

Toxicity and Safety Profiles of Methanolic Extract of *Pistacia integerrima* J. L. Stewart ex Brandis (PI) for Wistar Rats

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Key Words

acute toxicity, *Pistacia integerrima*, sub-acute toxicity, tropical biomedicine

Abstract

Objectives: The goals of this research were to evaluate acute (single-dose) and sub-acute (repeated-dose) toxicity profiles of methanolic extract of *Pistacia integerrima* J. L. Stewart ex Brandis (PI) for Wistar rats and to assess the safety profile of PI by observing physiological changes, mortality, changes in body weight, the histopathology of body organs, the hematology and the biochemistry of the animals.

Methods: The toxicity profile of PI was evaluated using Wistar rats of both sexes. Animals were divided into four groups: Group 1; control group (normal saline), Group 2; PI-1 (250 mg/kg), Group 3; PI-2 (500 mg/kg), Group 4; PL-3 (1,000 mg/kg). An acute-toxicity study in which animals received a single dose of PI extract (2,000 mg/kg) and were then observed for 14 days for changes in skin, fur, eye color, mucous membrane secretions and excretions, gait, posture, and tonic or clonic movements was performed according to guideline 425 of the Organization of Economic and Corporation Development (OECD). In the repeated-dose toxicity study (OECD –

407) animals received a daily dose of PI extract for 28 days (4 weeks). The parameters observed in this study include body weight, hematology and biochemistry of the animals.

Results: In the acute toxicity study, no mortalities or changes in behavior were noted in the animals. The repeated-dose toxicity study was also devoid of any toxicity in the animals during the 28 days of testing with PI extract. The extract did not alter the body weight, hematology or biochemistry of the animals. The methanolic extract of PI was to be found safe to the no-observed-adverse-effect-level (NOAEL) for the single-dose and repeated-dose toxicity tests in rats.

Conclusion: The methanolic extract of PI was devoid of toxicity; hence, it can be used for various ayurvedic preparations and treatments of diseases.

1. Introduction

Herbal plants have been used as medicines for thousands of years, and; some specific plants have been used for particular ailments [1]. Medicinal plants are sources of raw materials for both traditional systems of medicine and modern medicine. These medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and fewer lesser side effects. Traditionally, herbs and

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herbal products have been considered to be nontoxic and have been used by the general public and traditional medicinal doctors worldwide to treat a range of ailments. However, the fact that something is natural does not necessarily make it safe or effective. The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects. Whilst the literature documents severe toxicity resulting from the use of herbs, on many occasions the potential toxicities of herbs and herbal products have not been recognized [2].

Pistacia integerrima J. L. Stewart ex Brandis (PI) is a species of pistachio tree, commonly called zebrawood, native to Asia. It is one of the important plants of Indian traditional medicine and belongs to the family Anacardiaceae. PI is often classified as *Pistacia chinensis* sp. *integerrima*. The phytochemical constituents of PI have been reported to be tannins, essential oils and resins. The oil contains alpha-pinene (25%), camphene (27%), di-limonene (4% – 5%), 1:8-cineol (10%), caprylic acid (15%), alpha-terpineol (20%) and aromadendrene (4% – 5%). They also contain a small percentage of a lactonic stearoptene. The two triterpenic acids are ketocarboxylic and appear to be identical to the alpha and the beta acids [3, 4]. PI is used for a variety of purposes in India, including timber, dye, and fodder; it is also used as a herbal remedy for many ailments including coughing, asthma, fever, vomiting, diarrhea, loss of appetite, nose bleeds, snakebites, and gastrointestinal and liver disorders [5-8]. PI is reported to be useful as an anti-inflammatory, and an anti-diabetic agent and as a blood purifier [9-11]. Galls excrescences are formed by insects on the leaves, petioles and branches of plants. These leaf galls are harvested and used to make kakad shringi; a herbal medicine used to treat diarrhea in northern India.

2. Material and Methods

PI leaf galls were collected in the month of July from the Nadaun block of the Hamirpur district, Himachal Pradesh. The leaf galls were identified and authenticated by Dr. Ravindra Pandey, Department of Natural Products Research, Columbia Institute of Pharmacy, Raipur, and a voucher specimen, i.e., leaf galls, with voucher number 0342, was deposited in the herbarium of the institute. The plant material was washed with water and dried at room temperature. The dried plant material was crushed to make a coarse powder. The powdered material was soaked in methanol, distilled water, chloroform, ethyl acetate and n-hexane for one week and subjected to extraction until exhaustion of the plant material. The extract was then concentrated in a water bath at a temperature below 60°C.

