ORIGINAL ARTICLE

# Toxicological investigation of *Spondias pinnata* (Linn. F.) Kurz. (Family: Anacardiaceae) bark extract in Wistar rats

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**Context:** *Spondias pinnata* (Linn. F.) Kurz. (Family: Anacardiaceae) is widely used in Sri Lankan traditional medicine for the treatment of diabetes mellitus. **Aims:** The aim was to investigate acute and subchronic toxicity of the aqueous bark extract of *S. pinnata* in healthy male Wistar rats. **Materials and Methods:** Wistar rats were administered the *S. pinnata* bark extract (0.25–2.00 g/kg) and observed for 14 days. Subchronic dose toxicity was evaluated by daily administration of the plant extract (1.00 g/kg; optimum effective antihyperglycaemic dose in diabetic rats) to healthy Wistar rats for 30 days. **Results:** The extract neither produced significant changes in the consumption of food and intake of water nor affected biochemical parameters (serum lipid profile and activities of liver enzymes), hematological parameters (full blood count) and histopathology in healthy rats ($P > 0.05$). **Conclusions:** The results revealed that *S. pinnata* extract at a dose of 1.00 g/kg was found to be toxicologically safe as a potent antihyperglycemic agent in Wistar rats.

**Key words:** Acute toxicity, biochemical assessment, haematological assessment, organ histopathology, repeated dose toxicity

## INTRODUCTION

The use of medicinal plants for healing purposes has been increasingly popular as they are believed as beneficial and free of side effects.[1] However, the rationale for the utilization of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on safety. With the upsurge in the use of herbal medicines for various diseases, toxicological investigation of medicinal plants is imperative based on the need to validate their folkloric usage.

*Spondias pinnata* (Linn. F.) Kurz (Family: *Anacardiaceae*) is a deciduous tree distributed in Sri Lanka, India and other South-East Asian countries. The bark of the tree is used for treating dysentery, muscular rheumatism and diabetes mellitus. Leaves are used for earache. The fruit is an antiscorbutic and used for the bilious dyspepsia.[2,3] The phytochemistry of this plant has studied previously. The isolation of 24-methylene cycloartanone, stigma-4en-3one, lignoceric acid, β-sitosterol and its β-D-glucoside from *S. pinnata* have described.[4] The gum exudates of the species have been found to contain acidic polysaccharides.[5] Different extracts of *S. pinnata* are reported to show antibacterial,[6] hypoglycaemic,[7] antioxidant and free radical scavenging activities.[8] We studied the antidiabetic mechanisms of the bark extract of *S. pinnata* and the optimum effective therapeutic dose was found to be 1.00 g/kg in streptozotocin-induced diabetic rats (unpublished data). Despite the wide use of *S. pinnata* bark for various diseases in traditional medicine, no detailed study has been published regarding its complete toxicological profile. Therefore, this study was designed to investigate acute and sub chronic toxicity of the aqueous stem bark extract of *S. pinnata* in experimental Wistar rats.

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## MATERIALS AND METHODS

### Plant Material

Stem bark of *S. pinnata* was collected during May to June 2013 from the Southern region of Sri Lanka. Botanical identity was determined according to the descriptions given by Jayaweera[2] and confirmed by comparing authentic samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (Attanayake/2011/01).

### Preparation of the Aqueous Plant Extract

The bark parts of *S. pinnata* were cut into small pieces, dried at 40°C until a constant weight was reached and coarsely ground. Powdered plant material (50.00 g) was dissolved in 400.0 mL of distilled water and refluxed for

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4 h. The mixture was strained, and the final volume was adjusted to 50.0 mL. A single dose of 0.25, 0.50, 0.75, 1.00, 1.25 and 2.00 g/kg was administered orally to healthy Wistar rats in the acute toxicity study. The optimum effective antihyperglycemic dose of *S. pinnata* bark extract (1.00 g/kg) was administered orally to healthy Wistar rats in the sub-chronic toxicity study.

### Animals

Healthy adult male rats of Wistar strain (200 ± 25 g, body weight) were used to carry out experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (Tem: 25 ± 2°C, relative humidity: 55–65% and 12 ± 1 h light/dark cycle). Rats were fed with a standard diet (Medical Research Institute rat formulae, Sri Lanka) with free access to water before and during the experiment. The rats were randomized into various groups and allowed to acclimatize for a period of 7-day under standard environmental conditions before the commencement of the experiment. The animals described as fasting was deprived of food and water for 12 h *ad libitum*. All protocols used in this study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka guided by the Council for International Organization of Medical Sciences international guiding principles of biomedical research involving animals.

