RESEARCH ARTICLE
Acute toxicity study and phytochemical screening of *Pterocarpus marsupium* heartwood

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Received: October 12, 2021; Accepted: November 07, 2021

**ABSTRACT**

**Background:** Conventionally, Ayurvedic herbs are being used to treat various diseases. These medicinal compounds have to be evaluated for their safety and presence of therapeutic compounds for the clinical application. **Aims and Objectives:** The present study was designed to obtain the scientific knowledge on the safety profile as well as to assess the presence of pharmacologically active principles in the *Pterocarpus marsupium* heartwood. **Materials and methods:** The aqueous extract of *P. marsupium* heartwood was subjected to an acute toxicity in albino rats. The animals were divided into four groups (n = 6) and fed with graded doses (1000, 2000, and 5000 mg/kg p.o.) of plant extract, respectively, whereas control group had received 2 ml distilled water orally. Animals were continuously observed for the toxicological symptoms for 2 h and intermittently for 48 h and latter once in a day for 14 days. The body weight of the animals was recorded. In addition, the qualitative phytochemical investigations were conducted to identify the presence of active principles. **Results:** The animals fed with aqueous extract of *P. marsupium* heartwood did not exhibit any toxic symptoms and the mortality. However, there was a significant (P < 0.05) dose-dependent change in the weight gain observed in comparison to the control group. The median lethal dose (LD₅₀) of the plant extract was considered as >5000 mg/kg. Furthermore, the phytochemical investigations of the plant extract showed the presence of carbohydrates, flavonoids, triterpenoids, saponins, tannins and phenols. **Conclusion:** The aqueous extract of *P. marsupium* heartwood was found to be safe and well tolerated even at a large dose of 5000 mg/kg. Furthermore, the plant extract found to possess pharmacologically active principles having wide pharmacological spectrum. Hence, it can be preferred in various therapeutic conditions.

**KEY WORDS:** Acute Toxicity; *Pterocarpus marsupium*; Median Lethal Dose; Phytochemicals; Aqueous Extract

**INTRODUCTION**

The medicinal plants are rich source of phytochemicals which have contributed to the evolution of traditional Ayurveda system.¹ They are widely recognized therapeutic compounds as they produce lesser side effects compared to modern drugs. These compounds may also compensate the increased demand due to rise in human population.² It has been postulated that wide range of herbal plants is evaluated since ages and some still need to be explored for their safety and therapeutic role in the healing of various diseases.³

*Pterocarpus marsupium* Roxb. (*P. marsupium*) is known as Indian Kino tree, belonging to *Fabaceae* family. It is reported to have broad-spectrum medicinal properties. It is also familiar as Bija or Vijayasar in Hindi and Asana in Sanskrit, indigenous to Nepal, Sri Lanka, and India.⁴ The heartwood part of *P. marsupium* shown to possess antidiabetic,
astringent, anti-inflammatory properties, etc. The aim of the current study was to investigate the acute toxicity of *P. marsupium* heartwood and also to explore the potential compounds present in the plant which can be beneficial in the various therapeutic conditions.

**MATERIALS AND METHODS**

**Collection of Material**

The heartwood part of *P. marsupium* was obtained and authenticated by Dr. Sunil Kumar KN, Senior research officer, Department of Pharmacognosy, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi, Karnataka.

**Preparation of Plant Sample**

*P. marsupium* heartwood material was cleaned and made it into pieces. Then, it was dried under shade and pulverized into fine powder form using a mortar pestle. Later, it was subjected to extract preparation.

**Preparation of Extract**

The water extraction of *P. marsupium* heartwood was done using classical method. The heartwood material (800 g) was dispensed into a beaker which contains 24 L of distilled water. After 24 h, the mixture was filtered through Whatman No.1 filter paper and solvent was evaporated using water bath. The filtrate (106 g) was air-dried and dispensed in a sterile bottle for further use.

**Experimental Animals**

As per the CPCSEA guidelines, all the rats were isolated in individual polypropylene cages and provided adequate ventilation (23°C ± 2°C and 12 h light-dark cycle), under hygienic conditions. They were fed with rat pellets and given free access to water *ad libitum*. At the beginning of the study, the Institutional Ethics Committee approval was obtained (KSHEMA/IAEC/02/2013).

**Acute Toxicity Study**

The acute oral toxicity study was performed according to the recommendations of OECD guidelines-423. Twenty-four Albino rats (250–270 g) were recruited for this study. The control group rats received 2 ml distilled water orally. The remaining groups (2, 3, and 4) were fed with single oral dose of 1000, 2000, and 5000 mg/kg of aqueous extract of *P. marsupium*, respectively, through an oral gavage tube.

