Nephytoxic Activity of Methanolic Extract of *Sapindus Laurifolius* in Rats

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**ABSTRACT**

The *Sapindus laurifolius* methanolic leaf extract was evaluated for phytochemical analysis, acute and repeated dose 28-day oral toxicity study in Wistar rats. The phytochemical analysis revealed the presence of saponins, flavanoids and glycosides. In acute toxicity study, *S. laurifolius* leaf extract was found to be non toxic up to 2 g/kg. In repeated dose 28-day oral toxicity, leaf extract administered at the doses 50, 200 and 800 mg/kg and limit dose of 1000 mg/kg. There was a significant (P< 0.05) increase in AST, ALT, BUN and serum creatinine in rats administered with high dose of leaf extract. The histopathological changes confined to liver, kidney and intestine, which revealed mild to moderate hepatotoxicity, severe nephrotoxicity and increased goblet cell activity. The changes were found to correlate with increased dose of leaf extract. Satellite group administered with 800 mg/kg of leaf extract revealed damage to the kidney and liver continued even after the treatment has been stopped or animals may require still longer duration for recovery.

**Key words:** Sapindus laurifolius leaf extract, Wistar Rats, Phytochemical analysis, Saponin, Hepatotoxicity, Nephrotoxicity.

**INTRODUCTION**

Plants are commonly used for therapeutic purpose in human beings and animals. Some of the plants may be toxic to the animals which may result in morbidity or mortality. *Sapindus laurifolius* is one such plant used for fruits and the leaves which will be discarded. Cattle may have access to such leaves and suffer from the toxicity.

Plant *Sapindus laurifolius* also called as Indian soap nut or Reetha, belongs to the family Sapindaceae. Although it has so many medicinal properties, it was reported that consumption of *S. laurifolius* leaves caused toxicity in cattle (Shridhar and Narayana, 2004). Hence the present work was aimed to study the toxicological effect of the *S. laurifolius* leaves in a systematic way using rat as model.

**MATERIALS AND METHODS**

Healthy Wistar albino rats aged around 8-9 weeks weighing 160±10 g were procured from Central Animal Facility, Indian Institute of Science, Bangalore. The experiment was carried out for a period of 45 days with prior permission from Institutional Animal Ethics Committee.

Phytochemical analysis of the *Sapindus laurifolius* leaf extract was carried out using in High performance thin layer chromatography (HPTLC) technique (Wagner et al., 1984).

**Toxicity study:** Toxicity study was conducted in Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, Acute Oral Toxicity (OECD 423) and repeated dose 28-day oral toxicity study (OECD 407).

**Acute toxicity study:** Animals were divided into different group of three animals each of either sex used for determining LD50 cutoff value. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

**Repeated dose 28-day oral toxicity study:** Animals of either sex were divided into five groups
of six animals used for the study. The leaf extract of S. laurifolius was administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation for 28 days. Limit test at one dose level of 1000 mg/kg body weight/day was conducted.

The rats were divided into five different groups- Group I - normal control (distilled water), Group II- low dose (50 mg/kg), Group III - Medium dose (200 mg/kg), Group IV - High dose (800 mg/kg) and Group V - Satellite group (800 mg/kg). The satellite group rats were maintained for further two weeks after the 28 day period without administration of test sample to observe reversibility of any toxicity. All the animals were observed for health condition, morbidity and mortality twice a day throughout the study period of 28 days considering the period of anticipated effects after dosing.

**Serum biochemical parameters:** Blood samples were collected on day 0, 14 and 28 during the study period, the serum biochemical parameters AST, ALT, BUN and creatinine were determined by using the commercially available standard kits with the aid of clinical chemistry analyzer.

**Pathology:** At the end of study period, all the animals were humanely sacrificed and subjected to detailed gross necropsy and histopathology.

**Statistical analysis:** The data obtained were analyzed by using two-way ANOVA, Bonferroni post-test. Mean values and standard error of mean were calculated and expressed as Mean± SEM.

**RESULTS AND DISCUSSION**

The Sapindus laurifolius leaf extract was found positive for saponins, glycosides and flavonoids. Among the phytochemical constituents, saponins are the most important and it might be responsible for the biological activity of the S. laurifolius leaves which was further supported by the reports of Kasai et al. (1986) and Kishore et al. (2011), who isolated the saponins in higher concentration from the S. laurifolius leaf extract.

