RESEARCH ARTICLE

Acute Toxicity Test of Pomegranate (*Punica granatum L.*) Peel Extract Nanogel on the Liver and Kidneys of Wistar Rats

Armia Syahputra[1*], Hafid Syahputra[2], Rezky Nadira[1], Verina Angelique[1]

[1]*Departement of Periodontology, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia*
[2]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya-20155, South Sumatra, Indonesia*

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ABSTRACT

Introduction: Periodontal disease is a chronic inflammatory condition of the supporting tissues of the teeth caused primarily by biofilm-associated microbial infection. With a high global prevalence—including 74.1% in Indonesia as of 2022—conventional treatment relying on mechanical therapy and synthetic drugs often faces challenges such as adverse effects and the development of antibiotic resistance. As a safer alternative, herbal therapies using pomegranate (*Punica granatum L.*) peel extract, rich in bioactive compounds like flavonoids, tannins, and punicalagin, offer antioxidant, antibacterial, and anti-inflammatory benefits. However, safety evaluations, including acute toxicity testing, are essential prior to clinical application, especially in nanogel formulations designed for topical use.

Materials and methods: This laboratory-based experimental study used a post-test control group design involving 30 female Wistar rats, divided into one control group (0.5% Na-CMC) and four treatment groups receiving nanogel doses of 5, 50, 300, and 2000 mg/kg BW (BW). Parameters assessed included clinical signs of toxicity, mortality, body weight changes, organ weights, macroscopic appearance of the liver and kidneys, serum levels of creatinine, ALT, and AST, as well as histopathological evaluations of the liver and kidneys.

Results: No mortality or significant toxic symptoms were observed throughout the 14-day observation period. There were no statistically significant differences ($p > 0.05$) in body weight, relative organ weights, or biochemical markers (creatinine, ALT, AST) between the treatment and control groups. Histopathological analysis showed normal liver architecture across all groups, while mild inflammation in renal tissue was observed only at the highest dose (2000 mg/kg BW).

Conclusion: The administration of pomegranate peel extract nanogel up to a dose of 2000 mg/kg BW resulted in no systemic toxicity and only mild, non-lethal renal inflammation. These findings suggest that the formulation is well-tolerated in acute exposure and may be considered safe for further development as a topical adjunctive therapy in periodontal treatment.

Keywords: acute toxicity test, pomegranate peel, nanogel, liver, kidney.

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INTRODUCTION

Periodontal disease is a progressive inflammatory condition that affects the teeth-supporting tissues, including the gingiva, periodontal ligament, cementum, and alveolar bone. The inflammation is caused by microorganisms in the oral cavity that develop due to plaque buildup on the gingival margin, which is exacerbated by poor oral hygiene[1]. Other factors that influence the development of this disease include environmental, physical, social, stress, and systemic factors. Periodontal disease is one of the most prevalent oral diseases in the world, with a global prevalence of 19%, and 74.1% in Indonesia, according to the 2022 Riset Kesehatan Daerah Indonesia[2,3]. Common periodontal diseases found are gingivitis and periodontitis. Gingivitis, a mild form of periodontal disease, can progress to periodontitis if left untreated, which causes loss of attachment and damage to the supporting tissues of the teeth. If left untreated, this disease can lead to tooth loss, tooth mobility, impaired chewing function, and a decline in the patient's quality of life[4].

Treatment of periodontal disease is typically performed using mechanical therapy; however, its effectiveness is limited to removing biofilm in deep periodontal pockets[5]. Synthetic drugs are used as an additional therapy, but can cause side effects such as bacterial resistance[6]. As an alternative, herbal medicine has the advantage of minimal side effects and is safer than synthetic chemical drugs. One herbal medicine that can be used is pomegranate peel extract (*Punica granatum L.*), which contains active compounds such as flavonoids and tannins with demonstrated antioxidant and antibacterial activity[7]. Studies have shown that pomegranate peel extract effectively inhibits the proliferation of microorganisms responsible for periodontal disease, including *Enterococcus faecalis* and *Streptococcus mutans*[8].

**Author for Correspondence: armia.syahputra@usu.ac.id*

Table 1: Formulation of Pomegranate Peel Extract Nanogel Preparation

Material	Formulation
Pomegranate peel extract (g)	25
Karbopol 940 (g)	0.625
HPMC K4M (g)	0.625
TEA (g)	1
Gliserin (g)	10
Nipagin (g)	0.1
Nipasol (g)	0.1
Aquadest ad (l)	100

Table 2: Results of the Spreadability Test of Nanogel Preparation

Additional load (g)	Spreadability Diameter Results (cm)
100	6
150	7

The use of herbal medicine in the form of topical preparations for treating periodontal disease remains limited. Nanogel, a semi-solid preparation composed of nanoparticles within a hydrogel system, can be a viable solution due to its biocompatibility, biodegradability, and high drug loading capacity[9]. Research shows that pomegranate peel extract nanogel shows more effective anti-inflammatory and antibacterial effects at a concentration of 25%[10]. Before being used in humans, toxicity tests are needed to ensure the safety of using pomegranate peel extract nanogel. Acute toxicity tests were conducted on Wistar rats to detect toxic effects within a short timeframe and ensure a safe dose[11]. Toxicity tests are also important to identify potential damage to organs, such as the liver and kidneys, which play a role in the excretion of foreign chemicals[12]. This study aims to evaluate the acute toxicity of pomegranate peel extract nanogel as an adjunct therapy in periodontal treatment.

