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# Toxicity studies of *Sapindus laurifolius* methanolic leaf extract in Wistar rats

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## Abstract

Studies were aimed to evaluate the phytochemical composition of the *Sapindus laurifolius* leaves and toxicological effect of the *Sapindus laurifolius* leaf extract using Wistar albino rats as a model animal. The phytochemical analysis was performed using High Performance Thin Layer Chromatography (HPTLC). In toxicity studies, the acute oral toxicity study was 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 dose 28-day oral toxicity study (OECD 407), leaf extract administered at the dose of 50, 200 and 800 mg/kg and limit dose of 1000 mg/kg. The phytochemical analysis revealed the presence of saponins, flavanoids, glycosides and bitter principles. In acute toxicity study, the $LD_{50}$ cut-off values were found to be more than 2g/kg in leaf extract. In repeated dose 28-day oral toxicity, significant ($P<0.05$) increase in AST, ALT, BUN and creatinine, significant ($P<0.05$) increase in total protein was noticed. The histopathological changes confined to liver, kidney and intestine, revealed mild to moderate hepatotoxicity, severe nephrotoxicity and increased goblet cell activity. The changes were found to correlate with increased dose of leaf extract. Thus the phytochemical analysis of *S. laurifolius* revealed the presence of saponins, glycosides, flavonoids and bitter principles.The acute oral toxicity study of *S. laurifolius* methanolic leaf extract in rats resulted in no toxicity even at the highest dose, in repeated 28-day oral toxicity study revealed mild to moderate hepatotoxicity, severe nephrotoxicity and intestinal damage.

**Key words:** *Sapindus laurifolius*, phytochemical analysis, saponins, hepatotoxicity, nephrotoxicity

## Introduction

India has an ancient heritage of traditional medicine. Man and animals mostly depend on vegetable kingdom for their food. Many plants are categorized as poisonous plants. Plants by their metabolic activities besides being the source of feed and fodder also elaborate other substances, which are important from medicinal and toxicological point of view.

Plants are commonly used for therapeutic purpose in human beings and animals. However, their toxic feature has not been studied. One such plant *Sapindus laurifolius* (Indian soap nut)

belongs to the family Sapindaceae has antibacterial, exfoliant, expectorant, emetic properties, clears the skin problems like eczema, psoriasis, itchy skin (Saxena *et al.,* 2004; Shiau *et al.,* 2009), fruits and leaves of *Sapindus laurifolia* were acrid bitter, emetic, astringent, anthelmintic, abortifacient and tonic (Vaidyaratnam, 1996). Although it has so many medicinal properties, it was reported that consumption of fruits and leaves of the plant *Sapindus laurifolius* caused toxicity in cattle, there was severe diarrhoea, which was attributed to the saponin content of the plant leaf (Narayana, 2003). During the disease investigation process, it was noticed that the cattle that consumed the fresh leaves of the plant *Sapindus laurifolius* exhibited the clinical signs *viz*., excitation, diarrhoea and death (Shridhar, 2004). Hence the present work was aimed to study the phytochemical composition of the *Sapindus laurifolius* leaf extract, to conduct acute and sub-acute toxicity study of the *Sapindus laurifolius* in rats, to correlate the findings with histopathological and biochemical studies.

## Materials and Methods

### *Plant Extract*

*S. laurifolius* fresh leaves were collected from Western Ghats of Karnataka State, leaves were dried and methanolic extract of dried leaves obtained from rotary flash evaporator was used to conduct the experiment. Phytochemical analysis of the *S. laurifolius* leaf extract was carried out using High performance thin layer chromatography (HPTLC) technique (Wagner *et al.,* 1984).

### *Experimental design*

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

### *Acute toxicity study*

Acute toxicity was determined according to the OECD guidelines No.423. Female Wistar rats (n=3 per step) were selected by random sampling technique. The rats were kept fasting for overnight providing with water *ad libitum*. The 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 (Diener *et al.,* 1995).

