Modulatory effect of *Plectranthus amboinicus* Lour. on ethylene glycol-induced nephrolithiasis in rats

Sir,

Nephrolithiasis is worldwide in distribution and affecting 2% of the world population. In order to find a herbal remedy for this disease, the present study was undertaken to evaluate the antilithiatic activity of the concentrated fresh juice of the leaves of *Plectranthus amboinicus* Lour. It is popularly known in English as Indian borage, syn. *Coleus amboinicus* (Family: Lamiaceae). Indians use it widely for various illnesses, including kidney stone. It is scientifically evaluated for its antibacterial and antioxidant activity.

The fresh plants of *P. amboinicus* were collected from the herbal garden, Annasilingam Deemed University, Coimbatore and authenticated at the botany department. About 500 g of fresh leaves were cut to small pieces and fresh juice was prepared adding water (30 ml), with the help of mixer. The fresh juice was filtered and concentrated to a dry mass by vacuum distillation; black dry residue was obtained (16 g). The LD$_{50}$ was done using OECD guideline for testing of chemicals revised draft guideline 423. The one tenth of the LD$_{50}$ 500 mg/kg was chosen as a dose for the further study.

Adult male albino rats of the Wistar strain, weighing between 150-200 g were used for this study. Animals were acclimatized and maintained at 24°C±2°C, 70% RH and 12h/12h light and dark cycle throughout the study. They were fed with standard pellet diet (Hindustan liver limited, Bangalore, India) and water ad libitum. The CPCSEA and the local ethical committee approved the studies. The animals were divided into three groups G1, G2, and G3 of six animals each. Following the method of Tamilselvan, nephrolithiasis was induced. Group 1(G1; control) was fed with ordinary drinking water. Group 2(G2; lithiotic control) were fed with 1% ethylene glycolated water for 35 days. Group 3(G3; test group) were fed with 1% ethylene glycolated water and were simultaneously administered, by gastric tube. 500 mg/kg of concentrated fresh juice of the leaves of the *Plectranthus amboinicus* Lour. for 35 days. On the 34th day all the animals were placed in metabolic cages and urine was collected for 24 hours and urine was analyzed for the presence of calcium, oxalate and total protein. Calcium, and total protein were estimated by an auto analyzer (Logotech s.r.l, tecno-168). Oxalates were analyzed by the method of Hodgkinson and Williams. Day 35 the animals were sacrificed, the kidneys were excised, washed with cold saline, fixed in 10% formalin solution and subjected to histopathological studies.

The results are expressed as mean ± SEM. The data were subjected to one-way ANOVA followed by Turkey-Kramer multiple comparison tests. P values <0.05 were considered as significant.

Histopathological studies clearly revealed that the tissue samples from the control group (G1) shows tubules with single epithelial lining along the margin and were of normal size. In G2 (lithiotic control), all the tubules showed the presence of crystals, there was marked dilatation of the tubules and total degeneration of the epithelial lining with infiltration of inflammatory cells into the interstitial space. In G3 (test group) the specimen showed characters similar to the control group. Urine analysis showed a significant elevation of calcium, oxalates and total proteins level in the lithiotic control group (G2), when compared to normal control. The test group (G3), showed a significant reduction in all the parameters almost comparable with normal control. The urine and histopathological results clearly revealed the antilithiatic activity of *P. amboinicus*, particularly of calcium oxalate origin. Further research is needed to explore the exact active principle(s) responsible for the antilithiatic activity and the mechanism of action.

**Table 1**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose</th>
<th>Urinary calcium Level (mg/dl)</th>
<th>Urinary oxalate Level (mg/dl)</th>
<th>Urinary total protein level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal saline)</td>
<td>10 ml/kg</td>
<td>5.4±0.06</td>
<td>0.5±0.06</td>
<td>200±5.16</td>
</tr>
<tr>
<td>Lithiitc control (ethylene glycol)</td>
<td>1% ethylene glycolated water</td>
<td>11.2±0.12</td>
<td>1.5±0.07</td>
<td>280±5.77</td>
</tr>
<tr>
<td><em>P. amboinicus</em></td>
<td>500 mg/kg</td>
<td>5.6±0.58**</td>
<td>0.8±0.06**</td>
<td>200±8.56**</td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td>F</td>
<td>1613</td>
<td>65</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>df</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n=6 in each group. **P<0.001 when compared to lithiotic control.