MATERIALS AND METHODS

Preparation of Pomegranate Peel Extract

Pomegranate (*Punica granatum L.*) peel is washed thoroughly with water until it is dry, and then ground until it is smooth enough to obtain a powder. The pomegranate peel extraction is carried out using the maceration method, which involves placing pomegranate peel powder into an Erlenmeyer flask and then adding 5 liters of 70% ethanol. The solution is soaked for the first 6 hours with occasional stirring, and then left for an additional 18 hours. After that, the macerate is separated using centrifugation, decantation, or filtration. This extraction process can be repeated at least once using the same solvent as in the first extraction. The maceration results are then evaporated using a rotary evaporator to break down the ethanol dissolution of the extract. The yield obtained is calculated by determining the percentage weight (w/w) of the extract produced relative to the weight of the powdered simplicia used, which is determined by weighing. The yield produced must reach the minimum value listed in the monograph of each extract. Extraction can also be performed using other methods, such as percolation, Soxhlet, or counter-current extraction.

Preparation of Pomegranate Peel Extract Nanogel

Table 3: Results of Toxic Symptoms Observation After 14 Days of Pomegranate Peel Extract Nanogel Administration

Observation	Control	Preliminary Test				Main Test
	K	P1	P2	P3	P4	P5
Skin and fur	N	N	N	N	N	N
Eyes	N	+	+	+	+	+
Tremors	-	-	-	-	-	+
Seizures	-	-	-	-	-	-
Dhiarrhea	-	-	-	+	+	+
Lethargy	-	-	-	-	-	-
Stomach walk	-	-	-	-	-	-
Backward walking	-	-	-	-	-	-

Description: K:- Control group (Na-CMC 0.5%);
P1:- Pomegranate peel extract nanogel, 5 mg/kg BW;
P2:- Pomegranate peel extract nanogel, 50 mg/kg BW;
P3:- Pomegranate peel extract nanogel, 300 mg/kg BW;
P4:- Pomegranate peel extract nanogel, 2000 mg/kg BW;
P5:- Pomegranate peel extract nanogel, 2000 mg/kg BW;
N= Normal; - = No symptoms observed; + = Symptoms observed

The preparation of pomegranate peel extract nanogel was carried out using the polymer percussor method. The mortar and pestle are heated with hot water for 10 minutes. Then, when the outside of the mortar and pestle feels hot, the water is removed, and the items are dried in an oven. Hot water was added to the first mortar, and Carbopol 940 was added evenly. The mixture was then left to wait until the Carbopol 940 had expanded. After that, the same thing was done to the HPMC K4M developed in the second mortar. Carbopol 940 and HPMC K4M, which had expanded, were ground until homogeneous. TEA and glycerin were added to the first mortar and ground until a homogeneous mixture was formed. Nipagin and Nipasol were added to the second mortar and ground until a homogeneous mixture was formed.

Furthermore, the mixture from the second mortar was added to the first mortar little by little while still being ground until homogeneous. Then the remaining aquadest was added little by little up to 100 grams and ground until homogeneous. After that, the preparation was stirred at a speed of 2,500 rpm for 24 hours. In the final stage, the preparation was subjected to a sonication process for 3 hours and a centrifugation process. The formulation of the pomegranate peel extract nanogel preparation is described in Table 1.

Physical Characterization of Formulation

The physical characterization of the pomegranate peel extract nanogel was conducted to assess its suitability as a topical formulation. Particle size analysis using a Fritsch Analysette 22 NanoTec particle size analyzer showed that the nanogel particles fell within the appropriate nanometer scale, ensuring effective skin penetration, uniform drug distribution, and improved therapeutic efficiency.

Organoleptic testing revealed that the nanogel had a brown color, a characteristic odor, and a semi-solid consistency, indicating an acceptable physical appearance. Homogeneity

was confirmed by spreading the gel onto a transparent surface, where it appeared smooth and free of visible coarse particles, suggesting a uniform dispersion of the active ingredient within the gel matrix.

The pH of the nanogel was measured using a digital pH meter (AZ Instrument 86502) and was found to be within the physiological pH range, supporting its compatibility for topical application without irritation. Viscosity testing using an NDJ-8S viscometer with spindle 4 confirmed that the preparation had the appropriate consistency for a semi-solid gel, allowing for easy application and adherence to the skin surface.

Spreadability testing was conducted using a 20 × 20 cm glass plate with a millimeter grid. A 1-gram sample of the nanogel was placed at the center, allowed to settle for 60 seconds, and then compressed with weights of 100 g and

