2026, Scienceline Publication
World's Veterinary Journal
World Vet J, 16(2): 312-323.
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2026.wvj30
PII: S232245682600030-16

Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum)* Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice

Twahafifwa Nghuuluka Kanemo Jonas[1], Rebecca Wanjiku Waihenya[2], and Daniel Wainaina Kariuki[3]

[1]*Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya*
[2]*Zoology Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya*
[3]*Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

*Corresponding author's Email: twyfa633@gmail.com

ORIGINAL ARTICLE
Received: April 03, 2026
Revised: May 05, 2026
Accepted: May 30, 2026
Published: June 25, 2026

ABSTRACT

Pomegranate (*Punica granatum*) contains a high level of polyphenols and has anti-inflammatory properties. However, there is limited available data on the safety and effectiveness of pomegranate use during prolonged inflammatory conditions. The present study aimed to evaluate the anti-inflammatory properties and subacute toxicity profile of a 70% ethanolic extract of *Punica granatum* (*P. granatum*) rind in carrageenan-induced mice. A total of 30 male mice, aged five weeks and weighing 22-23 grams, were randomly assigned to five groups of six mice each. The study included a negative control group that received normal saline, a positive control group that received meloxicam at 2 mg/kg, and three treatment groups that received *P. granatum* rind extract at doses of 50, 100, and 200 mg/kg orally over 28 days. Inflammation was induced once weekly in all experimental groups by 1% carrageenan injection into the right hind paw, after which the edema was measured with a digital vernier caliper. The toxicity of the pomegranate extract was assessed using hematological and biochemical profiles of hepatic and renal parameters. Tissue samples from the heart, spleen, kidney, lungs, and liver were collected on day 29. To assess the inflammation status, concentrations of tumor necrosis factor-alpha (TNF-α) and IL-10 were quantified using enzyme-linked immunosorbent assay. The *P. granatum* rind extract significantly reduced paw edema without mortality or observable toxicity, compared to the negative control group. Mice maintained their normal feeding habits, and body weight increased normally across all groups. Hematological parameters, hepatic, and renal markers were within the normal physiological range across all groups. Histopathological analysis revealed no abnormalities in extract-treated groups. Extract-treated groups demonstrated a dose-dependent elevation of IL-10, peaking at 200 mg/kg, and a reduction in TNF-α level across all doses, with the highest suppression observed at 100 mg/kg compared to the negative control. Daily oral administration of *P. granatum* rind extract for 28 days exhibited dose-dependent anti-inflammatory effects, as evidenced by reduced paw edema and modulation of cytokines. The absence of systemic toxicity across all tested doses suggested that *P. granatum* rind extract can be a safe therapeutic agent for treating inflammatory conditions.

Keywords: Anti-inflammatory, Biochemical marker, Carrageenan, Cytokine, Hematological parameter, Inflammation, *Punica granatum*

INTRODUCTION

Inflammation is a complex biological response that occurs in reaction to infection, chemical irritation, tissue damage, or immune system dysregulation. While acute inflammation is essential for healing, incomplete healing can lead to persistent tissue damage, which may contribute to chronic metabolic diseases, including type 2 diabetes and cardiovascular disease (Medzhitov, 2008; Chen et al., 2018). Chronic inflammation primarily involves the sustained release of inflammatory mediators, including pro-inflammatory cytokines, reactive oxygen species, and inducible enzymes such as cyclooxygenase-2 (COX-2; Mittal et al., 2014). Collectively, inflammatory mediators exacerbate tissue damage, thereby contributing to disease progression. Conventional anti-inflammatory therapies, including nonsteroidal anti-inflammatory drugs (NSAIDs), are effective in controlling inflammation; however, their long-term use is limited by adverse effects, necessitating the search for safer alternatives (Bindu et al., 2020; Schjerning et al., 2020; Sohail et al., 2023).

Natural products are popular as alternatives or complementary options for addressing inflammation. *Punica granatum* (pomegranate) has been extensively documented in the literature for its anti-inflammatory and antioxidant properties. These properties are primarily attributed to its abundant polyphenolic content, which includes ellagitannins, flavonoids, and the hydrolyzable tannin punicalagin (Lansky and Newman, 2007; Viuda-Martos et al., 2010; Aviram and Rosenblat, 2012). The rind of the fruit, which is often discarded as a by-product of juice production, is the tissue with the highest polyphenol content. The rind contains higher concentrations of punicalagins, ellagic acid, and other bioactive ellagitannins than the seeds (Ismail et al., 2012; Kandylis and Kokkinomagoulos, 2020; Singh et al., 2023). Findings

To cite this paper: Jonas TNK, Waihenya RW, and Kariuki DW (2026). Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum*) Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice. *World Vet. J.*, 16(2): 312-323. DOI: https://dx.doi.org/10.54203/scil.2026.wvj30

from *in vitro* and *in vivo* studies have demonstrated that compounds derived from *Punica granatum* (*P. granatum*) rind can effectively regulate the expression and activity of key inflammatory mediators and pathways associated with oxidative stress (Ismail et al., 2012; Singh et al., 2023). Preclinical studies indicated that polyphenols derived from pomegranate peel can influence inflammatory and oxidative stress pathways, although the effects may vary depending on extract preparation, dosage, and study design (Singh et al., 2023).

