PHYTOCHEMICAL COMPOSITION AND TOXICOLOGICAL EFFECT OF SAPINDUS LAURIFOLIUS LEAF EXTRACT IN WISTAR RATS

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Abstract: Study was aimed to evaluate the phytochemical composition and toxicological effect of the Sapindus laurifolius methanolic leaf extract using Wistar albino rats as model animal. The phytochemical analysis was performed using high performance thin layer chromatography (HPTLC). Toxicity studies were conducted as per the guidelines of organization for economic co-operation and development (OECD 423 acute toxic class method) for testing of chemicals. In repeated 28-day oral toxicity study (OECD 407), leaf extract was administered at the dose of 50, 200 and 800 mg/kg fixing the limit dose of 1000 mg/kg. The phytochemical analysis revealed the presence of saponins, flavanoids, glycosides and bitter principles. In acute toxicity study, the LD50 cut-off values were found to be more than 2g/kg in leaf extract. In repeated 28 day oral toxicity study, significant (P<0.05) increase in AST, ALT, BUN, creatinine and total protein was noticed in the group administered with 800 mg/kg of plant extract. The histopathological changes were confined to liver, kidney and intestine revealing mild to moderate hepatotoxicity, severe nephrotoxicity and increased goblet cell activity with intestinal damage. The changes were correlating with increased dose of leaf extract. Thus S. laurifolius leaf extract was found to be non toxic in acute toxicity study as compared to repeated 28 day oral toxicity study, where in the plant extract was toxic in higher doses applied for longer duration.

Key words: Sapindus laurifolius, HPTLC, Hepatotoxicity, Nephrotoxicity.

INTRODUCTION

India has an ancient heritage of traditional medicine. Man and animals mostly depend on plant kingdom for their food. Some of the plants also have medicinal properties, mostly beneficial, but a few beside are toxic to animals. In present investigation one such plant viz., Sapindus laurifolius (Indian soap nut) belonging to the family Sapindaceae has studied for its acute toxicity to animals inspite of the fact that the plant also have important medicinal applications such as antibacterial, exfoliant, expectorant, emetic properties, clears the skin problems like eczema, psoriasis and itchy skin etc. [1,2]. Fruits and leaves of S. laurifolius were acrid bitter, emetic, astringent, anthelmintic, abortifacient and tonic [3]. Nevertheless, consumption of fruits and leaves (rich in saponin content [4]) of the plant causes toxicity to cattle, like severe diarrhoea, excitation and finally died [5]. The present work was aimed to study the phytochemical composition of the S. laurifolius leaf extract and examine the existing acute and sub-acute toxicity in experimental animals.

MATERIALS AND METHODS

Plant extract: S. laurifolius fresh leaves were collected from Western Ghats of Karnataka and
dried under the shade. Leaves were crushed into powder and extracted in methanol in Soxhelt extractor. The methanolic extract was dried to obtain semisolid content, which was kept in refrigerator for further use. Phytochemical analysis of extract was carried out using high performance thin layer chromatography (HPTLC) technique [6].

**Experimental design:** Healthy Wistar albino rats of either sex, aged around 8-9 weeks weighing 160±20 g were obtained from the stock of the animal house, Indian Institute of Sciences, Bangalore. Animals were acclimatized to the laboratory conditions for 7 days prior to the study and maintained on normal diet and water *ad libitum*. Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC).

**Acute toxicity study:** Acute toxicity was determined according to the OECD guideline No.423. The rats were kept fasting for overnight providing and methanolic extract of *S. laurifolius* was administered at a dose rate of 5, 50, 300 and 2000 mg/kg body weight. Food was withheld for further 3-4 hrs and observed once in 30 min during the first 24 hrs and daily thereafter, for a period of 14 days for any mortality [7].

**Repeated dose 28-day oral toxicity study:** The study was conducted according to the OECD guideline No.407. Wistar albino rats were divided into five groups (n=6) of either sex were used for the study. Group I served as control, which was gavaged with distilled water whereas group II, III and IV rats were given leaf extract at the dose rate of 50, 200, 800 mg/kg daily respectively for 28 days. Group V rats were administered with 800 mg/kg of leaf extract for 28 days and discontinued until 42nd day to observe any reversibility in toxicity. Limit test at one dose level of at least 1000 mg/kg was conducted up to 28 days. All through the experiment, rats were observed for the clinical signs of toxicity, morbidity and mortality.

**Haematology and clinical biochemistry:** The blood samples were obtained by retro-orbital plexus puncture method on day 0, 14 and 28 and the fresh blood was used to estimate haematological parameters like total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb) and packed cell volume (PCV). In clinical biochemistry, serum was used to estimate alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT) and blood urea nitrogen (BUN) concentrations using biochemical analyzer and commercially available diagnostic kits.

**Gross and histopathological studies:** Rats were sacrificed at the end of the study. Organs were weighed and representative tissue samples of various organs were collected in 10% normal buffer formalin solution (NBF) and were subjected to histopathology [8].

**Statistical analysis:** The data obtained were analyzed on graph pad prism 5.01 software and expressed as Mean ± SEM. The Statistical analysis was performed by using two-way ANOVA, Bonferroni post-test as per the standard procedures [9] to compare the treated and control groups.

**RESULTS**

The Phytochemical analysis of *S. laurifolius* leaf extract was found to be positive for saponins, glycosides, flavonoids and bitter principles in High performance thin layer chromatography.

**Acute oral toxicity study:** There were no deaths and clinical signs of toxicity in any of the test groups within 24 h after the administration of extract. The treated rats were kept for observation for a period of 14 days. No mortality and clinical signs of toxicity were observed in any of the groups in a given dose and duration. All the animals were sacrificed on day 15. Detailed post mortem examination was carried out and the experimental animals did not reveal any gross pathological changes. The histological examination of various organs of both male and female rats revealed normal architecture of all the organs.