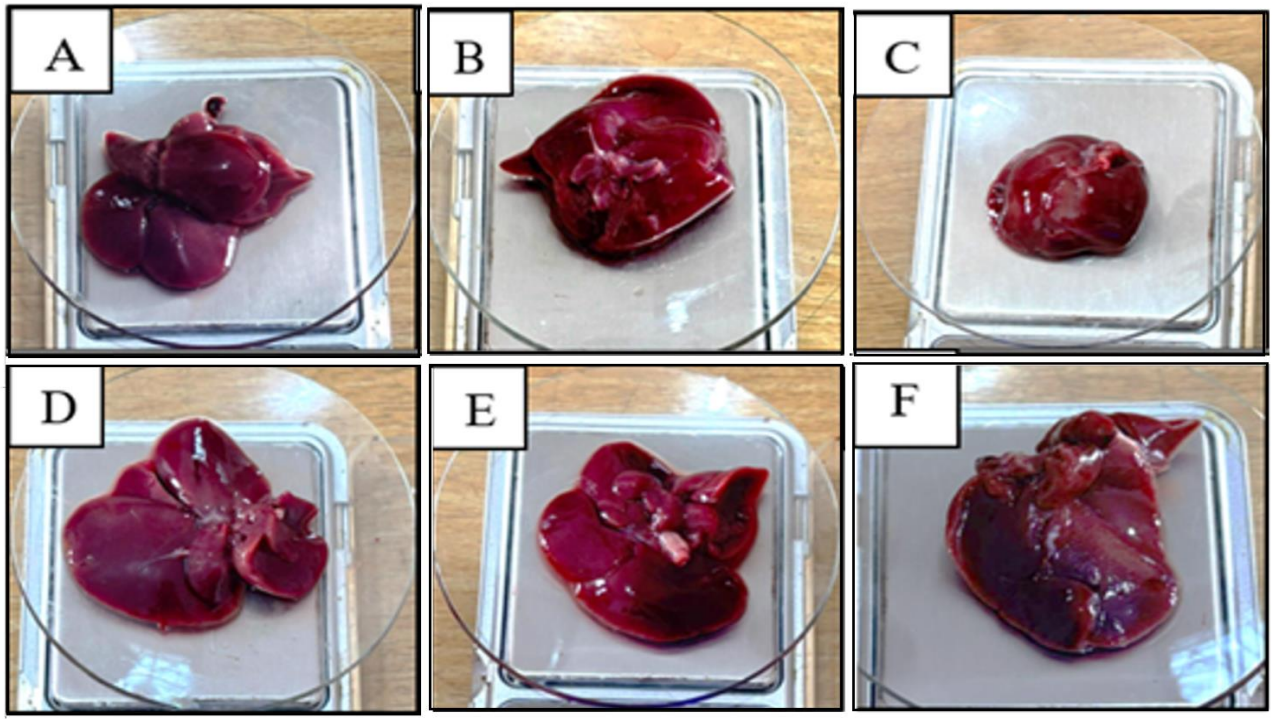

Figure 1: Macroscopic Appearance of the Liver: (A) Control (Na-CMC 0.5%), (B)-(E). Preliminary test (5, 30, 300, 2000 mg/kg BW), (F) Main Test (2000 mg/kg BW)

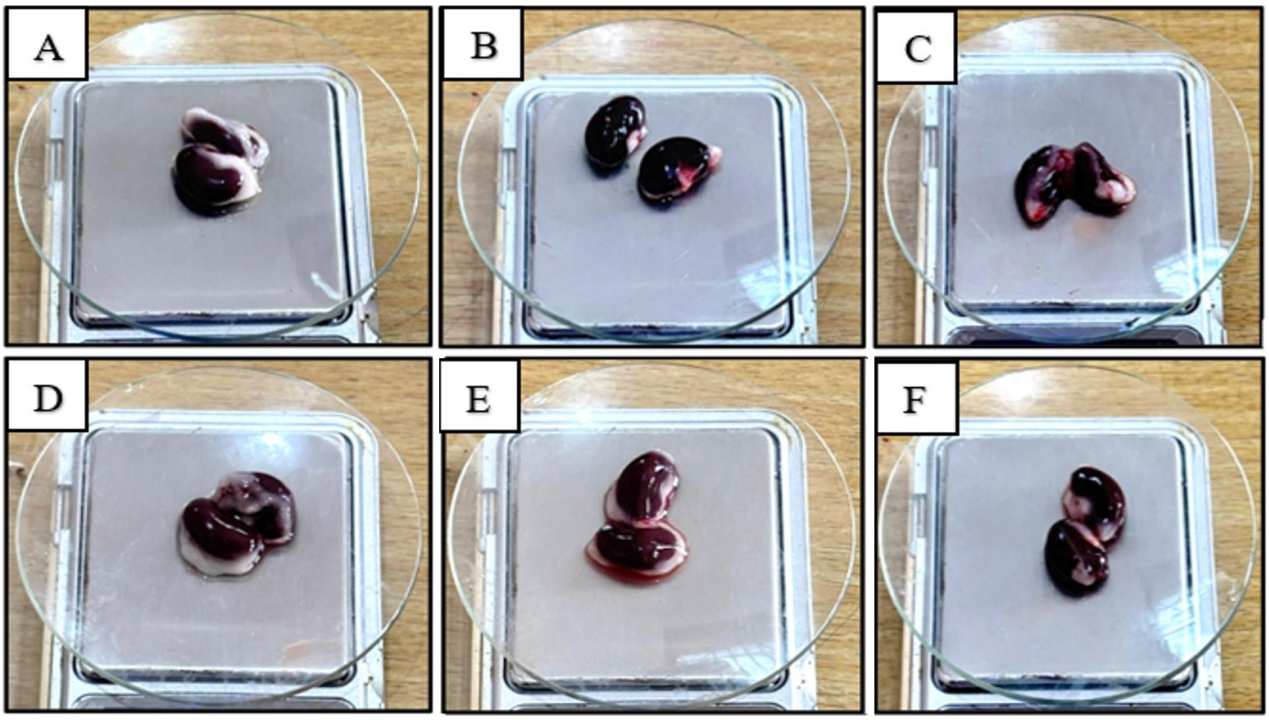

Figure 2: Macroscopic Appearance of the Kidney: (A) Control (Na-CMC 0.5%), (B)-(E) Preliminary test (5, 30, 300, 2000 mg/kg BW), (F) Main Test (2000 mg/kg BW)