Male Wistar rats with weights in the range of 160 – 200 g were used for the experiments, which were carried out according to a protocol approved by the Institutional Animal Ethics Committee, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India (1321/ac/10/CPCSEA, dated-01/28/2010). The animals were housed in polycarbonate cages in a room with a 12-hour day-night cycle, a temperature of 22 ± 2°C, and a humidity of 45% – 64%. During the entire experimental period, animals were fed

with a balanced commercial pellet diet (Ashirwad Industries, Mohali, India) and were allowed *ad libitum* access to water and normal saline.

The acute toxicity studies were conducted as per guideline 425 of the Organization of Economic and Cooperation Development (OECD), where a dose limit of 2,000 mg/kg of body weight was used. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Individual body weights were recorded once a week as well as on the day the experiment ended. Changes in skin, fur, eyes, mucous membranes secretions, excretions, and autonomic activity (e.g. lacrimation, pilo-erection, pupil size, unusual respiratory pattern) were recorded, as were changes in gait, posture, and response to handling, as well as the presence of clonic or tonic movements [12].

The sub-acute toxicity studies were conducted as per the guideline 407 of the OECD; this provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. Four groups of animals were selected, group 1 being the normal control and groups 2, 3 and 4 being treated daily with PI at doses of 250, 500, and 1,000 mg/kg of body weight, respectively, for 28 days. All rats were observed daily for physiological and behavioral changes. All animals were monitored for toxic manifestations such as change in body weight and mortality. After 28 days all surviving animals were fasted overnight, after which blood samples were collected for hematological and biochemical analyses. Animals were sacrificed after blood collection, and internal organ were removed and preserved in 10% formalin for histological examination [13].

Blood was collected from the retro orbital plexus. The hematology parameters measured for the sub-acute toxicity study of PI were white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and lymphocyte (LYMP) [14].

Clinical biochemistry analysed to investigate the major toxic effects on tissues and specifically, the effects on the kidneys and the liver were performed on blood samples obtained from of all animals. Biochemical parameters measured for the sub-acute toxicity study of PI were glucose, albumin, urea, total protein (TP), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT).

After blood collection, the rats were sacrificed for tissue studies. Internal organs such as the kidneys, lungs and liver were isolated. Histological examinations were performed on the tissues, which had been preserved in 10% formalin solution.

In this study, all data are expressed as means ± standard errors of the mean (SEMs). The results were analyzed statistically through Graph pad prism 6.0 by using the two-way analysis of variance (ANOVA), followed by the Bonferroni post-test to calculate the level of significance. The number of animals was six (n = 6); Statistically significant

Table 1 Hematological parameters measured in the male Wistar rats

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
WBC ($10^3/\mu\text{L}$)	3.85 ± 0.03	6.6 ± 0.12 [‡]	2.35 ± 0.14	2.25 ± 0.09 [†]
RBC ($10^6/\mu\text{L}$)	5.35 ± 0.01	6.6 ± 0.12	4.65 ± 0.09	5.6 ± 0.12
HB (g/dL)	10.8 ± 0.06	13.2 ± 0.06 [‡]	10.95 ± 0.03	10.95 ± 0.03
HCT (%)	33.45 ± 0.61	37.65 ± 0.26 [‡]	31.9 ± 0.81 [†]	32.75 ± 0.43
MCV (fL)	60.85 ± 0.25	55.4 ± 0.40 [‡]	59.55 ± 0.14	56.15 ± 0.61 [‡]
MCH (pg)	21.7 ± 0.23	19.8 ± 0.17 [†]	21.1 ± 0.12	19.55 ± 0.32 [†]
MCHC (g/dL)	35 ± 0.06	35.95 ± 0.20	35.6 ± 0.17	34.8 ± 0.23
PLT ($10^3/\mu\text{L}$)	102.5 ± 1.44	85 ± 0.58 [‡]	76 ± 0.58 [‡]	67 ± 0.58 [‡]
LYMP (%)	3 ± 0.06	4.5 ± 0.12	2.3 ± 0.12	1.95 ± 0.03

Values are expressed as means ± SEMs (number of animals, n = 6); and were significantly different at [†] $P < 0.05$, [‡] $P < 0.01$, and [‡] $P < 0.001$, when compared with the control group.