### Acute Toxicity Study

Acute toxicity testing was performed for the *S. pinnata* extract following the Organization for Economic Cooperation and Development guideline 423, fixed dose procedure.[9] Six groups containing healthy male rats ($n$ = 6/group) received aqueous extract of *S. pinnata* at the doses of 0.25, 0.50, 0.75, 1.00, 1.25, 2.00 g/kg, orally while the untreated healthy control group received distilled water.

Animals were observed individually after dosing once during the first 30 min, periodically during the first 24 h, and daily for a total of 14 days. Observations included changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern. Special attention was directed to the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

### Sub-Chronic Toxicity Study

Rats were randomly allotted to two groups ($n$ = 6/group). The first group served as the untreated healthy control group received distilled water daily. The rats in the second group received the aqueous bark extract of *S. pinnata* at the optimum effective antihyperglycemic dose (1.00 g/kg). The fasted (12 h) animals were sacrificed on the 30$^{th}$ day of the experiment. Blood samples were collected for the assessment of serum biochemical and hematological parameters. The heart, lung, liver, small intestine, pancreas, spleen, and kidney were carefully isolated and fixed in buffered formalin for the assessment of histopathology.

### Body Weight, Food and Water Intake

The body weight of each rat was assessed before the commencement of dosing, during the experimental period at weekly intervals and on the day of sacrifice. The amount of food, water consumed were measured daily from the quantity of food, water supplied and the amount remaining after 24h.

### Assessment of Biochemical Parameters

Serum concentrations of fasting glucose,[10] total cholesterol,[11] high density lipoprotein cholesterol,[12] triglyceride,[13] were estimated using spectrophotometric enzyme assay kits (Prodia Int. Germany). The concentration of low-density lipoprotein cholesterol was calculated by Friedewald equation.[14] Serum activities of alanine aminotransferase, aspartate aminotransferase (AST), alkaline phosphatase (ALP) were estimated using spectrophotometric enzyme assay kits (Stanbio, USA).[15,16]

### Assessment of Hematological Parameters

Hematological parameters were estimated using a hematological analyzer (Sysmax KH21, Japan). Total hemoglobin, red blood corpuscles, platelet count, red cell indices including packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and white blood corpuscles of blood samples were recorded.

### Assessment of Histopathology in Body Tissues

The heart, lung, liver, pancreas, small intestine, spleen, and kidney were fixed in 10% formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections were stained with hematoxylin and eosin.

### Statistical Analysis

Results were expressed as mean ± standard error of mean. The toxicological data were analyzed by ANOVA, followed by Dunnett multiple comparison test and two-sample $t$-test using the Minitab statistical software respectively. Results were considered as significant at $P < 0.05$.

## RESULTS

### Acute Toxicity Study

There was no mortality or morbidity observed in rats through the 14-day period following single oral

administration of the selected doses of the extract of *S. pinnata*. The animals did not show any changes in general appearance during the 14-day period. Morphological characteristics (fur, skin, eyes, and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy and unusual behavior were observed.

### Sub-Chronic Toxicity Study

There was no significant difference ($P > 0.05$) in body weights of animals [Figure 1], consumption of food and intake of water between two groups [Figures 2 and 3]. The results of biochemical and hematological data are shown in Table 1. There was no statistical difference in the parameters listed in the plant treated rats compared with the control ($P > 0.05$). The light microscopic examination of the heart tissue of *S. pinnata* treated rats on H and E sections revealed that, there were very few scattered occasional lymphocytes and congested blood vessels with no edema [Figure 4a and b]. Further, mild congestion with no edema was noted in the lung tissue of treated rats [Figure 4c and d]. The assessment of liver histopathology revealed few lymphocytic infiltrates around the central vein, in the portal tract and parenchyma in both control and plant treated rats [Figure 4e and f]. The histopathological examination of the tissues of kidney, small intestine, spleen and pancreas, showed no changes in cellular architecture in treated rats [Figure 4g-n]. Further, the findings were generally consistent with the expected pattern for Wistar rats of a particular age.