**Acute oral toxicity study:** There were no deaths and clinical signs of toxicity in any of the test groups within 24 h and for a period of 14 days after the administration of S. laurifolius leaf extract. S. laurifolius leaf extract was found to be non toxic up to the dose of more than 2 g/kg and it is categorized in unclassified group under Globally Harmonised System of Classification. These observations are similar to the findings of Jeyabalan and Palayan (2009) and Kishore et al. (2011), who identified that the methanolic extract of S. laurifolius was non-toxic up to the dose of 2 g/kg body weight in Albino mice and rats respectively.

**Repeated dose 28-day oral toxicity study:** The determination of oral toxicity using repeated doses was carried out after initial information on toxicity was obtained by acute oral toxicity testing. There were no deaths but rats administered with high dose (800 mg/kg) and limit dose (1000 mg/kg) of leaf extract exhibited clinical signs such as depression, salivation, diarrhea and decreased body weight gain. These clinical signs of toxicity are in accordance with the study of Witthawaskul et al. (2003) who observed similar toxic manifestation in rats feeding with saponin mixture.

In the present study, leaf extract at the dose of 800 mg/kg (Group IV & Group V) and limit dose group (1000 mg/kg) showed significant (P< 0.05) increase in ALT and AST (Table 1). These findings are in correlation with the results of Diwan et al. (2000), who identified saponin solution at the dose rate of 600mg/kg caused significant increase in the level of ALT and AST in the mice.

The significant (P< 0.01) increase in concentration of BUN and serum creatinine

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I Control (50mg/kg)</th>
<th>Group II Low dose (50mg/kg)</th>
<th>Group III Medium dose (200mg/kg)</th>
<th>Group IV High dose (800mg/kg)</th>
<th>Group V Satellite (800mg/kg)</th>
<th>Limit dose (1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.50±2.97</td>
<td>26.22±2.39</td>
<td>27.60±2.89</td>
<td>27.82±5.53</td>
<td>26.52±2.36</td>
<td>26.77±1.65</td>
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<td>14</td>
<td>27.68±2.76</td>
<td>28.10±1.76</td>
<td>29.30±1.73</td>
<td>29.27±1.89</td>
<td>28.53±1.24</td>
<td>34.52±1.53*</td>
</tr>
<tr>
<td>28</td>
<td>27.35±2.16</td>
<td>28.80±1.25</td>
<td>28.65±1.21</td>
<td>35.30±3.99</td>
<td>35.63±2.18</td>
<td>36.53±0.72**</td>
</tr>
<tr>
<td>42</td>
<td>28.88±2.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36.12±0.88*</td>
<td>-</td>
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</table>

**TABLE 1:** Effect of S. laurifolius leaf extract on alanine aminotransferase ALT (U/L) in rats in repeated dose 28 day oral toxicity study.
observed in 800 mg/kg and 1000 mg/kg leaf extract treated groups (Table 3, 4) indicated the possible role of toxins in causing kidney damage. Wisloff et al. (2008) showed the toxic effects of saponin containing plants in lambs, serum biochemistry revealed increased concentration of serum creatinine and BUN.

There was significant (P<0.05) difference between the satellite group and control satellite group ALT, BUN and serum creatinine values at day 42, this indicated the injury to the kidney was continued even after the administration of S. laurifolius leaf extract was stopped or kidney damage caused by the S. laurifolius leaf extract in the present study might take still longer time to get recovered or it might be irreversible in nature.

**Gross and histopathological examination:** At necropsy none of the treated and control rats showed any gross pathological lesions, varying degree of histopathological lesions in liver, kidney and intestine in all the treated groups except in low dose group where, organs retained their normal architecture. In medium and high dose group, liver showed granular appearance and swollen hepatocytes, congestion of vessels, decreased sinusoidal spaces, vacuolated cytoplasm and focal necrosis of individual hepatocytes (Fig 1). Kidney revealed haemorrhages and distension of tubular and glomerular epithelium, desquamation of tubular epithelium, hypercellularity of tubular and inter tubular spaces (Fig 2). In intestine there was increased goblet cell activity, broadening of villus structure, infiltration of inflammatory cells in lamina propria and submucosa (Fig 3).