SEMs, standard errors of the mean; WBC, white blood cell; RBC, red blood cell, HB, haemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYMP, lymphocyte.

Table 2 Hematological parameters measured in the female Wistar rats

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
WBC ($10^3/\mu\text{L}$)	3.6 ± 0.25	6.63 ± 0.12	2.17 ± 0.23	2.33 ± 0.12
RBC ($10^6/\mu\text{L}$)	5.38 ± 0.03	6.5 ± 0.15	4.69 ± 0.09	5.5 ± 0.15
HB (g/dL)	10.9 ± 0.11	13.13 ± 0.9	10.77 ± 0.19	11.03 ± 0.09
HCT (%)	33.1 ± 0.7	37.73 ± 0.27	30.83 ± 1.34	33.53 ± 0.89
MCV (fL)	60.43 ± 0.49	55.43 ± 0.41	59.77 ± 0.26	56.97 ± 1.02
MCH (pg)	21.3 ± 0.46	20.27 ± 0.49	21.33 ± 0.26	19.9 ± 0.47
MCHC (g/dL)	34.8 ± 0.21	35.93 ± 0.20	35.83 ± 0.29	34.83 ± 0.23
PLT ($10^3/\mu\text{L}$)	100.33 ± 2.60	86.33 ± 1.45 [‡]	74.67 ± 1.45 [‡]	67.33 ± 0.67
LYMP (%)	2.93 ± 0.09	4.53 ± 0.12	2.07 ± 0.26	2 ± 0.06

Values are expressed as means ± SEMs (number of animals, n = 6); and were significantly different at [†] $P < 0.05$, [‡] $P < 0.01$, and [‡] $P < 0.001$, when compared with the control group.

SEMs, standard errors of the mean; WBC, white blood cell; RBC, red blood cell, HB, haemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYMP, lymphocyte.

difference in the outcomes, when compared to the control group, were indicated as follows: [†] $P < 0.05$, [‡] $P < 0.01$, [‡] $P < 0.001$.

3. Results

An investigation of acute toxicity is an initial step in the characterization of the biological effect of any substance and is necessary for conducting any biological experiment. No deaths or hazardous signs were recorded in the rats

during the 14 days of observation after acute treatment by an oral route with PI at doses of 50, 300, and 2,000 mg/kg of body weight.

In sub-acute toxicity investigation, no toxicity and no deaths were recorded during the 28 days of treatment by an oral route with PI in doses of 250, 500, and 1,000 mg/kg of body weight. No significant changes were observed in the body weights of the both control and the experimental groups of animals (Figs. 1, 2).

The hematological profiles of the animals revealed no significant change in any of the clinical parameters (Tables 1,

Table 3 Biochemical parameters measured in the male Wistar rats

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
GLU (mg/dL)	61.33 ± 0.88	62.33 ± 0.88	71.33 ± 0.88 [‡]	82.67 ± 0.88 [‡]
UREA (mg/dL)	50.33 ± 0.88	54 ± 0.58 [†]	59.33 ± 0.88 [‡]	60.33 ± 0.88 [‡]
TP (g/dL)	10.29 ± 0.03	10.18 ± 0.01	10.03 ± 0.02	9.42 ± 0.02
ALB (g/dL)	4.61 ± 0.02	4.57 ± 0.02	4.59 ± 0.01	4.63 ± 0.01
SGPT (IU/L)	64.67 ± 0.88	55.33 ± 0.88 [‡]	88.67 ± 0.88 [‡]	92.67 ± 0.88 [‡]
SGOT (IU/L)	152.33 ± 1.45	168.67 ± 0.88 [‡]	179.67 ± 0.88 [‡]	181.67 ± 0.88 [‡]

Values were expressed as means ± SEMs (n = 6) and were significantly different at **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001, when compared with control group.

SEMs, standard errors of the mean; GLU, Glucose; TP, total protein; ALB, albumin; SGPT, serum glutamate oxaloacetate transaminase; SGOT, serum glutamate pyruvate transaminase.