Table 4: Average Body Weight Calculation Between Groups on Week 0, 1, and 2

Group	Intervention	Average body weight (g) ± SD		
		Week 0	Week 1	Week 2
Control	Na-CMC 0.5%	149 ± 13.40	152 ± 11.70	155 ± 12.45
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	180 ± 11.76	172 ± 15.51	171 ± 15.75
	Pomegranate peel extract nanogel 50 mg/kg BW	176 ± 17.85	175 ± 22.30	173 ± 21.86
	Pomegranate peel extract nanogel 300 mg/kg BW	158 ± 20.36	149 ± 22.37	151 ± 19.48
	Pomegranate peel extract nanogel 2000 mg/kg BW	179 ± 7.08	174 ± 4.56	176 ± 5.78
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	179 ± 7.13	174 ± 4.43	175 ± 6.13
p-value		0.018*	0.053	0.097

Description: Kruskal-Wallis test, * = significant difference with control group ($p < 0.05$).

Table 5: Animal Mortality Observations After Administration of Pomegranate Peel Extract Nanogel

Group	Intervention	Number of Rats	Number of Deaths	Mortality Percentage (%)
Control	Na-CMC 0.5%	5	0	0%
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	5	0	0%
	Pomegranate peel extract nanogel 50 mg/kg BW	5	0	0%
	Pomegranate peel extract nanogel 300 mg/kg BW	5	0	0%
	Pomegranate peel extract nanogel 2000 mg/kg BW	5	0	0%
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	5	1	20%

Description: Kruskal-Wallis test

150 g. The resulting diameter of spread was measured, indicating that the gel had satisfactory spreadability, a crucial parameter for ensuring uniform application across the target area.

Acute Toxicity Oral Test

The method used is the fixed dose method, as per BPOM No. 10 tahun 2022. Nanogel will be administered using an oral probe. The doses used in acute toxicity tests are based on the OECD guidelines and the BPOM standards. A dose of 300 mg/kg body weight (BW) is used as the standard to determine the LD50, but other doses, such as 5, 50, and 2000 mg/kg BW, are also used to determine toxic effects at various concentration levels. The rats were observed individually for the first 30 minutes after injection of the test preparation, followed by monitoring at four-hour intervals for the initial 24 hours and subsequently once daily for 14 days. Pathological exams, animal behavior, and weight monitoring are crucial during observation.

Observation of Acute Toxicity

Observation of acute toxicity was conducted over 14 days to evaluate the potential toxicological effects of the pomegranate (*Punica granatum L.*) peel extract nanogel (PPE nanogel) in female Wistar rats. The animals were monitored daily for clinical signs of toxicity, including changes in fur condition, alterations in eye mucosa, abnormal gait (such as backward walking or abdominal dragging), general weakness, and seizures. These behavioral and physiological indicators were selected to detect early manifestations of systemic toxicity following single-dose administration of the test formulation.

Body weight measurements were recorded on days 0, 7, and 14 to assess any deviations from normal growth patterns and to identify potential adverse effects on nutritional status. At the end of the observation period, surviving animals were euthanized using 10% chloroform for organ collection and further biochemical and histopathological evaluations. Animal mortality was recorded continuously throughout the study. In the event of death, necropsy was immediately performed to extract the liver and kidneys for analysis.

To evaluate hepatic and renal function, blood samples were collected via cardiac puncture on day 14. Approximately 0.5 mL of blood was drawn from each rat, allowed to rest at room temperature for five minutes, and centrifuged at 3000 rpm for 10 minutes to separate the serum. The resulting serum was analyzed using spectrophotometry to measure creatinine levels, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, which serve as established biochemical indicators of kidney and liver function, respectively.

Following euthanasia, the liver and kidneys were carefully excised, rinsed with 0.9% sodium chloride solution to remove residual blood, blotted dry with absorbent paper, and weighed. Absolute organ weights were recorded, and relative organ weights were calculated by dividing the organ weight by the corresponding body weight. These

parameters provide insight into organ hypertrophy or atrophy that may result from a toxic insult.

Gross pathological examination was conducted by visually assessing the color, surface characteristics, and consistency of the liver and kidneys. Abnormalities, such as discoloration or textural changes, were documented as potential signs of organ toxicity. This macroscopic evaluation identified anatomical deviations associated with the administration of the nanogel formulation.

For microscopic assessment, the organs were fixed in 10% neutral buffered formalin, processed for paraffin embedding, sectioned using a microtome, and stained with hematoxylin and eosin. Histopathological examination under a light microscope was conducted to identify cellular and structural alterations in the liver and kidneys, including inflammation, necrosis, degeneration, and other indicators of tissue damage. These findings were essential in confirming the presence or absence of organ-specific toxicity induced by the PPE nanogel.