The animals were continuously observed for their behavioral pattern and signs of toxicity for 30 min post-extract administration. Then, they were monitored intermittently for the 2, 4, 24, and 48 h and latter once in a day for up to 14 days. The rats were observed for the onset of clinical and toxicological symptoms such as changes in behavioral pattern, skin fur and color, eyes and mucous membrane, sleep, salivation, diarrhea, tremors, coma, and mortality. The body weight was recorded before the extract treatment, at 48 h, day 7 and day 14 using electronic weight balance.

**Phytochemical Analysis**

The qualitative phytochemical screening of the aqueous extract of *P. marsupium* heartwood was performed by following standard methods.

**Tests for Carbohydrates**

*Molisch’s test*

To the extract, 1 ml of α-naphthol solution and 0.2 ml concentrated sulfuric acid were added along the wall of the test tube. Purple to the violet color ring was formed at the junction of the two liquids proves the presence of carbohydrates.

*Fehling’s test*

Two milligrams of plant extract were treated with Fehling’s solution A and B in equal concentration. Then, the solution was boiled on a water bath. Appearance of a brick red precipitate shows the presence of carbohydrates.

*Benedict’s test*

The extract was mixed in a 5 ml of Benedict’s reagent and boiled for 2–3 min and then cooled. The carbohydrates were said to be present on the development of a red precipitate.

**Test for Steroids**

*Salkowski test*

Two milliliters of chloroform were mixed with plant extract and added an equal amount of sulfuric acid. Observation of cherry red color in the chloroform layer and green color in the acid layer reflects the presence of sterols.

*Liebermann–Burchard test*

The extract was dissolved in chloroform; 1 ml of acetic anhydride was added, then warmed in a water bath and cooled. A little amount of concentrated sulfuric acid was poured along the wall of the test tube. Development of bluish-green color shows the presence of sterols.

**Tests for Alkaloids**

*Dragendorff’s test*

To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendorff’s reagent were added and shaken...
well. Formation of an orange-red precipitate reflects the presence of alkaloids.

**Wagner’s test**

Two to three drops of Wagner’s reagent were poured into a mixture containing the plant extract and acetic acid. Alkaloids were said to be present on the appearance of a reddish-brown precipitate.

**Hager’s test**

Few mg of the extract was dissolved in acetic acid, 3 ml of Hager’s reagent was added, and the formation of a yellow precipitate indicates the presence of alkaloids.

**Test for Flavonoids**

**Shinoda’s test**

To the extract, a pinch of magnesium powder and 8–10 drops of concentrated hydrochloric acid were added and heated on a water bath. Appearance of red color shows the presence of flavonoids.

**Test for Triterpenoids**

**Salkowski test**

The extract was treated with 2 ml of chloroform and 3 ml of sulfuric acid. Appearance of a reddish-brown color layer at the interface reflects the presence of triterpenoids.

**Test for Saponins**

**Froth test**

Distilled water was collected into a test tube containing plant extract and then it was shaken for a brief period. Appearance of stable froth reflects the presence of saponins.

**Test for Tannins**

**Ferric chloride test**

To the extract, two drops of a dilute solution of ferric chloride were added; the formation of dark blue color reflects the presence of tannins.

**Test for Coumarins**

The extract was mixed with 3–4 drops of 2 N sodium hydroxide solution. Appearance of a dark yellow color reflects the presence of coumarins.

**Test for Carboxylic Acids**

The extract dissolved in water was treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acids.

**Test for Resins**

Few mg of the extract was mixed with water and acetone. Turbidity indicates the presence of resins.

**Test for Quinones**

A few mg of alcohol extract was treated with 0.5% NaOH. Deep coloration such as pink, purple, or red indicates the presence of quinones.

**Test for Phenols**

The two drops of ferric chloride reagent were added to the extract. Formation of blue to blue-black indicates the presence of phenols.

**Statistics**

The results were analyzed using one-way analysis of variance method followed by Scheffe’s multiple comparison post hoc test. The significance level was assumed as $P < 0.05$. The SPSS statistics 20 software was used for the statistical analysis.

**RESULTS**

**Acute Toxicity Study**

The animals fed with graded doses (1000, 2000, and 5000 mg/kg p.o.) of aqueous extract of *P. marsupium* did not manifest any pharmacotoxicity or significant clinical changes. None of the animals died during the study period. The moribund status was noticed in the rats administered with 5000 mg/kg plant extract for a brief period of 4 h but normalized later. However, there was a significant ($P < 0.05$) dose-dependent alteration in the weight gain observed in between plant extract fed rats to normal control group rats at the end of the study [Table 1].

**Phytochemical Analysis**

The aqueous extract of *P. marsupium* heartwood was subjected to preliminary phytochemical investigations for the presence of various chemical compounds. It has revealed that the extract contains carbohydrates, flavonoids, triterpenoids, saponins, tannins, and phenols [Table 2].