**Table 1: Biochemical and haematological parameters in Wistar rats**

| Parameters studied | Healthy control | *Spondias pinnata* treated rats |
|---|---|---|
| Fasting blood glucose (mmol/L) | 5.20±0.70 | 4.94±1.01 |
| Total cholesterol (mmol/L) | 3.70±0.18 | 3.66±0.25 |
| HDL-C (mmol/L) | 1.39±0.02 | 1.38±0.05 |
| LDL-C (mmol/L) | 1.86±0.10 | 2.08±0.20 |
| Triglyceride (mmol/L) | 0.99±0.02 | 1.00±0.06 |
| Alanine aminotransferase (U/L) | 12.39±0.79 | 11.62±1.20 |
| Aspartate aminotransferase (U/L) | 44.21±1.75 | 42.54±1.80 |
| Alkaline phosphatase (U/L) | 61.48±1.50 | 62.08±1.01 |
| Total haemoglobin (g/dL) | 15.25±0.80 | 14.30±0.40 |
| Red blood corpuscles ($10^6$/mm$^3$) | 8.27±1.31 | 7.98±0.33 |
| Platelet count ($10^3$/mm$^3$) | 1096.33±93.87 | 1095±40.23 |
| Pack cell volume (%) | 47.95±2.38 | 48.90±2.05 |
| Mean corpuscular volume (fL) | 61.45±1.28 | 60.76±2.50 |
| Mean corpuscular haemoglobin (pg) | 19.53±0.60 | 18.08±0.83 |
| Mean corpuscular haemoglobin concentration (g/dL) | 31.77±0.90 | 31.26±0.89 |
| White blood corpuscles ($10^3$/mm$^3$) | 4.82±1.60 | 5.68±1.99 |

The values are expressed as mean±SEM ($n$=6/group). The two sample $t$-test at α=0.05 showed no statistical difference between the biochemical and haematological parameters in *Spondias pinnata* treated healthy rats compared to untreated healthy control rats. HDL-C – High density lipoprotein cholesterol, LDL-C – Low density lipoprotein cholesterol, SEM – Standard error of mean

## DISCUSSION

The present study reports the acute and sub-chronic toxicological effects of aqueous bark extract of *S. pinnata* in healthy Wistar rats for the first time.

Toxicity screening of new pharmacological agents, plant products and crude extracts are generally performed in rodents. It is reported that sub-chronic and chronic

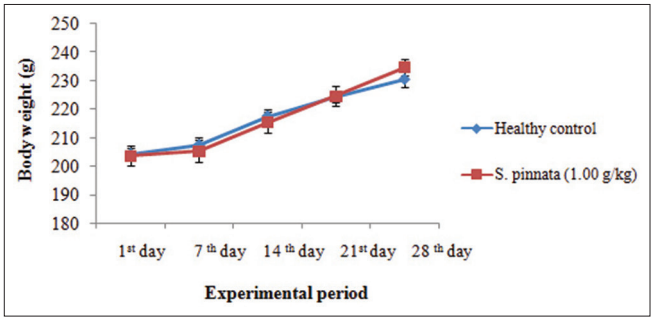


**Figure 1:** Effect of bark extract of *Spondias pinnata* (1.00 g/kg) on body weight of healthy rats at specific intervals for 28 days. Each data point is expressed as mean ± standard error of mean ($n$ = 6/group). The two sample $t$-test at α = 0.05 showed no statistical difference between mean body weight of plant treated rats compared to untreated healthy control rats

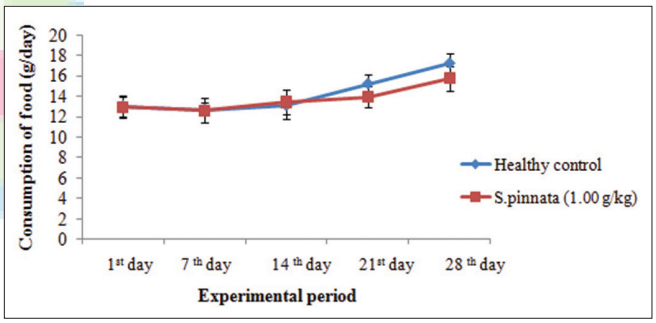


**Figure 2:** Effect of bark extract of *Spondias pinnata* (1.00 g/kg) on consumption of food in healthy rats at specific intervals for 28 days. The values are expressed as mean ± standard error of mean ($n$ = 6/group). The two sample $t$-test at α = 0.05 showed no statistical difference between consumption of food in plant treated rats compared to untreated healthy control rats