RESULTS AND DISCUSSION

Particle Size of Pomegranate Peel Nanogel Extract

Nanogel refers to hydrogel nanoparticles with a size range of 1–1000 nm, formed through physical or chemical cross-linking networks. The small size enhances drug penetration through physiological barriers and ensures sustained drug release. Smaller particles have a higher dissolution rate due to the larger surface area-to-volume ratio, allowing faster drug absorption. A larger surface area increases interaction with the solvent, improving drug solubility in the blood. Nanogels are an effective drug delivery system due to their good biocompatibility and high water content, which enables drug diffusion into the blood and release from the polymer, thereby reducing toxicity. Particle size analysis

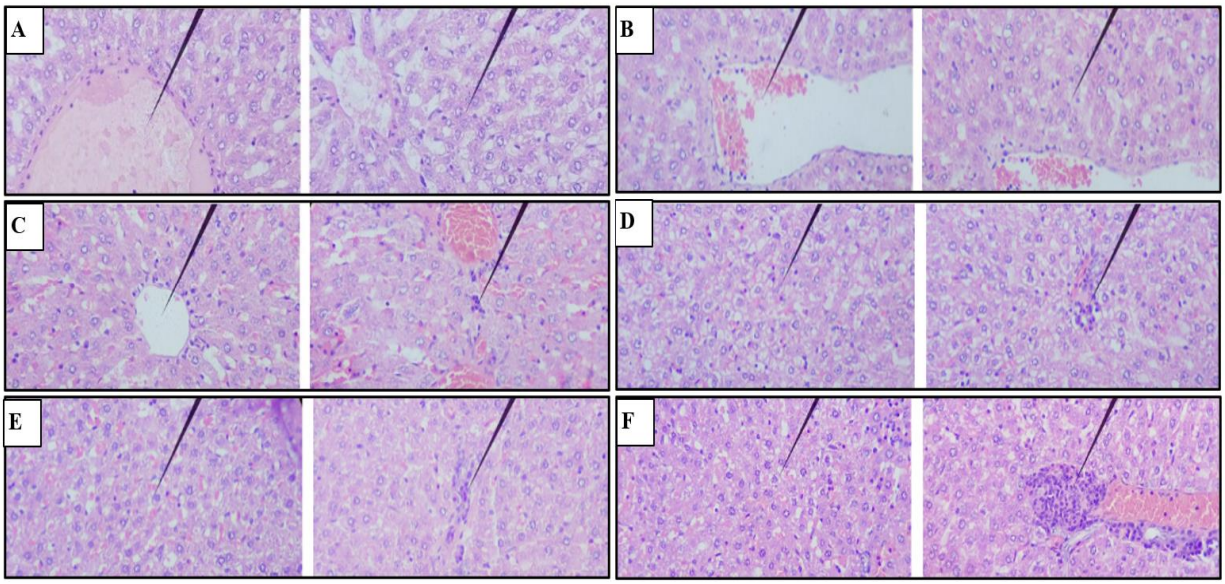

Figure 3: Liver Histopathology in Rats: (A) Control (Na-CMC 0.5%), (B)-(E) Preliminary test (5, 30, 300, 2000 mg/kg BW), (F) Main Test (2000 mg/kg BW)

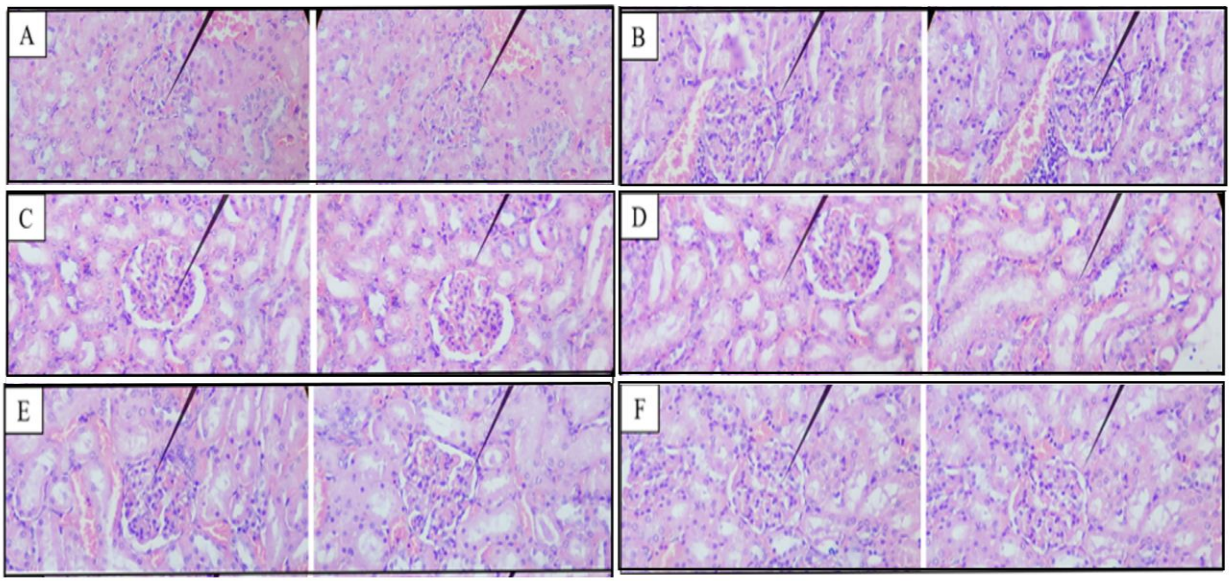

Figure 4: Kidney Histopathology in Rats: (A) Control (Na-CMC 0.5%), (B)-(E) Preliminary test (5, 30, 300, 2000 mg/kg BW), (F) Main Test (2000 mg/kg BW)