**Repeated dose 28-day oral toxicity study:** The study was carried out after initial information obtained by acute oral toxicity testing. There were no deaths but animals exhibited clinical signs such as depression, weakness, salivation, diarrhoea and decreased body weight in rats administered with 800 and 1000 mg/kg (limit dose group) of *S. laurifolius* leaf extract.

During the analysis of bio-chemical parameters, there was a significant (P<0.01) increase in serum ALT and AST concentrations in rats treated with 800 and 1000 mg/kg on day 14 and 28 (Table1, 2), also a
Table 1: Effect of *S. laurifolius* leaf extract on alanine aminotransferase ALT (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 (1000mg/kg)</th>
<th>Limit dose (1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.50±2.97</td>
<td>26.22±2.89</td>
<td>27.60±2.89</td>
<td>27.82±2.53</td>
<td>26.52±2.36</td>
<td>26.77±1.65</td>
</tr>
<tr>
<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>28.34±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±1.39**</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>
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</tr>
</tbody>
</table>

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>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>
</tr>
<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 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>0.73±0.02</td>
<td>0.73±0.02</td>
<td>0.74±0.03</td>
<td>0.71±0.03</td>
<td>0.75±0.03</td>
<td>0.72±0.02</td>
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<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>

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>0.74±0.02</td>
<td>0.73±0.02</td>
<td>0.74±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>

Significant (P<0.05) increase in the serum total protein level on the day 28. The serum BUN and creatinine concentration increased significantly (P<0.001) from day 14 to 28 in rats administered with 800 and 1000 mg/kg of leaf extract (Tables 3,4).

There was significant (P<0.05) increase of ALT, BUN and creatinine levels in group V animals as compared to control (Tables 1,3,4). This indicated the injury in kidney and liver was continued even after the administration of *S. laurifolius* leaf extract was stopped. This indicates that kidney damage caused by intoxication might take still longer time to get recovered or it might be irreversible in nature.

Haemopoetic parameters such as TEC, TLC, Hb and PCV values were found to be non significant in any of the rat groups administered with *S. laurifolius* leaf extract. This implied that *S. laurifolius* do not affect haemopoetic system even at the high dose level.

In the histopathological study, all the rats administered with leaf extract showed varying degree of micro lesions in liver, kidney and intestine, where as remaining organs showed normal architecture. Liver showed swollen hepatocytes, vacuolar degeneration, congestion of central veins, necrosis of individual hepatocytes, prominent bile duct hyperplasia compare to histological findings of normal control group animals (Figs.1,2). Kidney showed distension of tubular and glomerular epithelium, desquamation of tubular epithelium, intertubular and glomerular hemorrhagic areas, vacuolations of the glomerular...
Fig. 1: Section of liver showing normal architecture in control group rat in repeated 28 days oral toxicity study (H&E 200).

Fig. 2: Section of liver from rat treated with 800 mg/kg dose of *S. laurifolius* leaf extract showing swollen and granular hepatocytes, congestion, and prominent biliary hyperplasia in periportal region in subacute toxicity study (H&E 200).

Fig. 3: Section of kidney showing normal architecture in control group rat in repeated 28 days oral toxicity study (H&E 200).

Fig. 4: 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).

Fig. 5: Section of intestine from rat treated with 800 mg/kg dose of *S. laurifolius* leaf extract showing increased goblet cell activity, destruction and shortening of the villi in sub acute toxicity study (H&E 100).
epithelium, hypercellularity of tubular and inter tubular spaces as compare to control animals (Figs. 3,4). In intestine, there was increased goblet cells, broadening of villus structure, infiltration of inflammatory cells in lamina propria and submucosa indicating intestinal inflammation in rats administered with high dose of *S. laurifolius* leaf extract (Fig. 5).

**DISCUSSION**

The *S. laurifolius* leaf extract consist of saponins, glycosides, flavonoids and bitter principles. Among these saponin is one of the most important biologically active constituents [10-12]. In earlier investigations Kishore et al. [12] and Jeyabalana and Palayan [13] found that *Sapindus* did not showed any signs of toxicity and minimum lethal dose was applied greater than 2 g/kg given orally in Albino mice and rats. The clinical symptoms observed in repeated 28 days doses of extract reveal weakness, anorexia and weight loss which is also observed by Witthawaskul et al. [14].

The elevated serum AST and ALT concentration and increased total protein compared to control group animals shows liver damage as evident in histopathological study. Changes in these constituents due to saponins present in the leaf extract is also reported by Lakmichi et al. [15]. This increase may be due to their release from damaged liver cells. Ozer et al. [16] claimed that altered membrane permeability or liver cell necrosis and cytosol leakage increase the enzymes in serum. Increased enzyme synthesis or decreased catabolism, may also result the release of intracellular enzymes into the blood stream [17]. The altered level of AST, ALT, serum creatinine and BUN was further supported by the gross and histopathological lesions in high dose group.

The elevated serum creatinine and urea nitrogen indicates the possible role of *S. laurifolius* leaf extract causing kidney damage as also reported in lambs [20]. The histopathological changes observed in repeated 28 days doses of extract correlate with the increased dose of saponin. Thus saponin present in plant *S. laurifolius* might be responsible for the changes in liver, kidney and intestine. Saponin present in the plant affects absorptive cells of the intestinal mucosa, especially those near the tips of the villi with severe intestinal inflammation and increased goblet cell activity [22]. It is concluded from over all study that *S. laurifolius* leaves are toxic in high doses if consumed for long duration, therefore, some suitable therapy is required. The work in this direction is in progress.

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