Original Article

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Acute Oral Toxicity of Aqueous Alcoholic Extract of *Punica granatum* Fruit Pulp in Female Swiss Albino Mice: An Experimental Study

Pharmacology Section

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ABSTRACT

Introduction: *Punica granatum* L. (pomegranate) is broadly consumed as a dietary fruit and has a long history of use in traditional health practices. The edible fruit pulp, comprising the fleshy arils, has been incorporated into diets across regions for its taste and nutritional value.

Aim: To assess the acute oral toxicity of an aqueous-alcoholic extract of *Punica granatum* fruit pulp in female Swiss albino mice in accordance with Organisation for Economic Cooperation and Development (OECD) Guideline 423.

Materials and Methods: An experimental study was carried out in centre for Laboratory Animal Research (CLAR) Saveetha Medical College and University, Chennai, Tamil Nadu, India. The animals were procured from Biogen laboratory, animal facility, Bangalore, Karnataka, India. The study was conducted from 8th to 23rd April 2025. Healthy, adult female Swiss albino mice aged 8 to 12 weeks were included in the study. A limit test at one dose level of 2000 mg/kg body weight was carried out with six animals (3 animals per step). According to OECD 423, mortality and toxicity, clinical signs for exclusively initial few hours. Body mass, food and water intake, psychological behavioural changes were assessed and later for a period of 14 days. At study termination, haematological indices, serum biochemical parameters and urine profiles were assessed. Histopathological examination of major organs was performed to evaluate tissue architecture. Statistical analysis was done to analyse significance level in the study.

Results: No mortality or treatment-related clinical signs were noticed throughout the observation period. Body weight progression, food and water intake remained comparable to controls. Haematological parameters, serum liver enzymes {Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-glutamyl Transferase (GGT)}, renal markers {urea, creatinine, Blood Urea Nitrogen (BUN), uric acid} and lipid profile values showed no significant differences between treated and control groups (p-value >0.05). Urinalysis and histopathology revealed normal structural features without evidence of cellular degeneration, inflammation, or organ-specific toxicity.

Conclusion: The aqueous-alcoholic extract of *Punica granatum* fruit pulp did not elicit acute toxic effects at a limit dose of 2000 mg/kg in mice, indicating a high margin of safety. These results provide baseline toxicological support for the safe practice of pulp-derived preparations; however, additionally subchronic and chronic studies are required to establish long-term safety thresholds.

Keywords: Enzymes, Histopathology, Pomegranate, Toxicology

INTRODUCTION

Punica granatum L. (pomegranate) is broadly consumed as a dietary fruit and has a long history of use in traditional health practices. The edible fruit pulp, comprising the fleshy arils, has been incorporated into diets across regions for its taste and nutritional value and has traditionally been utilised in formulations intended to support general wellbeing and gastrointestinal comfort [1,2]. In contemporary dietary settings, pomegranate pulp and its juice are commonly marketed as functional food products, reflecting increasing interest in naturally derived plant materials for health maintenance [3].

The pulp contains a diverse profile of phenolic compounds, including anthocyanins, ellagic acid derivatives, hydrolysable tannins and flavonoids, which contribute to its chemical stability and redox activity [4]. These constituents have been investigated in preclinical research for their capacity to modulate oxidative stress pathways; however, the presence of bioactive phenolics also underscores the need for systematic toxicological evaluation, as phytochemical richness alone does not guarantee safety at concentrated or supplemental doses.

Current toxicological evidence indicates that standardised pomegranate fruit extracts derived largely from pulp or juice exhibit a wide margin of safety in rodent models, with oral LD50 (Lethal Dose 50%) values exceeding 5 g/kg and no adverse effects reported during repeated-dose exposure up to 600 mg/kg/day over 90 days. Protein fractions isolated specifically from the pulp have also shown no acute toxicity or genotoxicity in mice at doses up to 2000 mg/kg [5]. However, documented variability in extraction methods, cultivar chemistry and phytochemical concentration complicates direct extrapolation across preparations. In addition, some in-vitro observations and isolated livestock toxicity cases indicate that safety cannot be universally assumed across contexts or exposure levels [6].