Table 6: Results of the average ALT levels in the blood of Wistar rats

Group	Intervention	Average ALT levels (mg/dL) ± SD	*P value*
Control	Na-CMC 0.5%	21.01 ± 4.49	
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	20.02 ± 2.735	
	Pomegranate peel extract nanogel 50 mg/kg BW	26.95 ± 9.140	0.096
	Pomegranate peel extract nanogel 300 mg/kg BW	20.12 ± 4.302	
	Pomegranate peel extract nanogel 2000 mg/kg BW	23.25 ± 2.855	
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	29.75 ± 8.71*	

Table 7: Results of the average AST levels in the blood of Wistar rats

Group	Intervention	Average AST levels (mg/dL) ± SD	*P value*
Control	Na-CMC 0.5%	0.596000 ± 0.0415933	
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	0.622320 ± 0.0606375	
	Pomegranate peel extract nanogel 50 mg/kg BW	0.658000 ± 0.0614003	0.293
	Pomegranate peel extract nanogel 300 mg/kg BW	0.668732 ± 0.1072139	
	Pomegranate peel extract nanogel 2000 mg/kg BW	0.695024 ± 0.0489318	
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	0.666005 ± 0.0785599	

Description: One-way ANOVA test with a confidence level of 95%

Table 8: Results of the average creatinine levels in the blood of Wistar rats

Group	Intervention	Average creatinine levels (mg/dL) ± SD	*P value*
Control	Na-CMC 0.5%	0.596000 ± 0.0415933	
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	0.622320 ± 0.0606375	
	Pomegranate peel extract nanogel 50 mg/kg BW	0.658000 ± 0.0614003	0.293
	Pomegranate peel extract nanogel 300 mg/kg BW	0.668732 ± 0.1072139	
	Pomegranate peel extract nanogel 2000 mg/kg BW	0.695024 ± 0.0489318	
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	0.666005 ± 0.0785599	

Table 9: Results of Average Relative Liver Organ Weight

Group	Intervention	Average Relative Liver Organ Weight (Mean ± SD)	*P value*
Control	Na-CMC 0.5%	0.0410 ± 0.0012	
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	0.0394 ± 0.0043	
	Pomegranate peel extract nanogel 50 mg/kg BW	0.0450 ± 0.0030	0.160
	Pomegranate peel extract nanogel 300 mg/kg BW	0.0454 ± 0.0040	
	Pomegranate peel extract nanogel 2000 mg/kg BW	0.0422 ± 0.0035	
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	0.0425 ± 0.0035	

Description: Kruskal-Wallis test

Table 10: Results of Average Relative Kidney Organ Weight

Group	Intervention	Average Relative Kidney Organ Weight (Mean ± SD)	*P value*
Control	Na-CMC 0.5%	0.008595 ± 0.0010340	
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	0.009911 ± 0.0010079	
	Pomegranate peel extract nanogel 50 mg/kg BW	0.009357 ± 0.0014769	0.160
	Pomegranate peel extract nanogel 300 mg/kg BW	0.008847 ± 0.0007339	
	Pomegranate peel extract nanogel 2000 mg/kg BW	0.009052 ± 0.0010768	
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	0.008715 ± 0.0007720	

Description: One-way ANOVA test with a confidence level of 95%

using a particle size analyzer (Fritsch Analysette 22 NanoTec) showed a size of 589 nm, which falls within the required range of 1 to 1000 nm for nanogel preparations.

Organoleptic

The organoleptic evaluation of pomegranate peel extract nanogel demonstrated a brown color, a distinct aroma, and a semi-solid consistency.

Homogenity

The pomegranate peel extract nanogel preparations were homogeneous and free from grains or coarse particles.

pH

The pH values of the pomegranate peel extract nanogels were 6.56. These pH values fall within the physiological pH range of 4.5–7.5, indicating compatibility with physiological conditions.

Viscosity

Viscosity is a crucial metric that signifies the consistency of a formulation and its capacity to flow. The viscosity assessment was performed with an NDJ-8S viscometer with a spindle operating at a velocity of 30 rpm. The viscosity test findings for the pomegranate peel extract nanogel preparation yielded a viscosity value of 2821 mPas, categorising it into the semi-solid range of 2,000-50,000 mPas.

Spreadability Test

The distribution capacity of the preparation was assessed by applying loads of 100 and 150 grams and measuring the distribution diameter. The results of the spreadability test for the nanogel preparations, as presented in Table 2, indicate that the spreadability increases with the applied load, remaining within the acceptable range for semi-solid preparations (5–7 cm).

Acute Toxicity Oral Test

The toxic effect test of pomegranate peel extract nanogel was conducted on female rats. The doses used were 5, 50, 300, and 2000 mg/kg BW. Observations were made over 14 days, covering toxic symptoms, body weight, animal mortality, relative organ weight, macropathology, and histopathology of the liver and kidney, as well as measurements of ALT, AST, and creatinine levels.

Observation of Acute Toxicity

Toxic Symptoms

Toxic symptom observations were conducted every 30 minutes for the first 4 hours after administration of the test preparation, followed by daily observations for 14 days. Symptoms observed included seizures, weakness, changes in fur, changes in eye mucosa, backward walking, and walking with the abdomen, as shown in Table 3.