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Cigarette smoke condensate reduces the detoxifying capabilities of rat lens

Sir,

Epidemiological studies indicate that cigarette smoking increases the risk of developing cataract while cessation of smoking reduces the risk.\(^1\) It is postulated that the complex mixture of trace metals, polycyclic aromatic hydrocarbons and nitro compounds in cigarette smoke act as pro-oxidants that exert oxidative damage to lens and possibly initiate cataractogenesis.\(^2\) Thus, this may be a crucial mechanism of cataractogenesis in smokers. However, very few studies are available on the effects of cigarette smoking on the endogenous antioxidant mechanisms. Therefore, we conducted a preliminary study to record the effect of cigarette smoke on the detoxifying mechanisms of organ-cultured lens and validate a quick in vitro screening model for potential anti-cataract agents.

Adult Wistar rats (150-200 g) of either sex, were used in accordance with institutional ethical guidelines. They were sacrificed using anaesthetic ether and lens dissected (weight range of 0.02-0.04 g) for the present study. Cigarette smoke condensate (CSC) was prepared according to the method of Shalini et al.\(^3\) A leak proof system was improvised that burnt filter tipped cigarettes, in a steady stream of air. The smoke content from six cigarettes (Wills, ITC Ltd) was trapped by bubbling through distilled water (24 ml) containing dimethyl sulfoxide (DMSO, 50 µl). This was filtered through glasswool and centrifuged at 9000 rpm for 5 min to give CSC. The CSC was standardized by ensuring that the optical density (OD) was 0.6 at an absorption maximum of 270 nm (Beckman Spectrophotometer). A 1:1 dilution of CSC with Dulbecco’s Modified Eagle’s Medium (DMEM) was prepared and the capsulated rat lenses were incubated in CSC with Dulbecco’s Modified Eagle’s Medium (DMEM) was prepared and the capsulated rat lenses were incubated in CSC with Dulbecco’s Modified Eagle’s Medium (DMEM) was prepared and the capsulated rat lenses were incubated in CSC with Dulbecco’s Modified Eagle’s Medium (DMEM). The control rat lens was incubated as such with DMEM. Ambient light and temperature conditions (37° C) were maintained. Rat lenses were homogenized in 1 ml of 5% trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 15 min (Eltek refrigerated centrifuge, RC 4100 D). The as-
say procedure of Ellman was followed for estimation of reduced glutathione (GSH).\(^4\) The lens was homogenized in phosphate buffered saline (PBS) to prepare a 10% homogenate. Glutathione-S-transferase (GST) activity in the homogenate was estimated according to the method of Habig, et al.\(^5\) The kinetic profile was read at 340 nm for every 30 s up to 180 s. To elucidate the effect of various concentrations of CSC on GST activity in homogenate, increasing volume of CSC (10, 20, 40 and 80 µl) were added to normal. The enzyme catalyzes the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with GSH to form a complex and the product is measured spectrophotometrically. The result is expressed as nmol of CDNB conjugated/min/mg protein. The protein levels of rat lens were estimated by the standard Lowry’s method.\(^6\)

Two tailed unpaired t-test was applied to compare the level of significance between the means of treated and control group for corresponding time points. In GST estimation each concentration of CSC was compared to normal levels and \(P<0.05\) was considered statistically significant.

There was a significant fall in normal GSH level in rat lens (5.98 ± 0.41 µl) as the duration of CSC exposure was increased from 0.5 to 2 h (0.49 ± 0.10 µl) (Figure 1). In comparison to normal activity of GST in rat lenses, there was a significant fall in its activity with 20, 40 and 80 µl of CSC (Figure 2).

Epidemiological studies, in vivo studies alongwith in vitro studies, provide a correlation between cigarette smoking and cataractogenesis.\(^7\) As the lens is an immunologically isolated

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References


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Figure 1: GSH levels in normal and CSC exposed rat lens. Data represented as mean±SEM. *\(P<0.05\), **\(P<0.001\) significantly different from control group; n=6

Figure 2: GST activity in normal and CSC exposed rat lens. Each point is the representation of mean±SEM. *\(P<0.05\), **\(P<0.01\) significantly different from the normal value; n=6