Despite broad consumption and generally favourable toxicological findings, critical gaps remain regarding the long-term safety and high-dose tolerability of pulp-derived extracts, as well as the potential for reproductive or genotoxic effects under prolonged exposure [7]. Human safety data at supplemental dosages also remain limited. Accordingly, controlled acute toxicity studies are necessary to establish foundational safety parameters and determine whether concentrated pulp extracts can be used without eliciting systemic or organ-specific toxic effects.

The present investigation evaluates the acute oral toxicity of an aqueous-alcoholic extract of *Punica granatum* fruit pulp in female Swiss albino mice, following OECD Guideline 423 [8]. The aim was to determine the safety profile at a limit dose and to provide initial toxicological reference data to support future investigations into dosage, tolerability and long-term exposure properties.

MATERIALS AND METHODS

The present experimental study was conducted in accordance with OECD 423 test guidelines between 8th to 23rd April 2025 in Centre for Laboratory Animal Research (IAEC (Registration number: 1183/PO/Re/S/08/CPCSEA,) Saveetha Medical College and Hospital, Chennai, Tamil Nadu, India on approval from animal of institutional animal ethics committee, meeting held in March 2025 approval no: (SU/CLAR/RD/01/2025). All experimental procedures were carried out in compliance with the guidelines outlined in the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and followed the Organisation for Economic Co-operation and Development (OECD) Guideline 423 for acute oral toxicity testing in rodents [8].

Inclusion criteria: Healthy adult female Swiss albino mice aged 8 to 12 weeks, weighing 25 to 35 g ±20%, non-pregnant, nulliparous which were clinically normal with no visible signs of disease or abnormality from Animal House Facility, Saveetha Institute of Medical and Technical Sciences, Chennai, India (CPCSEA Registration No. 1183/PO/Re/S/08/CPCSEA, dated 20 April 2018) were included in the study.

Exclusion criteria: The study excludes animals demonstrating any signs of illness, unusual behaviour, body weight variation, pregnant, lactating, previously used for experiments or any other condition found abnormal on veterinarian evaluation.

Sample size: Total sample size is 6.

The animal requirement criteria were in accordance with the OECD 2001 guidance and reduction principle [9].

Extraction Procedure

Fresh fruits of *Punica granatum* L. were collected from local agricultural markets in Bengaluru, Karnataka, India, during September 2024. The botanical identity of the plant material was confirmed and deposited in the Herbarium of the Faculty of Pharmaceutical Research and Pharmacognosy, Sri Dharmasthala Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, for future reference and authentication records. Approximately 1kg of fresh fruit was manually peeled and the pulp was separated from the seeds. The pulp was homogenised using a mechanical blender to obtain a uniform slurry. The homogenised material was subjected to maceration in a hydroethanolic solvent system (distilled water:ethanol, 1:1 v/v) at a ratio of 1:1 (w/v) using 1 L of solvent. The mixture was kept at room temperature for 48 hours with intermittent stirring to enhance solvent penetration and extraction efficiency [10-12].

Following maceration, the mixture was filtered through muslin cloth to remove coarse plant debris and the filtrate was further clarified using Whatman No. 1 filter paper. The resulting extract was concentrated using a water bath maintained at 45-50°C until a semi-solid mass was obtained. The concentrate was then dried to a constant weight and stored in airtight, light-protected containers under refrigeration until further use. This extraction approach was consistent with established phytochemical extraction protocols employing maceration followed by low-temperature solvent removal to minimise degradation of thermolabile constituents [13-15].