The histopathological changes observed in the present study had similarities to observations in earlier experiments with dosing of plant leaf extract containing saponin to mice and lambs (Diwan et

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**TABLE 2:** Effect of S. laurifolius leaf extract on aspartate aminotransferase AST (U/L) in rats in repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I Control</th>
<th>Group II Low dose (50mg/kg)</th>
<th>Group III Medium dose (200mg/kg)</th>
<th>Group IV High dose (800mg/kg)</th>
<th>Group V Satellite (800mg/kg)</th>
<th>Limit dose (1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>56.48±2.17</td>
<td>55.13±2.47</td>
<td>57.78±1.53</td>
<td>56.78±2.27</td>
<td>55.25±2.13</td>
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<tr>
<td>14</td>
<td>57.67±1.98</td>
<td>56.97±2.06</td>
<td>57.33±2.97</td>
<td>56.83±1.49</td>
<td>58.48±1.54</td>
<td>59.75±1.04</td>
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<tr>
<td>28</td>
<td>56.53±1.73</td>
<td>57.05±2.31</td>
<td>56.85±2.17</td>
<td>64.15±1.50**</td>
<td>64.07±1.66**</td>
<td>64.58±1.52**</td>
</tr>
<tr>
<td>42</td>
<td>57.32±1.67</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>58.57±0.53</td>
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</tr>
</tbody>
</table>

**TABLE 3:** Effect of S. laurifolius leaf extract on blood urea nitrogen BUN (mg/dl) in rats in repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I Control</th>
<th>Group II Low dose (50mg/kg)</th>
<th>Group III Medium dose (200mg/kg)</th>
<th>Group IV High dose (800mg/kg)</th>
<th>Group V Satellite (800mg/kg)</th>
<th>Limit dose (1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.90±1.61</td>
<td>20.38±1.63</td>
<td>21.15±0.26</td>
<td>19.10±1.39</td>
<td>17.42±1.57</td>
<td>19.00±1.08</td>
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<tr>
<td>14</td>
<td>18.85±0.95</td>
<td>20.40±0.66</td>
<td>21.07±0.52</td>
<td>23.95±0.83**</td>
<td>23.58±1.02**</td>
<td>23.25±0.68*</td>
</tr>
<tr>
<td>28</td>
<td>18.98±1.66</td>
<td>18.78±0.94</td>
<td>20.08±1.23</td>
<td>24.63±1.27**</td>
<td>23.98±1.02**</td>
<td>27.25±0.42**</td>
</tr>
<tr>
<td>42</td>
<td>20.40±0.66</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24.62±0.51*</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 4:** Effect of S. laurifolius leaf extract on serum creatinine (mg/dl) in rats in repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I Control</th>
<th>Group II Low dose (50mg/kg)</th>
<th>Group III Medium dose (200mg/kg)</th>
<th>Group IV High dose (800mg/kg)</th>
<th>Group V Satellite (800mg/kg)</th>
<th>Limit dose (1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.73±0.02</td>
<td>0.73±0.02</td>
<td>0.73±0.03</td>
<td>0.71±0.03</td>
<td>0.75±0.03</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>14</td>
<td>0.76±0.03</td>
<td>0.79±0.03</td>
<td>0.78±0.02</td>
<td>0.94±0.04*</td>
<td>0.94±0.03*</td>
<td>0.98±0.09**</td>
</tr>
<tr>
<td>28</td>
<td>0.78±0.03</td>
<td>0.83±0.04</td>
<td>0.90±0.03*</td>
<td>1.14±0.09**</td>
<td>1.13±0.11**</td>
<td>1.08±0.08**</td>
</tr>
<tr>
<td>42</td>
<td>0.77±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.95±0.04*</td>
<td>-</td>
</tr>
</tbody>
</table>

Compared with the control group values of respective days
Values are Mean±SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 42 pertain to satellite
FIG 1: Section of liver from rat treated with 800 mg/kg dose of S. laurifolius leaf extract showing swollen and granular hepatocytes, congestion, prominent biliary hyperplasia in periportal region in subacute toxicity study. (H&E 200).

FIG 2: Section of kidney from rat treated with 800 mg/kg dose of S. laurifolius leaf extract showing desquamation of tubular epithelial cells, tubular necrosis in subacute toxicity study. (H&E 200).

Thus, the possible role of plant S. laurifolius in causing renal damage might be due to membrane permeabilising effect which might be detrimental to the renal epithelium as well as intestinal epithelial cells. Active ingredients saponins present in the plant also inhibits the active tubular transport and thus affect the kidney, other active ingredients glycosides and flavonoids also causes hepatic and renal damage. (Cheeke, 1998; Wisloff et al., 2008).

CONCLUSION
The acute oral toxicity study of Sapindus laurifolius methanolic leaf extract in rats resulted in no toxicity even at the highest dose. The repeated 28-day oral toxicity study revealed mild to moderate hepatotoxicity, severe nephrotoxicity and intestinal damage. The changes were found to correlate with the increased dose of methanolic leaf extract.

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REFERENCES