### *Repeated dose 28-day oral toxicity study*

Repeated dose 28-day oral toxicity was determined according to the OECD guidelines No.407. Wistar 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 were gavaged with *S. laurifolius* methanolic leaf extract at the dose level of 50, 200, 800 mg/kg daily respectively for 28 days. Satellite group rats (group V) administered with 800 mg/kg of leaf extract for 28 days and discontinued until 42$^{nd}$ day to observe any reversibility in toxicity. Limit test at one dose level of at least 1000 mg/kg body weight/day was conducted. The rats were weighed individually at the beginning of the study and at weekly interval till day 28. All the rats were observed daily 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 Leucocyte Count (TLC), haemoglobin (Hb) and Packed Cell Volume (PCV). In clinical biochemistry, serum was used to estimate serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT) and blood urea nitrogen (BUN) 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 (Luna, 1968).

## 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 (Stee and Torrie, 1996) to compare the treated and control groups.

## Results

The Phytochemical analysis of *S. laurifolius* leaf extract was found 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 *S. laurifolius* leaf extract. The treated groups were kept for observation for a period of 14 days. No mortality and clinical signs of toxicity were observed in any of the tested groups in a given dose and duration.

All the tested groups of animals were sacrificed on day 15. Detailed post mortem examination was carried out and all 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 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 animals exhibited clinical signs such as depression, weakness, salivation, diarrhoea and decreased body weight gain in 800 mg/kg and limit dose group (1000 mg/kg) rats administered with methanolic leaf extract of *S. laurifolius.*

In the present study, there was a significant increase (P<0.01) in serum ALT and AST concentrations in the serum samples of 800 and 1000 mg/kg treated groups on day 14 and 28 (Table1, 2) and significant (P<0.05) increase in the serum total protein level on the day 28.The serum urea nitrogen and serum creatinine concentration in rats increased significantly (P<0.001) from day 14 to 28 in groups treated with 800 and 1000 mg/kg of leaf extract (Table 3, 4).

**Table 1: Effect of *S. laurifolius* leaf extract on alanine aminotransferase ALT (U/L) in rats in repeated dose 28 day oral toxicity study**

| Day | Group I Control | Group II Low dose (50mg/kg) | Group III Medium dose (200mg/kg) | Group IV High dose (800mg/kg) | Group V Satellite (800mg/kg) | Limit dose (1000mg/kg) |
|---|---|---|---|---|---|---|
| 0 | 27.50±2.97 | 26.22±2.39 | 27.60±2.89 | 27.82±2.53 | 26.52±2.36 | 26.77±1.65 |
| 14 | 27.68±2.76 | 28.10±1.76 | 29.30±1.73 | 29.27±1.89 | 28.53±1.24 | 34.52±1.53* |
| 28 | 27.35±2.16 | 28.80±1.25 | 28.65±1.21 | 35.30±1.39** | 35.63±2.18** | 36.53±0.72** |
| 42 | 28.88±2.19 | - | - | - | 36.12±0.88* | - |

Compared with the control group values of respective days
Values are Mean±SEM, * P<0.05, ** P<0.01, n=6, the values on day 42 pertain to satellite group

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

| Day | Group I Control | Group II Low dose (50mg/kg) | Group III Medium dose (200mg/kg) | Group IV High dose (800mg/kg) | Group V Satellite (800mg/kg) | Limit dose (1000mg/kg) |
|---|---|---|---|---|---|---|
| 0 | 56.48±2.17 | 55.13±2.47 | 57.78±1.53 | 56.78±2.27 | 55.25±2.13 | 54.13±1.13 |
| 14 | 57.67±1.98 | 56.97±2.06 | 57.33±2.97 | 56.83±1.49 | 58.48±1.54 | 59.75±1.04 |
| 28 | 56.53±1.73 | 57.05±2.31 | 56.85±2.17 | 64.15±1.50** | 64.07±1.66** | 64.58±1.52** |
| 42 | 57.32±1.67 | - | - | - | 58.57±0.53 | - |

Compared with the control group values of respective days
Values are Mean±SEM, ** P<0.01, n=6, the values on day 42 pertain to satellite group.