Toxicity Studies

Acute toxicity study: A limit test accordance with OECD Guideline 423 was followed to conduct the acute toxicity study. A total of six female Swiss albino mice were used in this experimental study. All the animals were housed for seven days prior to the drug administration for acclimatisation in a polypropylene cage at 22±3°C, relative humidity of 40-70% and a 12 hour light-dark cycle and animals were fed with standard laboratory pellet diet and water was supplied ad libitum. Prior to the experiment, the animals were fasted overnight and water was withheld for three to four hours and weighed before administration of test compound. Initially a set of three animals were given a single oral dose of 2000 mg/kg body weight of the aqueous-alcoholic *P. granatum* fruit pulp extract by oral gavage and were observed initially for first few hours intensely and continued for 24 hours [Table/Fig-1]. If no mortality occurs the limit test dosing was stopped and the remaining animals were kept as control group for observed for any signs of mortality alterations in posture and locomotion, behavioural changes, autonomic responses, appearance of convulsions or tremors, grooming behaviour, respiratory pattern changes and any other clinical signs indicative of toxicity. Body weight, food and water intake were recorded at baseline and monitored throughout the study. At the end of the 14-day observation period, all animals were euthanised for evaluation of relative organ weights, haematological and biochemical parameters, followed by histopathological examination of major organs [16].

General appearance	Fur condition, posture, grooming
Behavioural signs	Alertness, locomotion, response to handling
Autonomic responses	Secretions, voluntary and involuntary reflexes defecation
Neurological signs	Tremors, convulsions, gait abnormalities, righting reflex
Respiratory and circulatory signs	RR, HR, colour change in mucous membrane

[Table/Fig-1]: Clinical observations.

Body weight was recorded on days 0, 7 and 14. Morbidity, mortality and any visible indicators of distress were documented throughout the observation period to ensure compliance with humane endpoint criteria.

Necropsy and histopathology: At the termination of the 14-day observation time, all experimental animals were euthanised and a complete gross necropsy was performed. Major organs including the liver, spleen, kidneys, heart and ovaries were dissected, blotted to remove excess moisture and weighed to determine relative organ weights. Each organ was examined macroscopically for evidence of congestion, discolouration, hypertrophy, atrophy, or any other pathological alterations [17,18].

Biopsy from these organs were fixed in 10% neutral buffered formalin for at least 24-48 hours, followed by routine processing using the paraffin-embedding technique. Approximately, 5 µm thickness sections were prepared and Haematoxylin and Eosin (H&E) staining was performed. Histological evaluation was conducted under light microscopy to assess cellular morphology, tissue architecture, inflammatory changes, degenerative lesions, or necrotic features [19].

Haematological and biochemical analyses: At the end of the study, blood was collected from each mouse via the retro-orbital sinus under light anaesthesia. Approximately 1 mL of blood was collected into EDTA-coated tubes for haematological evaluation and an additional 1-2 mL was collected into plain tubes for serum separation. Haematological parameters, including Red Blood Cell count (RBC), White Blood Cell count (WBC), Haemoglobin Concentration (HGB), Haematocrit (HCT), Platelet Count (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Neutrophils (NEUT), Lymphocytes (LYM) and Mixed Cell Fraction (MXD: monocytes, eosinophils and basophils), were quantified using an automated haematology analyser (Sysmex XT-1800, Germany).

Serum was obtained by centrifuging blood collected in plain tubes at 3000 rpm for 10 minutes and stored at -20°C until biochemical analysis. Serum biochemical parameters were assessed using a fully automated biochemical analyser (Olympus AU 640, Japan). Liver function markers included ALT, AST and Albumin (ALB). Cardiac biomarkers assessed were Creatine Kinase-MB (CK-MB)

and troponin I. Renal function was evaluated by measuring serum urea and creatinine. Lipid profile analysis included total cholesterol, triglycerides, Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL), determined using serum aliquots obtained from heparinised collection [20-23].

STATISTICAL ANALYSIS

All quantitative data are presented as mean±Standard Error of the Mean (SEM), with six animals per group (n=6). Students unpaired t-test was performed to compare two group mean data. A p-value <0.05 was considered statistically significant

RESULTS

Acute toxicity observations: Administration of a single oral dose of 2000 mg/kg body weight of the aqueous-alcoholic extract of *Punica granatum* fruit pulp produced no mortality or treatment-related adverse effects in female Swiss albino mice throughout the 14-day observation period. All animals remained active and alert, exhibiting normal grooming, locomotor behaviour and responsiveness to external stimuli. There were no observable abnormalities in posture, fur condition, or mucosal colouration. Autonomic responses such as salivation, lacrimation, defecation pattern and piloerection remained within normal physiological limits. No neurological signs, including tremors, convulsions, gait disturbance, or altered reflex responses, were noted.