Table 4 Biochemical parameters measured in the female Wistar rats

Parameters	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
GLU (mg/dL)	59.67 ± 0.88	62.33 ± 0.88 [†]	72.67 ± 0.88 [‡]	82.67 ± 0.88 [‡]
UREA (mg/dL)	49 ± 0.58	52.67 ± 0.88 [‡]	57.67 ± 0.88 [‡]	60 ± 0.58 [‡]
TP (g/dL)	10.32 ± 0.01	10.17 ± 0.01	10.06 ± 0.01	9.43 ± 0.01
ALB (g/dL)	4.62 ± 0.01	4.54 ± 0.01	4.59 ± 0.01	4.63 ± 0.01
SGPT (IU/L)	64.67 ± 0.88	53.67 ± 0.88 [‡]	87.33 ± 0.88 [‡]	92.67 ± 0.88 [‡]
SGOT (IU/L)	151.67 ± 0.88	168.67 ± 0.88 [‡]	179 ± 0.58 [‡]	181 ± 0.58 [‡]

Values were expressed as means ± SEMs (n = 6) and were significantly different at **P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001, when compared with control group.

SEMs, standard errors of the mean; GLU, Glucose; TP, total protein; ALB, albumin; SGPT, serum glutamate oxaloacetate transaminase; SGOT, serum glutamate pyruvate transaminase

2). The MCHCs for the experimental animals of both sexes were higher than those for the control animals, but the values were within the normal range, demonstrating the methanolic extract of PI exhibited no toxicity.

During the biochemical analyses, all the parameters were in the normal range, and no significant changes were noted when the values of the parameters for the different dose groups were compared with the values for the control group. Slight variations were observed in parameters such as Glucose (GLU), urea, SGPT, SGOT in the 1,000 mg/kg of body weight dose group, but the values were in the normal range (Tables 3, 4).

Different body organs of the male and the female animals were weighed and examined. The organs were found to be devoid of any sign of toxicity, and no significant changes in any of the organ weights were observed (Tables 5, 6).

The histopathological examination of the kidney, lungs and liver were normal in both the control and the treated groups.

4. Discussion

The research protocol was envisaged and designed to evaluate the toxicity profile of methanolic extract of PI with a main focus on observing the normal physiological conditions of experimental animals after treatment with extract. Thus, because of the advancement of research in this area, a wide range of parameters and experimental targets were studied to assess the overall profile of the PI extract to determine if any toxicity was present. The various hematological and biochemical parameters evaluated for the rats showed no abnormalities, and the values were within normal physiological limits and were comparable to the values in the vehicle-treated (control) animals.

Fig. 1 presents the toxicity profile for the PL extract. The PI extract is seen to have had no, toxic effect on any of the observed blood parameters, WBC, RBC, HB, HCT, MCH, MCHC, PLT and LYMP, for the different dose levels. Regardless of the doses of the PI extract the blood parameters were within the normal range. In Fig. 2, small differenc-

Table 5 Organ body weights of the male Wistar rats

Organs	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Testes	2.33 ± 0.31	2.41 ± 0.31	2.49 ± 0.31	2.83 ± 0.31
Epididymis	1.12 ± 0.12	1.38 ± 0.16	1.47 ± 0.11	1.88 ± 0.13
Urinary bladder	0.21 ± 0.01	0.25 ± 0.01	0.28 ± 0.03	0.30 ± 0.02
Seminal vesicles	1.24 ± 0.21	1.34 ± 0.28	1.41 ± 0.28	1.58 ± 0.27
Prostate	0.67 ± 0.05	0.89 ± 0.06	0.99 ± 0.06	1.05 ± 0.05
Spleen	0.89 ± 0.01	0.91 ± 0.02	0.98 ± 0.02	1.18 ± 0.03
Kidney	2.49 ± 0.06	2.63 ± 0.06	2.89 ± 0.05	2.98 ± 0.06
Liver	10.15 ± 1.25	10.23 ± 1.73	10.49 ± 1.81	10.88 ± 1.71

Values were expressed as means ± SEMs (n = 6) and were significantly different at * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$, when compared with control group.

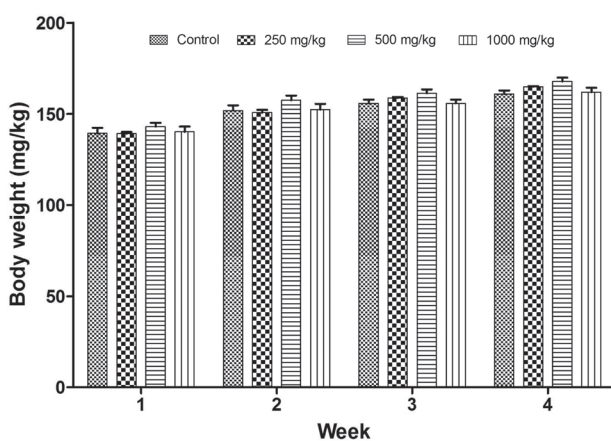
SEMs, standard errors of the mean.