**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**

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

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 group.

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

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

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 group

There was significant (P<0.05) difference between the satellite group and control satellite group ALT, BUN and serum creatinine values at day 42 (Table 1, 3, 4), this indicated the injury to the kidney and liver was continued even after the administration of *S. laurifolius* leaf extract was stopped. The 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.

In the present study, there was no significant change in TEC, TLC, Hb and PCV values 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 present study, the rats administered with methanolic leaf extract of *S. laurifolius* showed varying degree of histopatholigical lesions in liver, kidney and intestine in all the treated groups, whereas remaining organs retained their normal architecture. This altered level of AST,ALT, serum creatinine and BUN was further supported by the gross and histopathological lesions in high dose groups, by the presence of swollen hepatocytes vacuolar degeneration, congestion of central veins and liver sinusoids, necrosis of individual hepatocytes, prominent bile duct hyperplasia in liver (Fig. 1), kidney showed haemorrhages and distension of tubular and glomerular epithelium, desquamation of tubular epithelium, intertubular and glomerular hemorrhagic areas, vacuolations of the glomerular epithelium, hypercellularity of tubular and inter tubular spaces (Fig. 2). In intestine increased goblet cell activity, broadening of villus structure, focal areas of inflammatory cells infiltration in lamina propria and submucosa, oedema of the submucosa (Fig. 3).

**Fig. 1**: 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)

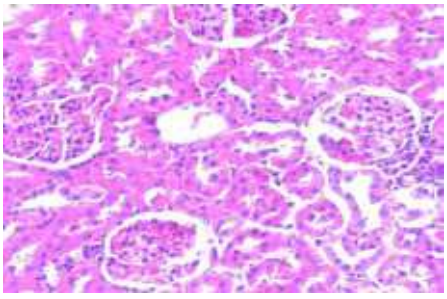


**Fig. 2**: Section of kidney from rat treated with 800 mg/kg dose of *S. laurifolius* leaf extract showing desquamation of tubular epithelial cells, tubular necrosis in subacute toxicity study. (H&E 200)

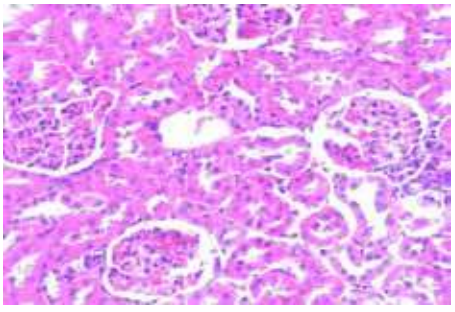
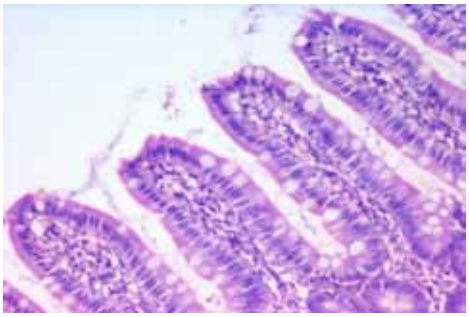


**Fig. 3**: Section of intestine from rat treated with 800 mg/kg dose of *S. laurifolius* leaf extract showing increased goblet cell activity, destruction and shortening of the villi in subacute toxicity study (H&E 200)



## Discussion

The *S. laurifolius* leaf extract consist of saponins, glycosides, flavonoids and bitter principles. These phytochemical constituents might be responsible for the biological activity of the *S. laurifolius* leaves, among the chemical constituents, saponins are the most important biologically (Kasai *et al.,* 1986; Vaghasiya *et al.,* 2009; Kishore *et al.,* 2011).