Mortality and morbidity: No morbidity or mortality was recorded in any group over the course of the study, confirming the absence of acute systemic toxicity at the administered dose.

Serum Biochemistry

Effect on cardiac markers: Serum biochemical markers of hepatic function displayed no statistically significant differences between the extract-treated and control groups. Levels of cardiac markers CKMB and Troponin I remained within physiological ranges, indicating an absence of cardiomyocytes injury following oral administration of the extract [Table/Fig-2].

Parameter	Control (Mean±SEM)	Treated (Mean±SEM)	t-value	p-value	Significance
CKMB (IU/L)	13.45±1.1	14.21±1.2	1.13	0.56	Not significant
Troponin I (ng/mL)	35.45±2.4	30.56±2.7	1.34	0.59	Not significant

[Table/Fig-2]: Cardiac markers.
CKMB: Creatinine kinase myocardial band; Values represent the mean±SEM (n=3 per group); Significance in comparison to control group was obtained using unpaired students t test; The p-value >0.05 was considered not significant

Effect on liver function parameters: Serum biochemical markers of hepatic function displayed no statistically significant differences between the extract-treated and control groups. Levels of ALT, AST, ALP and GGT remained within physiological ranges, indicating an absence of hepatocellular injury or cholestatic changes following oral administration of the extract [Table/Fig-3].

Effect on serum kidney function parameters: Renal function was estimated by measuring serum concentrations of urea, creatinine, BUN and uric acid. No statistically significant differences were detected between the extract-treated and control groups. All values remained within established physiological ranges for female Swiss albino mice, indicating that administration of the aqueous-alcoholic *Punica granatum* extract at 2000 mg/kg did not induce renal impairment [Table/Fig-4].

Histopathological study of different tissue samples: The tissues samples colour and size remained normal in all the sacrificed mice. All the collected tissues undertaken for H&E staining under 400x magnification. This experimental study revealed that all the samples were normal, no abnormalities were observed in [Table/Fig-5-7].

Parameter (IU/L)	Control (Mean±SEM)	Treated (Mean±SEM)	t-value	p-value	Significance
AST	40.3±1.6	42.1±1.8	1.29	0.49	Not significant
ALT	40.1±2.4	42.3±2.6	1.07	0.57	Not significant
ALP	59.2±4.3	67.2±4.8	2.15	0.28	Not significant
GGT	40.5±1.6	43.5±1.9	2.09	0.29	Not significant

[Table/Fig-3]: Serum liver function parameters in control and extract-treated mice.
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; Values represent the mean±SEM (n=3 per group); Significance in comparison to control group was obtained using unpaired students t-test; The p-value >0.05 was considered significant

Parameter (mg/dL)	Control (Mean±SEM)	Treated (Mean±SEM)	t-value	p-value	Significance
Urea	31.5±2.4	30.9±2.2	0.31	0.86	Not significant
Creatinine	0.56±0.06	0.52±0.05	0.88	0.64	Not significant
BUN	13.8±0.4	14.3±0.5	1.35	0.48	Not significant
Uric Acid	3.6±0.3	3.8±0.2	0.96	0.60	Not significant

[Table/Fig-4]: Serum renal function parameters in control and extract-treated mice.
Values represent the mean±SEM (n=3 per group); Significance in comparison to control group was obtained using unpaired students t-test; p>0.05 was considered significant. The p-value >0.05, No significant differences were found in comparison with control