Table 6 Organ body weights of the female Wistar rats

Organs	Control	250 mg/kg	500 mg/kg	1,000mg/kg
Liver	9.02 ± 0.31	9.98 ± 0.09 [†]	10.21 ± 0.35 [†]	10.64 ± 0.36 [†]
Kidney	1.64 ± 0.23	1.51 ± 0.001	1.55 ± 0.005	1.59 ± 0.03
Spleen	0.53 ± 0.004	0.59 ± 0.02	0.60 ± 0.03	0.61 ± 0.03
Urinary bladder	0.17 ± 0.006	0.18 ± 0.007	0.19 ± 0.04	0.21 ± 0.03
Uterus	0.18 ± 0.007	0.37 ± 0.01	0.38 ± 0.05	0.40 ± 0.03
Ovary	0.19 ± 0.06	0.20 ± 0.01	0.21 ± 0.04	0.23 ± 0.02

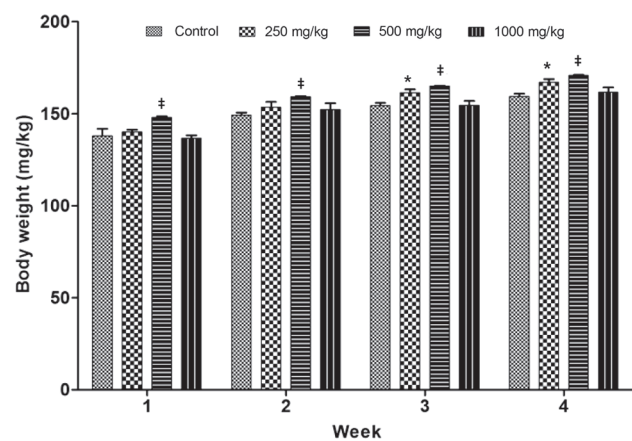
Values were expressed as means ± SEMs (n = 6) and were significantly different at * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$, when compared with control group.

SEMs, standard errors of the mean.

**Figure 1** Mean body weights of male Wistar rats.

Values are expressed as means ± SEMs (number of animals, n = 6) and were; significantly different at * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$, when compared with the control group.

SEMs, standard errors of the mean.

**Figure 2** Mean body weights of female Wistar rats.

Values are expressed as means ± SEMs (number of animals, n = 6) and were; significantly different at * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$, when compared with the control group.

SEMs, standard errors of the mean.

es in the levels of the biochemical parameters SGPT and SGOT can be seen. Higher doses of the PI extract, i.e. 500 and 1,000 mg/kg of body weight, both caused increase in the SGPT and the SGOT levels, but these increases did not take the levels outside the normal range. Histopathological evaluation showed no adverse effects of PI extract on the dissected organs; swelling, atrophy, and hypertrophy were not observed. The results of the urine analysis showed no signs of toxicity in the urine; the parameters being within the normal range; creatinine, pH, color, quantity of urine. However, pus cells, sperms, bacteria, and ketone bodies were found, but rarely. No statistically significant differences were found in any of the parameters between the control and the treated groups with respect to various vital organs, and no visceral abnormalities were seen in any of the groups. All animals survived until the scheduled euthanasia and no significant gross pathological alterations were found in the internal organs.

5. Conclusion

In summary, our acute and sub-acute toxicity studies of Wistar rats revealed no toxicological symptoms or mortality at any of the dose levels of methanolic extract of PI used in this research. Daily treatments with PI at intervals of 24 hours in doses of 250, 500, and 1,000 mg/kg of body weight p.o. had no adverse effect on body weight. The various hematological and biochemical parameters evaluated in the rats showed no abnormalities, and the values were within the normal physiological limits and were comparable to the values for the vehicle-treated (control) animals. The methanolic extract of PI was found to be non-toxic based on the results of the oral acute and sub-acute toxicity tests performed on rats in this research. However, an evaluation of chronic toxicity is needed to determine the long-term safety of the extract.

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Conflict of interest

The authors declare that there are no conflict of interest.

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