Body Weight

Body weight was measured daily until day 14 to determine the volume of the test preparation administered, and statistical analysis was performed on a weekly basis. The data were not normally distributed and not homogeneous, as indicated by the Shapiro-Wilk normality test and Levene's homogeneity test. Therefore, the Kruskal-Wallis test was used to assess differences between groups. The results of body weight observations after the administration of pomegranate peel extract nanogel are shown in Table 4.

Statistical analysis using the Kruskal–Wallis test revealed a significant difference in baseline body weight between the control and treatment groups at week 0 ($p = 0.018$, $p < 0.05$). However, no significant differences were observed at week 1 ($p = 0.053$) or week 2 ($p = 0.097$), indicating that the administration of pomegranate peel extract nanogel did not produce a significant impact on body weight throughout the 14-day observation period.

Observation of Animal Mortality

The mortality observations during the administration of the test preparation are presented in Table 5.

Based on the table above, it can be observed that no rats died in the control group, as well as in the 5, 50, and 300 mg/kg BW groups, with a mortality percentage of 0%. However, in the 2000 mg/kg BW group, one rat died on day 4, resulting in a mortality percentage of 20%.

Measuring ALT, AST, and Creatinine Levels

Measurement of ALT, AST, and creatinine levels was performed on the serum of rats, which was separated from blood collected from the heart on day 15. The data were found to be normally distributed and homogeneous after performing the Shapiro-Wilk normality test and Levene's homogeneity of variance test. Therefore, to assess the differences between groups, an ANOVA test was conducted. The results of the average ALT and AST levels after administration of pomegranate peel extract nanogel are presented in Tables 6 and 7.

The results of the Shapiro-Wilk test confirmed that ALT levels were normally distributed, allowing for further analysis using one-way ANOVA. The ANOVA results showed no statistically significant difference in ALT levels among the groups ($p = 0.096$, $p > 0.05$). However, Post Hoc analysis using the Least Significant Difference (LSD) test revealed significant differences in ALT levels between the control group and the 2000 mg/kg BW group in the main study.

Additionally, significant differences were observed in the preliminary test between the 5 mg/kg BW and 2000 mg/kg BW groups, as well as between the 300 mg/kg BW and 2000 mg/kg BW groups.

The AST levels data exhibited a normal distribution, as indicated by the Shapiro-Wilk test results; therefore, the difference was evaluated using ANOVA. The ANOVA analysis revealed a significant difference in AST levels among groups, with a p-value of 0.0931 ($p > 0.05$). The Post Hoc LSD test findings indicated no significant variations in AST levels among the groups ($p > 0.05$).

The data had a normal distribution, and one-way ANOVA revealed no significant difference in creatinine levels across the groups ($p = 0.293$, $p > 0.05$). Post Hoc LSD test findings revealed a substantial disparity in creatinine levels between the control group and the pomegranate peel extract nanogel at 2000 mg/kg BW in the preliminary test.

Weighing the Organs

The liver and kidneys were cleaned with 0.9% NaCl, dried with absorbent paper, and weighed to determine their absolute organ weights. Divide the absolute organ weight by body weight to get the relative organ weight. Due to the non-normal distribution and heterogeneity of the data, a Kruskal-Wallis test was used to compare the groups. Table 9 shows relative liver organ weight.

Kruskal-Wallis statistical analysis (SPSS) revealed no significant difference in relative liver organ weight between the control group and the treatment groups administered 5, 50, 300, and 2000 mg/kg BW of pomegranate peel extract nanogel, with a p-value of 0.160 ($p > 0.05$). The oral administration of pomegranate peel extract nanogel at various dosages did not affect the increase in liver organ weight. The results of the relative kidney organ weight are presented in Table 10.

The data were normally distributed. Based on statistical analysis using one-way ANOVA, the results indicated no significant difference in kidney weight between groups, with a p-value of 0.417 ($p > 0.05$). Post hoc LSD test results showed no significant difference in kidney weight among any of the groups.

Table 11: Results of Macroscopic Observation of the Liver

Group	Intervention	Observation		
		Color	Texture	Consistency
Control	Na-CMC 0.5%	Reddish-brown	Smooth	Elastic
Preliminary Test	Pomegranate peel extract nanogel 5 mg/kg BW	Reddish-brown	Smooth	Elastic
	Pomegranate peel extract nanogel 50 mg/kg BW	Reddish-brown	Smooth	Elastic
	Pomegranate peel extract nanogel 300 mg/kg BW	Reddish-brown	Smooth	Elastic
	Pomegranate peel extract nanogel 2000 mg/kg BW	Reddish-brown	Smooth	Elastic
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	Reddish-brown	Smooth	Elastic

Table 12: Results of Macroscopic Observation of the Kidney

Group	Intervention	Observation		
		Color	Texture	Consistency
Control	Na-CMC 0.5%	Reddish-brown	Smooth	Elastic
Preliminary	Pomegranate peel extract nanogel 5 mg/kg BW	Reddish-brown	Smooth	Elastic
Test	Pomegranate peel extract nanogel 50 mg/kg BW	Reddish-brown	Smooth	Elastic
	Pomegranate peel extract nanogel 300 mg/kg BW	Reddish-brown	Smooth	Elastic
	Pomegranate peel extract nanogel 2000 mg/kg BW	Reddish-brown	Smooth	Elastic
Main Test	Pomegranate peel extract nanogel 2000 mg/kg BW	Reddish-brown	Smooth	Elastic

Macropathology of the Organs

The organs of the test animals were collected on the 14th day of the administration period for the test substance. Macropathological examination of the liver included observations of color, consistency, and surface, as presented in Figure 1 and Table 11.