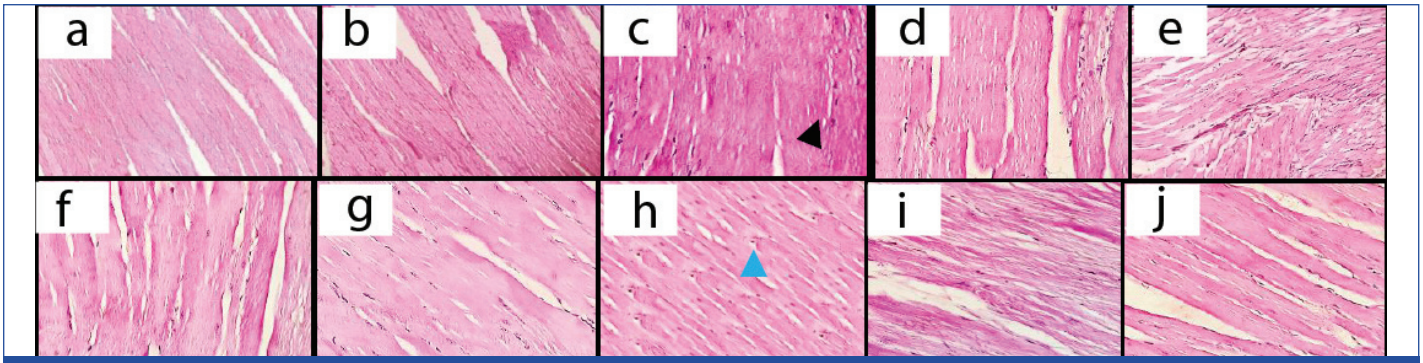
Effect on serum lipid profile: Serum lipid profile parameters, including total cholesterol, triglycerides, HDL and LDL, were measured to evaluate the effect of the extract on lipid metabolism. There were no statistically significant differences between the control and extract-treated groups for any of the lipid parameters assessed, with all values remaining within normal physiological ranges [Table/Fig-8].

Body weight monitoring: Body weights were documented on days 0, 7 and 14 to evaluate the effect of the extract on growth and general physiological status. Both control and extract-treated groups showed a steady rise in body mass over the study time. No statistically significant differences were noticed between groups at any time point (p-value >0.05), indicating that administration of the *Punica granatum* extract at 2000 mg/kg did not affect normal growth patterns [Table/Fig-9].

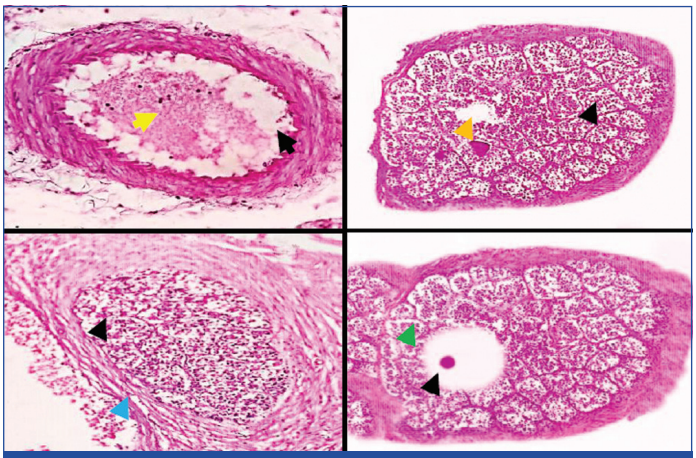
Food intake: Daily food intake remained comparable between the control and extract-treated sets throughout the 14-day observation period. Although a slight upward trend was observed in the treated animals over time, the differences were not statistically significant (p-value >0.05), indicating that administration of the extract did not interfere with feeding behaviour or appetite regulation [Table/Fig-10].

Water intake: Water consumption was monitored throughout the 14-day observation period to assess any extract-induced changes in hydration status or metabolic demand. No statistically significant differences in daily water intake were detected between the control and treated groups at any time point (p-value >0.05), indicating that administration of the *Punica granatum* extract did not affect drinking behaviour or fluid balance [Table/Fig-11].