Acute toxicity study gives a clue on the range of doses that could be used in subsequent toxicity testing and estimating the therapeutic index of drugs and xenobiotics (Jeyabalan *et al.,* 2009). In present 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 nontoxic up to the dose of more than 2 g/kg which was similar to previous reports where it was observed that the plant did not show any signs of toxicity up to 2 g/kg and minimum lethal dose was greater than 2 g/kg when given orally in Albino mice and rats (Kishore *et al.,* 2011; Jeyabalan *et al.,* 2009). Based on these observations plant *S. laurifolius* is categorized in unclassified group under Globally Harmonised System of Classification.

The clinical findings observed in repeated 28 days oral toxicity study might be attributed to biological contents of the plant and were similar to the earlier findings of Witthawaskul *et al.,* (2003) who reported that saponins gave rise to toxic manifestation in rats, symptoms included weakness, anorexia, weight loss.

The elevated serum AST and ALT concentration and increased total protein level compared to control group is suggestive of the possible role of saponins present in gavaged extract in liver damage (Lakmichi *et al.,* 2011). The increase in the concentrations of serum ALT and AST can be specifically attributed to liver damage caused by the *S. laurifolius* leaf extract, which could have caused altered membrane permeability or liver cell necrosis and cytosol leakage in to the serum (Ozer *et al.,* 2008). Other possible causes are increased enzyme synthesis or decreased catabolism, which result in the release of intracellular enzymes into the blood stream (Rouiller, 1964). The results of present study showed that AST and ALT levels were elevated in the treated rats which indicate hepatocellular damage or injury caused by high doses of methanolic leaf extract of *S. laurifolius*.

The altered protein concentration might be attributed to functional nephrotoxicity and hepatotoxicity, the total protein increased in high dose group compare to control group, an increase in total protein might be due to their increased synthesis in liver (Banaee *et al.,* 2008). It was also reported that in various liver dysfunctions, generally the protein content increases so as to maintain the protein concentration in the liver (Sagar *et al.,* 2010). The altered level of serum total protein also noticed in rats administered with plant extract containing saponin at doses of 5, 70 and 2000 mg/kg per day a forty-day toxicity study (Lakmichi *et al.,* 2011). Sub-acute toxicity of the saponin mixture was evaluated with the dose of 1000 mg/kg orally for 14 days, AST, ALT and alkaline phospatase activity were increased in saponin-received rats and it was found that the saponin mixture directly impacts on the liver (Witthawaskul, 2003). Saponin was one of the important ingredients of *S. laurifolius* leaves, which might be responsible for the observations in the present study.

The elevated serum creatinine and urea nitrogen in comparison to control concentration is suggestive of the possible role of saponins in causing kidney damage. Such type of kidney damage was also reported with elevation of serum creatinine and urea nitrogen concentration (Wisloff *et al.,* 2008).

The functional studies in toxicology should be coupled with the appropriate histological studies, because appropriate morphological studies are useful for anatomical localization of action of toxin (Sagar *et al.,* 2010). In our study, the findings observed at the higher dose of 800 mg/kg/day and lethal dose of 1000 mg/kg/day were well corroborated by histological outcomes of liver, kidney and intestine. The histopathological changes of intestine also correlated with the earlier findings of Cherian *et al.,* (1996), who had reported the toxic action of saponins in rats. It was found that saponin affects absorptive cells of the intestinal mucosa, especially those near the tips of the villi, there was severe intestinal inflammation and increased goblet cell activity.

The histopathological changes noted in the present study are in accordance with Diwan *et al.,* (2000), who had reported the effect of saponins on histopathological changes in mice. Administered 0.5 ml of saponin solution to six groups containing 50, 100, 150, 250, 350 and 600 mg/kg, control group received 0.5 ml of saline solution. The histopathological changes were found to correlate with the increased dose of saponin. Thus saponin present in plant *S. laurifolius* might be responsible for the histopathological lesions observed in liver, kidney and intestine.

Thus methanolic leaf extract of *S. laurifolius* is nontoxic and $LD_{50}$ cut off value is more than 2 g/kg and it has toxic potential only when administered for longer duration in high concentration. It was also observed that oral administration of methanolic extract of *S. laurifolius* had adverse biochemical and histological effects when ingested for a longer duration.

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