Based on Table 11 above, it can be observed that the organs remain in normal condition, characterized by a reddish-brown color, a chewy consistency, and a smooth surface. The normal criteria for liver organs are the absence of color changes, surface structural alterations, and consistency changes. The results for the kidneys are consistent with those observed in the liver, as presented in Figure 2 and Table 12.

Histopathology of Organs

To observe the histopathological results of the liver and kidneys in rats, tissue sections from each group were examined using a Zeiss Primostar microscope (Zeiss, Germany) with a 10x ocular lens and a 40x objective lens. Each preparation was examined for cells exhibiting pathological changes (Figures 3 and 4).

Histopathological examination of the liver in the control group revealed no signs of damage, including necrosis, hydropic degeneration, hemorrhage, or inflammation, indicating a normal liver condition. In the 5 mg/kg BW group, a single focus of hydropic degeneration was observed, but no necrosis, hemorrhage, or inflammation was found. In the 50 mg/kg BW group, inflammation was detected in one focus, but other damage was absent. The 300 mg/kg BW group showed one focus each of hydropic degeneration and inflammation, while necrosis and hemorrhage were not present. Similarly, the 2000 mg/kg BW group showed hydropic degeneration and inflammation in one focus, without necrosis or hemorrhage. Overall, while mild changes were observed, no severe liver damage, such as necrosis or hemorrhage, was found in any of the groups.

Microscopic examination with Hematoxylin-Eosin (HE) staining in the control group and the groups treated with 5 mg/kg BW, 50 mg/kg BW, and 300 mg/kg BW showed no evidence of acute toxicity in the kidney organs of the rats, as no changes were observed in parameters such as inflammation, necrosis, degeneration, congestion, or hemorrhage. In the 2000 mg/kg BW group, inflammation was observed, characterized by endothelial swelling and the presence of interstitial nephritis.

DISCUSSION

Herbal medicines are widely utilized in healthcare worldwide, often under the assumption that they are inherently safe due to their natural origin[13]. However, adverse effects may occur, typically being mild and affecting a small proportion of individuals. Pomegranate peel extract is employed in various cultures not only as a culinary ingredient but also in traditional medicine for the treatment of several ailments. Despite limited experimental data on its toxicity, studies have indicated that high doses of its active compounds, particularly ellagitannins and punicalagin, may be associated with hepatotoxicity and nephrotoxicity[14].

Observations over 14 days revealed weight loss in all treatment groups during the first week, which was most likely attributed to stress induced by the initiation of the experiment. However, the weight loss did not exceed 20%, indicating that the test animals did not experience significant stress or suffering[15]. In the second week, most treatment groups exhibited a recovery in body weight, suggesting that the rats had acclimated to the treatment and experimental environment. Various internal and external factors, including diet and the genetic characteristics of the test animals, could influence these changes in body weight[16].

Toxic symptoms were predominantly observed in the high-dose groups, specifically 300 mg/kg BW and 2000 mg/kg BW.

The symptoms included weakness, tremors, erythema around the eyes, and diarrhea, which were likely caused by the interaction of pomegranate peel extract nanogel nanoparticles with the rats' biological systems. This interaction may have led to gastrointestinal irritation and effects on the central nervous system[17,18]. In contrast, no significant toxic symptoms were observed in the lower-dose

groups (5 mg/kg BW and 50 mg/kg BW), indicating that the nanogel is safe at lower doses.

Although the majority of the rats survived until the end of the observation period, one rat from the 2000 mg/kg BW group died on day 4 of the experiment. This mortality was likely due to a combination of the observed toxic symptoms and prolonged stress, leading to a compromised immune system[19].

Macroscopic examination of the liver revealed no significant changes in size or weight in any of the treatment groups. The liver retained its normal reddish-brown color and firm consistency, indicating that there was no substantial structural damage[20]. Histopathological examination of the liver revealed mild hydropic degeneration and localized inflammation at certain foci, but no signs of necrosis or hemorrhage. These findings suggest that administering pomegranate peel extract nanogel at low to moderate doses does not induce significant liver damage. Similarly, macroscopic examination of the kidneys showed no significant changes in color or size in the low- to moderate-dose groups. However, in the high-dose group (2000 mg/kg BW), endothelial swelling and interstitial nephritis were observed, indicating mild inflammation. Despite the presence of inflammatory changes, no necrotic damage was observed in the kidneys. The serum creatinine levels increased in the high-dose treatment group; however, these levels remained within the normal range for rats, indicating that pomegranate peel extract nanogels did not cause significant renal dysfunction.

CONCLUSIONS

Pomegranate (*Punica granatum L.*) peel extract nanogel did not exhibit any toxic effects on Wistar rats, as observed at the functional and macroscopic levels of the liver and kidney after 14 days. There was no visible weight loss, behavioral changes, or damage to organ function and structure. The histopathological analysis also revealed no indications of liver damage. Additionally, it showed no signs of kidney damage at the low dose, but inflammation was observed at the 2000 mg/kg BW dose. The lower dose group (5, 50, 300 mg/kg body weight) showed no toxic symptoms. While the highest dose group (2000 mg/kgBW) showed toxic symptoms that caused the death of one rat, the pomegranate peel extract nanogel is classified as a preparation with a mild toxicity level based on the LD50 classification in the acute toxicity test.

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