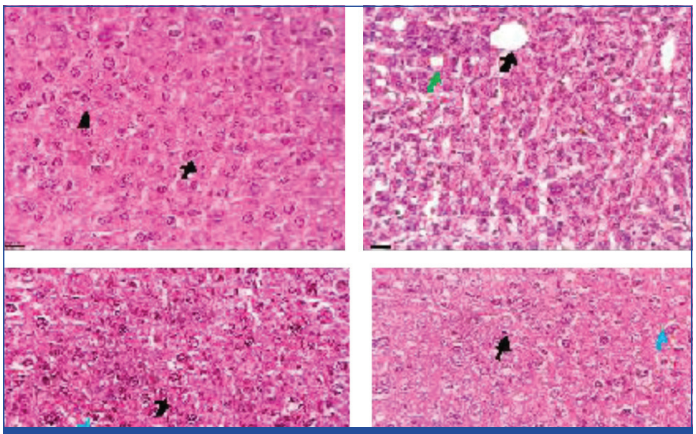
Haematological parameters: Haematological indices were evaluated to determine whether administration of the aqueous-alcoholic *Punica granatum* extract influenced erythropoietic, leukocytic, or thrombocytic profiles. No statistically significant differences (p-value >0.05) were noticed between the treated and control groups for haemoglobin concentration (Hb), RBC count, WBC count, PLT, or Packed Cell Volume (PCV). All values remained within established physiological ranges for female Swiss albino mice, indicating the absence of haematopoietic toxicity [Table/Fig-12].



[Table/Fig-5]: a-e) Heart tissue (arrows) showed no gross morphological adverse changes, tissues are intact cardiomyocytes and intercalated disc longitudinal section of tissue architecture, cytoplasm and cardiomyocytes and showed normal tissue architecture in the atrium region, centrally placed nucleus and absence of pathological changes. f-j) Heart tissue showed arranged muscle fibers and adverse changes are not seen. No inflammation in cardiac cells and absence of signs of damage.



[Table/Fig-6]: Photomicrograph mouse ovary tissue showed (yellow and black coloured arrows denote developed oocyte with no gross morphological changes, green and blue denoting shows many folliculi distributed between deeply basophilic stained granulosa cells and also mature oocyte, corpus luteum and basophilic granulosa cells) under 400x by H&E staining.



[Table/Fig-7]: Histopathological study of female Swiss albino mice liver. H&E staining under 400x magnification.
Liver section indicated the presence of hepatic cells (arrow) with prominent nucleus, hepatic lobules and central vein (blue arrow). Portal lobules (arrow head) and central vein in the central of the lobules (arrowhead). The liver tissues revealed with no inflammation. The section showed binucleate hepatocyte, granular cytoplasm and prominent nucleoli. A clear central vein is lined by a single layer of endothelial cells, sinusoidal capillaries and normal hepatocytes with round nuclei. Hepatocytes are arranged in plates

No deviations indicative of anaemia, leukocytosis/leukopenia, thrombocytopenia, or haemoconcentration were observed [Table/Fig-13].

Urine analysis: Urinalysis was performed to further evaluate renal function and hydration status. No abnormalities were observed in urine volume, pH, or physical appearance between control and treated groups. Both groups showed clear, pale-yellow urine with comparable output volumes, indicating preserved renal concentrating ability and normal fluid balance [Table/Fig-14].

These results support the absence of extract-related renal impairment or osmotic stress.

Parameter (mg/dL)	Control (Mean±SEM)	Treated (Mean±SEM)	T-value	p-value	Significance
Total cholesterol	79.6±2.3	81.2±2.7	0.78	0.68	Not significant
Triglycerides	51.7±4.3	54.7±4.6	0.82	0.43	Not significant
HDL	42.9±1.0	42.2±0.5	1.08	0.57	Not significant
LDL	66.5±2.9	64.6±2.2	0.90	0.63	Not significant

[Table/Fig-8]: Serum lipid profile in control and extract-treated mice.
Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t-test. The p-value >0.05 was considered not significant. (HDL: High density lipoproteins, LDL: Low density lipoprotein)