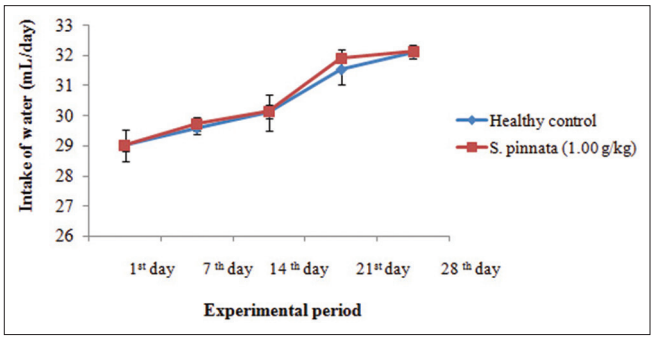


**Figure 3:** Effect of bark extract of *Spondias pinnata* (1.00 g/kg) on intake of water in healthy rats at specific intervals for 28 days. The values are expressed as mean ± standard error of mean ($n$ = 6/group). The two sample $t$-test at α = 0.05 showed no statistical difference between intake of water in plant treated rats compared to untreated healthy control rats

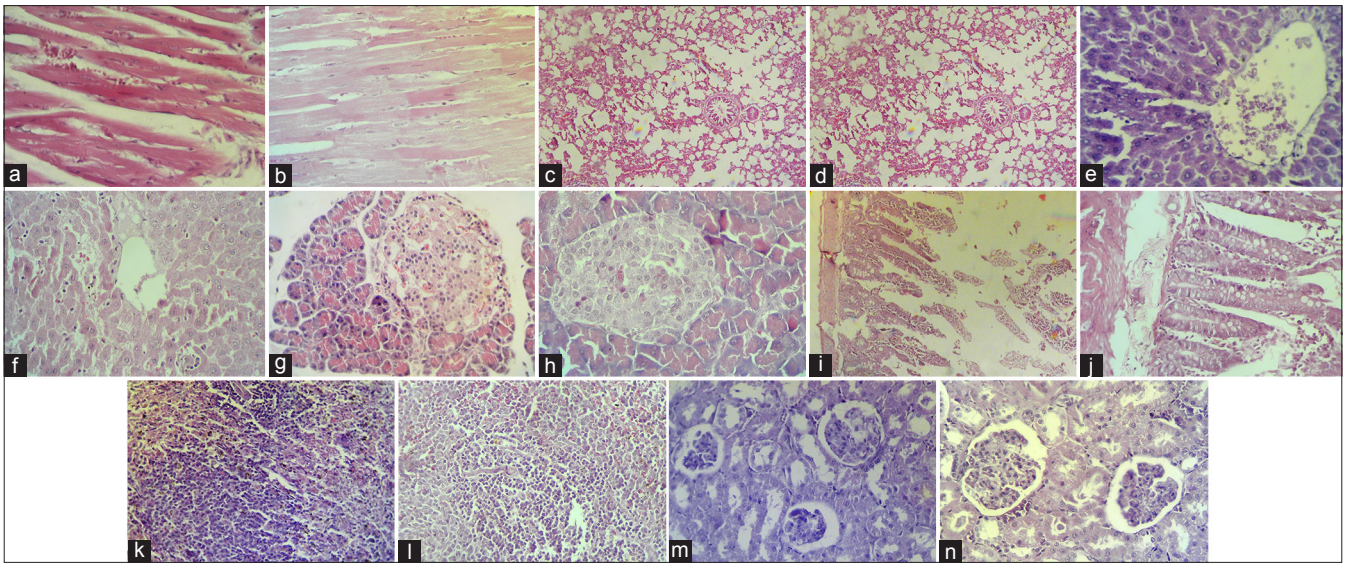


**Figure 4:** Photomicrographs (×400) of heart, lung, liver, small intestine, pancreas, spleen and kidney histopathology from representative rats of healthy control rats (a, c, e, g, i, k, m) and *Spondias pinnata* treated (1.00 g/kg) rats (b, d, f, h, j, l, n)

toxicological effects of herbal extracts including the doses potentially usable in humans are usually tested in rats.[17] Accordingly Wistar rats were used in the present investigation. Furthermore, a better correlation has been reported between the toxicological results in rats and humans than the correlation between mice and humans.[17,18] The oral route is commonly used for screening of toxicological effects of plant extracts in laboratory animals.[19,20] The rate of absorption might be slow but is painless to animals. Further, the same route has been used for the administration of herbal remedies as aqueous extracts for patients by Ayurvedic physicians since time immemorial.

