± 1.25</td>
<td>242.14 ± 8.63</td>
</tr>
<tr>
<td>III</td>
<td>44.52 ± 1.43 a*</td>
<td>39.42 ± 1.20 a*</td>
<td>427.63 ± 8.53 a*</td>
</tr>
<tr>
<td>IV</td>
<td>33.24 ± 1.27 b*</td>
<td>22.54 ± 1.33 b*</td>
<td>262.75 ± 7.45 b*</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7.72 ± 0.34</td>
<td>6.67 ± 0.29</td>
<td>64.06 ± 3.23</td>
</tr>
<tr>
<td>II</td>
<td>7.64 ± 0.38</td>
<td>6.60 ± 0.35</td>
<td>60.74 ± 3.47</td>
</tr>
<tr>
<td>III</td>
<td>16.74 ± 0.53 a*</td>
<td>14.63 ± 0.47 a*</td>
<td>102.98 ± 3.36 a*</td>
</tr>
<tr>
<td>IV</td>
<td>9.45 ± 0.45 b*</td>
<td>6.94 ± 0.29 b*</td>
<td>68.86 ± 4.19 b*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>10.14 ± 0.47</td>
<td>8.11 ± 0.22</td>
<td>67.12 ± 3.56</td>
</tr>
<tr>
<td>II</td>
<td>10.12 ± 0.52</td>
<td>8.01 ± 0.37</td>
<td>63.17 ± 3.74</td>
</tr>
<tr>
<td>III</td>
<td>22.16 ± 0.17 a*</td>
<td>17.64 ± 0.49 a*</td>
<td>103.09 ± 3.86 a*</td>
</tr>
<tr>
<td>IV</td>
<td>11.66 ± 0.42 b*</td>
<td>10.09 ± 0.27 b*</td>
<td>75.13 ± 2.86 b*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group. 
a as compared with group I, b as compared with group III. *P<0.001.

Table 2. Effect of *A. lanata* on concentration of phospholipids in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (mg/gm tissue)</th>
<th>Kidney (mg/gm tissue)</th>
<th>Serum (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9.53 ± 0.46</td>
<td>4.47 ± 0.12</td>
<td>87.93 ± 4.65</td>
</tr>
<tr>
<td>II</td>
<td>9.57 ± 0.47</td>
<td>4.47 ± 0.14</td>
<td>86.12 ± 3.82</td>
</tr>
<tr>
<td>III</td>
<td>4.63 ± 0.36 a*</td>
<td>2.23 ± 0.10 a*</td>
<td>147.38 ± 4.66 a*</td>
</tr>
<tr>
<td>IV</td>
<td>9.36 ± 0.38 b*</td>
<td>4.29 ± 0.11 b*</td>
<td>89.13 ± 4.52 b*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group. 
a as compared with group I, b as compared with group III. *P<0.001.
In urolithic rats (Group III), the increased VLDL, LDL and HDL-cholesterols and LDL/HDL ratio were observed (Table 3). The increased levels were reduced to normalcy in A. lanata supplemented rats (Group IV). Treatment with A. lanata aqueous suspension alone to drug control (Group II) did not alter the levels compared to group I.

Table 3. Effect of A. lanata on concentrations of serum LDL, VLDL, HDL and LDL/HDL in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.82 ± 0.71</td>
<td>13.42 ± 0.53</td>
<td>35.82 ± 1.19</td>
<td>0.4137 ± 0.02</td>
</tr>
<tr>
<td>II</td>
<td>13.99 ± 0.74</td>
<td>12.63 ± 0.72</td>
<td>34.12 ± 1.82</td>
<td>0.3699 ± 0.04</td>
</tr>
<tr>
<td>III</td>
<td>34.64 ± 0.63 a*</td>
<td>20.62 ± 0.64 a*</td>
<td>47.72 ± 1.64 a*</td>
<td>0.7259 ± 0.04 a*</td>
</tr>
<tr>
<td>IV</td>
<td>18.09 ± 0.54 b*</td>
<td>15.03 ± 0.58 b*</td>
<td>5.74 ± 1.03 b*</td>
<td>0.5062 ± 0.02 b*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group. a as compared with group I, b as compared with group III. *P<0.001.

Discussion

Lipids are an important group of components involved in cellular function, making substantial contribution to the surface properties of the cell [14]. Lipids are seen to accumulate in blood and in both glomeruli and tubulo-interstitial tissue that facilitate oxidative damage [15]. In the present study, total lipids content was significantly elevated in serum, liver and kidney of ethylene glycol induced urolithic rats. The serum lipid profile is generally considered as a reflection of the tissue metabolism and the permeability of cell membrane to various ions, which inturn depends on lipid composition [16]. If has been reported that hyperlipidemia secondary to nephrosis can aggravate the primary renal disease [17]. Hyperlipidemia has an adverse effect on glomerular function in normal and experimental animals [18]. A. lanata aqueous suspension was reduced the lipids accumulation in ethylene glycol induced urolithic rats.

Increased plasma concentration of cholesterol is the major lipid abnormality in the nephritic syndrome in man. Serum total cholesterol was raised in the stone forming rats [19]. The present study also observed high concentration of total cholesterol in serum, liver and kidney of ethylene glycol induced urolithic rats. This might be attributed to suppress cholesterol-degrading enzymes such as cholesterol-7-alpha-hydroxylase activities, the key enzyme in the conversion of cholesterol to bile acids. The accumulated cholesterol was diminished on treatment with A. lanata aqueous suspension in ethylene glycol induced urolithic rats.
Stone formers showed elevated serum triglycerides concentration [19]. The present study also observed an increased level of triglycerides in serum, liver and kidney of ethylene glycol induced urolithic rats. This may be attributed to decreased activity of lipoprotein lipase. Lipoprotein lipase is involved in the uptake of triglyceride rich lipoproteins by the extra hepatic tissues. Decreased activity of lipoprotein lipase was probably indicated lower uptake of triglyceride rich lipoproteins causes hypertriglyceridemia [20]. *A. lanata* aqueous suspension was reduced the triglycerides level in serum, liver and kidney of ethylene glycol induced urolithic rats.

Phospholipids are essential structural components of animal cell membranes and cytoskeletons [21]. The present study observed an increase in the content of phospholipids in serum while decrease in liver and kidney of ethylene glycol induced urolithic rats. An analogous increase in serum concentration of phospholipids has been reported in glycollate-fed rats [22]. The decrease in the tissue phospholipids may perhaps be due to increased activity of phospholipase. On administration of *A. lanata* aqueous suspension, the altered phospholipids level was reverted to near normal in ethylene glycol induced urolithic rats.

Lipoproteins in plasma exist as an emulsion by associating with non-polar lipids, amphipathic lipids and proteins, to make water miscible compounds. In the present study ethylene glycol induced urolithic rats showed a significant increases in LDL-cholesterol, VLDL-cholesterol and a marginal elevation in HDL-cholesterol levels associated with an increase in the LDL/HDL ratio. Hyperlipidemia is often present in patients with a nephritic syndrome [23]. The lipoprotein pattern associated with the nephritic syndrome is quite variable. LDL and VLDL levels are often elevated [24]. Whether HDL levels are also altered is controversial. The elevated level of HDL might be attributed to increased activity of lecithin cholesterol acyl transferase (LCAT). Indeed HDL has been reported to be decreased, normal or even elevated [25]. The present results corroborate with the above reports. *A. lanata* aqueous suspension was brought the lipoproteins concentration to near normal in ethylene glycol induced urolithic rats. The ability of *A. lanata* with its ameliorating activity on ethylene glycol induced hyperlipidemic urolithiasis in rats has been highlighted.

References