Day	Control (Mean±SEM) (g)	Treated (Mean±SEM) (g)	t-value	p-value
0	23.67±1.47	23.33±1.40	0.29	0.88
7	24.16±1.27	22.33±1.98	1.34	0.48
14	26.33±1.61	25.83±1.60	0.38	0.84

[Table/Fig-9]: Body weight changes in control and extract-treated mice.
Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t-test. The p-value >0.05 was considered not significant. No significance difference between control and test group

Day	Control (g/day)	Treated (g/day)	t-value	p-value
0	4.12±0.25	4.18±0.22	0.31	0.87
7	4.35±0.30	4.42±0.28	0.29	0.87
14	4.50±0.27	4.63±0.31	0.54	0.77

[Table/Fig-10]: Daily food intake in control and extract-treated mice.
Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t-test. The p-value >0.05 was considered not significant. Significant differences were not found in comparison with control

Day	Control (mL/day) (Mean±SEM)	Treated (mL/day) (Mean±SEM)	t-value	p-value
0	4.37±0.34	4.53±0.74	0.34	0.85
7	4.56±0.12	4.61±0.29	0.16	0.87
14	4.63±0.46	3.93±0.34	2.11	0.29

[Table/Fig-11]: Daily water intake in control and extract-treated mice.
Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t-test. The p-value >0.05 was considered not significant

DISCUSSION

Absence of apparent impact on cardiovascular, hepatic, renal, reproductive and neurological functions during the 14-day observation period following limit dose administration confirmed the safety of the aqueous-alcoholic extract of *Punica granatum* fruit pulp, indicating that the extract is likely safe when administered orally.

The current experimental study explored the acute oral toxicity profile of an aqueous-alcoholic extract of *Punica granatum* fruit pulp in female Swiss albino mice following OECD Guideline 423.

Parameter	Control (Mean±SEM)	Treated (Mean±SEM)	t-value	p-value	Significance
Haemoglobin (g/dL)	11.5±0.3	11.8±0.3	1.22	0.52	Not significant
RBC (×10⁶/µL)	3.98±0.05	3.87±0.04	2.97	0.16	Not significant
WBC (×10³/µL)	6.18±0.16	6.16±0.18	0.14	0.93	Not significant
Platelets (×10³/µL)	393.2±8.3	383.6±7.9	1.45	0.44	Not significant

[Table/Fig-12]: Haematological parameters in control and extract-treated mice. Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t test. The p-value >0.05 was considered not significant. Results between test and control are not significant. (RBC: Red blood cells; WBC: White blood cells).

Parameter	Control (Mean±SEM)	Treated (Mean±SEM)	T-value	p-value	Significance
PCV (%)	37.0±1.4	36.0±1.3	0.90	0.63	Not significant

[Table/Fig-13]: Packed Cell Volume (PCV) in control and extract-treated mice. Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t test. The p-value >0.05 was considered not significant. Results between test and control are not significant. (PCV: Packed cell volume)

Parameter	Control	Treated	T-value	Interpretation
Urine volume (mL/24 h) mean±SEM	1.48±0.26	1.53±0.31	0.21	No significant difference (p=0.91)
pH	7.0	7.0	-	Neutral pH maintained
Appearance	Clear, pale yellow	Clear, pale yellow	-	Normal

[Table/Fig-14]: Urine analysis parameters in control and extract-treated mice. Values represent the mean±SEM (n=3 per group). Significance in comparison to control group was obtained using unpaired students t test. The p-value >0.05 was considered not significant

Administration of a single 2000 mg/kg dose did not result in death, behavioural abnormalities, or clinical signs indicative of systemic toxicity during the 14-day observation period. Body mass progression, food and water intake remained comparable to the control group, suggesting that the extract did not interfere with metabolic homeostasis or general physiological function. Haematological indices, serum biochemical markers of hepatic and renal function and lipid profile parameters were within normal biological variation, further supporting the absence of acute toxic effects [24,25].