**DISCUSSION**

The herbal plants have been extensively evaluated for their active principles in Ayurveda.\[11,12\] However, still there is a lack of safety and toxicological profile data for many of the manufactured natural products.\[13\] The present study mainly focused on the proper scientific knowledge on the safety profile as well as the therapeutic principles of *P. marsupium*.
Acute toxicity study of *Pterocarpus marsupium* heartwood

Table 1: Body weight (g) of the rats treated with aqueous extract of *Pterocarpus marsupium* (*n=6*)

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Normal control</th>
<th>1000 mg/kg</th>
<th>2000 mg/kg</th>
<th>5000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>252.7±5.0</td>
<td>251.3±3.2</td>
<td>250.5±2.9</td>
<td>255.3±5.4</td>
</tr>
<tr>
<td>At 48 h</td>
<td>254.3±4.9</td>
<td>246.8±3.4</td>
<td>241.0±3.5</td>
<td>246.8±4.8</td>
</tr>
<tr>
<td>Day 7</td>
<td>262.2±7.3</td>
<td>257.3±4.4</td>
<td>251.0±2.8</td>
<td>252.3±4.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>277.2±8.4</td>
<td>266.3±3.7</td>
<td>261.5±2.7</td>
<td>259.5±5.5</td>
</tr>
<tr>
<td>Weight gain</td>
<td>24.5±5.8</td>
<td>15±1.7*</td>
<td>11±1.4*</td>
<td>4.2±1.2*</td>
</tr>
</tbody>
</table>

*Significant difference compared to normal control group (*P* < 0.05). All the resulting values are expressed as mean±standard deviation.

Table 2: Phytochemical constituents of aqueous extract of *Pterocarpus marsupium* heartwood

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Absent</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Absent</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Absent</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Absent</td>
</tr>
<tr>
<td>Saponins</td>
<td>Absent</td>
</tr>
<tr>
<td>Tannins</td>
<td>Absent</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Absent</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>Absent</td>
</tr>
<tr>
<td>Resins</td>
<td>Absent</td>
</tr>
<tr>
<td>Quinones</td>
<td>Absent</td>
</tr>
<tr>
<td>Phenols</td>
<td>Present</td>
</tr>
</tbody>
</table>

hearthwood extract. Pharmacological extracts are essential to be evaluated for their benefits, safety, and efficacy for the broad therapeutic application. The current study did not manifest any clinical signs of toxicity and mortality in rats administered with single graded doses of the aqueous extract of *P. marsupium* heartwood for a period of 14 days. There was a significant (*P* < 0.05) dose-dependent decrease in weight gain observed in rats fed with plant extract compared to control group. There may be possible alteration in the metabolism which causes reduction in the food intake and consequently affecting the body weight.[9] The present study suggests that the aqueous extract of *P. marsupium* heartwood was safe when given orally and no drug-related toxicity was observed even at the highest dose tested. It is, therefore, considered as median lethal dose (LD<sub>50</sub>) of the orally given plant extract >5000 mg/kg.

Our study also investigated the presence of phytochemical compounds in the aqueous extract of *P. marsupium* heartwood. It was revealed that the plant extract contains active phytochemicals such as carbohydrates, flavonoids, triterpenoids, saponins, tannins, and phenols. It is proved that *P. marsupium* heartwood can be a promising source of biologically active compounds. Phenolic component of plant extract is potentially toxic component to inhibit the microbial growth and multiplication.[14] Tannins were reported to possess anti-diarrheal, antimicrobial, antisecretolytic, and anthelmintic effects.[15] Flavonoids have been evaluated for their antidiabetic, antioxidant, anti-inflammatory, anticaner, and antiviral activities.[16] All these bioactive agents found to possess various therapeutic properties which could be beneficial in the clinical application for various disorders.[17]

According to the results obtained from the current study, the aqueous extract of *P. marsupium* heartwood was considered to be well tolerated since no mortality or other severe side effects observed.

**Limitations**

It is recommended to analyze the hematological and serum enzyme markers by performing subacute and chronic toxicity study to obtain adequate safety profile for long-term and repeated use.

**CONCLUSION**

The aqueous extract of *P. marsupium* heartwood was found to be safe and well tolerated even at a large dose of 5000 mg/kg. Furthermore, the plant extract found to possess pharmacologically active principles having wide pharmacological spectrum. Hence, it can be preferred in various therapeutic conditions.

**ACKNOWLEDGMENTS**

Authors are thankful to Dr. Goparaju Anumolu, Statistician, RVM Institute of Medical Sciences and Research Center, Siddipet, Telangana, for his guidance in statistics.

**REFERENCES**


**How to cite this article:** Koyagura N, Munge B, Vedula P. Acute toxicity study and phytochemical screening of Pterocarpus marsupium heartwood. Natl J Physiol Pharm Pharmacol 2022;12 (Online First). DOI: 10.5455/njppp.2022.11400202107112021

**Source of Support:** Nil, **Conflicts of Interest:** None declared.