Acute toxicity study was conducted with a range of six doses; 0.25–2.00 g/kg including the optimum effective dose of *S. pinnata* (1.00 g/kg). The human therapeutic dose was considered in computing the range of doses, and this was done according to the standard guidelines.[21] The acute toxicity study indicated that administration of *S. pinnata* extract with the selected doses was well-tolerated by all test animals, suggesting its safety for long-term investigations. Accordingly, Mondal *et al*.[22] reported that the administration of methanol, petroleum ether and chloroform bark extracts of *S. pinnata* to rats did not produce any acute toxic effects in rats.

The optimum effective dose of *S. pinnata* (1.00g/kg) was selected for the sub-chronic toxicological assessment. The administration of the extract did not show any changes in skin, fur, eyes and behavior of animals throughout the 28-day study. In general, body weight is a simple and sensitive index of toxicity after exposure to potentially toxic substances.[23,24] In the present study, the plant extract did not significantly alter body or relative weight of organs as compared to the untreated healthy control group, which suggests that the extracts did not hinder the growth of healthy rats. Furthermore, the consumption of food and intake of water are important parameters in the safety of a natural product for any therapeutic purpose.[25] The proper intake of nutrients is essential to maintain the physiological state of the animals and to accomplish proper response to plant extract tested. In this study, the consumption of food and intake of water were not altered, suggesting that *S. pinnata* extract neither induced nor suppressed appetite in healthy rats.

The assessment of hematological parameters can be used to determine the extent of deleterious effects of foreign compounds including plant extracts on blood constituents of an animal. The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status.[26,27] Such toxicity testing is relevant for changes in the hemopoietic system that has a higher predictive value for human toxicity, when extrapolated from animal studies. Sub-chronic exposure of Wistar rats to the plant extract produced small and transient changes in some hematological parameters, e.g., white blood cell count, platelet count. However, the values were statistically not significant ($P > 0.05$). Therefore, the results revealed that the *S. pinnata* extract was not toxic to the circulatory red blood cells, white blood cells, platelets and did not interfere with their production. Therefore, it is plausible to assume the extract is not hematotoxic.

Even though there were minor alterations in the percentage of white blood cells, the assessment of histopathology of the spleen did not exhibit any abnormalities in rats treated

with the extract, implying absence of any abnormal effects on hematopoiesis and immunologic functions.

Liver plays an important role in the detoxification of toxic substances *in vivo*. Considering the key role in detoxification of natural toxic substances and xenobiotics, the liver was selected to conduct a detailed assessment of biochemical parameters and histology on hematoxylin and eosin stained sections. In addition, the morphological changes in kidney tissue were assessed on light microscopy to evaluate the toxic effects of *S. pinnata* extract on kidney. Usually, liver cell damage is characterized by a rise in hepatic enzymes like ALT, AST and ALP. The results are in accordance with the published experimental data for Wistar rats by other authors.[28] In addition, absence of any effects on fasting serum glucose concentration and serum lipid parameters suggest that carbohydrate and lipid metabolism in the animals were not altered by the *S. pinnata* extract.

The assessment of histopathology in the body tissues is the gold standard for evaluating treatment-related pathological changes in tissues.[19] In the present study, histopathological evaluation of repeated dose 28-day treatment indicated that the plant extract did not adversely affect heart, lung, small intestine, liver, pancreas and kidney of rats which corroborated the results of biochemical analysis. Most of the observed statistically nonsignificant variations were random, unrelated to dose and unaccompanied by any histologic correlations.

The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal and cardiovascular adverse effects,[18,29] while certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions are poorly correlated with toxicity observed in animals and humans.[30] The effects on cardiovascular, central nervous, respiratory systems and effects on fertility were not assessed during the study, and these should be evaluated prior to human exposure. Furthermore, it is quite difficult to ascertain certain adverse effects in animals, such as headache, abdominal pain, dizziness and visual disturbances which can be considered as limitations of the present study. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects directly from animals to humans. However, clinical evaluation may be required to define a safe dosage in humans precisely.[17]

## CONCLUSION

The acute toxicity study suggests that aqueous bark extract of *S. pinnata* is safe in healthy Wistar rats up to a dose of 2.00 g/kg. The oral administration of the extract to rats for 28-day were not associated with adverse effects reflected in the general condition, growth, relative weight of organs, clinical biochemical, hematological values and more importantly did not cause abnormalities in histopathology of body tissues. Therefore, bark extract of *S. pinnata* (1.00 g/kg), was found to be toxicologically safe as a potent antihyperglycaemic agent in healthy Wistar rats.

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