The absence of toxicity detected in the current experimental study is consistent with the known phytochemical composition of *P. granatum* pulp. The fruit pulp is rich in phenolic constituents, particularly punicalagin, ellagic acid derivatives, granatin-type tannins, anthocyanins such as cyanidin glycosides and flavonoids including quercetin and naringenin. These compounds have been widely reported to possess strong free radical–scavenging and metal-chelating properties, contributing to their chemical stability and low intrinsic reactivity in-vivo [26-28]. The predominance of such antioxidant polyphenols in the pulp may play a protective role in limiting lipid peroxidation, protein oxidation and oxidative stress-mediated cytotoxicity that typically underlies acute organ injury.

Previous studies evaluating pomegranate extracts in toxicant-induced organ injury models provide mechanistic context for the present findings [29]. Extracts from the peel, juice and other fruit fractions have been shown to preserve hepatocyte architecture and renal tubular integrity, with reported reductions in lipid peroxidation and improvements in antioxidant enzyme activities in models of carbon tetrachloride, vancomycin, lead acetate and monosodium glutamate exposure [30-32]. The preserved biochemical and histological profiles observed in the current experimental study align with these data, suggesting that the pulp extract does not impose oxidative burden under physiological conditions and lacks inherent hepatotoxic or nephrotoxic potential at the administered dose [14,33].

The safety outcomes reported herein are also consistent with prior acute and subacute toxicity evaluations of pomegranate fruit preparations. Oral LD50 values for standardised pomegranate extracts have been reported to exceed 5 g/kg in rodents, with no observed antagonistic effects at doses up to 600 mg/kg/day in 90-day toxicity studies [10]. Similarly, ethanolic and hydroalcoholic extracts of pomegranate peel, seeds and whole fruit have been shown to be well tolerated in mice at doses up to 2000 mg/kg, with no significant changes in clinical behaviour, haematology, or organ histopathology [12]. The present data therefore reinforce the existing evidence that *P. granatum* exhibits a wide margin of safety in rodent models.

Taken together, the discoveries from this study confirm that the aqueous–alcoholic extract of *P. granatum* fruit pulp does not elicit acute toxic effects at a limit dose of 2000 mg/kg, as demonstrated by normal behavioural responses, physiological indices, blood chemistry and histological architecture of major organs. These results contribute to the toxicological reference framework for *P. granatum* and support its broad safety margin for oral administration. However, safety outcomes under repeated dosing conditions and at higher exposure levels will require further assessment through subchronic and chronic toxicity studies to establish definitive No-Observed-Adverse-Effect Levels (NOAELs) for long-term use.

In this experimental study of the Aqueous alcoholic extract of *Punica granatum* fruit pulp shows that the LD50 was greater than 2000 mg/kg following single-dose oral administration in mice models indicating safety, tolerability and are supported by haematological and biochemical indicators, histopathological and other physical results. However, chronic, subchronic studies, sex-based comparison and advanced toxicological profiling are required for future pharmacological implication.

Limitation(s)

The animal toxicity studies are a crucial aspect of preclinical pharmaceutical assessment however; they pose a variety of known limitations that restrict its relevance in safety for humans. Integrative approaches are required to be adopted. The study employed only a single high-dose (2000 mg/kg) limit test, without evaluating multiple dose levels. Therefore, dose-response relationships and the precise threshold for toxicity could not be established. The study included only female Swiss albino mice and sex-based differences in toxicity were not assessed.

CONCLUSION(S)

The findings of this investigation demonstrated the aqueous–alcoholic extract of *Punica granatum* fruit pulp is well tolerated in female Swiss albino mice at an acute oral limit dose of 2000 mg/kg, with no mortality or clinical symptoms of toxicity observed throughout the 14-day monitoring period. Body mass progression, food and water intake patterns, haematological indices, serum biochemical markers of hepatic and renal function, lipid profile values and histopathological examinations of major organs remained within normal physiological ranges. These outcomes indicate the absence of systemic, metabolic, or organ-specific toxicity under the tested conditions and support a high protection margin for the extract. While the present findings establish its acute safety profile, additionally subchronic and chronic toxicity studies are warranted to define lasting tolerability and govern a definitive NOAEL for extended use.

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