Carrageenan-induced paw edema is a well-established model for evaluating anti-inflammatory agents in rodents, characterized by a biphasic inflammatory response, with the early release of histamine, serotonin, and bradykinin, followed by sustained production of cytokines and prostaglandins (Posadas et al., 2004; Woode et al., 2022). Repeated weekly administration of carrageenan extends the acute model to simulate recurrent inflammatory conditions, thereby offering a clinically relevant model for assessing sustained anti-inflammatory efficacy (Perrot et al., 1999).

There is considerable evidence supporting the anti-inflammatory effects of *P. granatum*; however, most studies have focused on short-term or acute outcomes. There was limited evidence concerning the long-term safety profile and biological efficacy of *P. granatum* during multiple dosing in chronic inflammation. The lack of data on subacute conditions limited the use of *P. granatum* in treating inflammation and antioxidant therapy. A comprehensive evaluation of inflammatory and toxic markers of *P. granatum* is required to determine whether ongoing administration of pomegranate extract is effective and safe. The present study aimed to evaluate the anti-inflammatory effects and the subacute safety of *P. granatum* rind ethanolic extract in mice using a recurrent carrageenan-induced inflammation model.

MATERIALS AND METHODS

Ethical approval

All the procedures used in the present study were reviewed and approved by the Mount Kenya University Institution Scientific Review Committee (ISER), Kenya (Ref: MKU/ISERC/5682). The present study was conducted in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All possible measures were taken to minimize animal suffering and distress during experimental procedures.

Punica granatum rind extract preparation

Fresh pomegranate fruits were collected from Karson Farm, Nyeri County, Kenya. Nine fruits were thoroughly rinsed with distilled water, the rinds were isolated and air-dried in the shade for three days, then freeze-dried for 48 hours. The dried rind powder (100 g) was extracted with 1 liter of 70% (v/v) aqueous ethanol at a 1:10 (w/v) solvent-to-solid ratio at 22°C ± 2 for 48 hours under continuous shaking (Sultana et al., 2009). The extract was filtered through Whatman filter paper, concentrated by rotary evaporation, and oven-dried, yielding 20 g of dried extract from 100 g of the initial powder (20% w/w extraction yield).

Animals and study design

A total of 30 male BALB/c mice, five weeks old, weighing from 22 to 23 g, were purchased from the Small Animal Facility for Research and Innovation (SAFARI), JKUAT, Kenya, and used as inflammatory disease models due to their well-characterized immune response and extensive use (Malm Tillgren et al., 2023). The mice were acclimatized in their cages for one week before the study began. The mice were maintained in clean plastic cages with a stainless-steel top grill that contained proper aeration and a 12-hour light/dark cycle (Larrosa et al., 2010). The cages were cleaned weekly to maintain a clean environment. The mice were provided with pelleted feed (Unga Feeds Limited, Nairobi, Kenya) and unrestricted access to water (Karwasra et al., 2019).

Pomegranate rind extract administration

For the subacute toxicity investigation, five groups, each with six mice, were designed. The first treatment group received 50 mg/kg of *P. granatum* rind extract, the second treatment group received 100 mg/kg of *P. granatum* rind extract, and the third treatment group received 200 mg/kg of *P. granatum* rind extract, according to the study of Patel et al. (2008) and Bhandary et al. (2013). Based on the study by Dudhgaonkar et al. (2006), meloxicam at 2 mg/kg was administered orally as the positive control, while normal saline at 10 mL/kg served as the negative control. Inflammation was induced once weekly for four weeks (28 days) using 1% carrageenan by injection into the right hind paw (Winter et al., 1962) to maintain a persistent inflammatory condition (Perrot et al., 1999). Paw swelling was measured hourly after carrageenan injection, for four hours, and at 4 hours post-treatment using a digital Vernier caliper. The reduction of edema percentage was calculated using Formula 1.

$$\text{Edema reduction (\%)} = [(\text{Negative control edema} - \text{Treated group edema}) \div \text{Negative control edema}] \times 100 \quad \text{(Formula 1)}$$

where NC is the negative control group mean edema, T is the treated group mean edema, and edema is the mean paw thickness at 5:00 PM minus the mean paw thickness at 9:00 AM. Treatment was administered daily by oral gavage.

Clinical observations

The mice were observed throughout the 28-day study for any signs of toxicity, including ataxia, diarrhea, lacrimation, ptosis, tremors, convulsions, and coma (Parasuraman, 2011). The mice were monitored four hours after daily oral treatment. The body weights of the mice were measured at the beginning of the study and thereafter weekly to track the changes in body weight. Food and water intake were measured daily by weighing the remaining feed and recording the residual water volume per group.

Sample collection

On day 29, mice were humanely euthanized with carbon dioxide asphyxiation. Blood samples (1.8-1.9 mL) were collected via cardiac puncture into micro tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological assessment and into plain tubes for biochemical analyses. Vital organs, including the spleen, lungs, liver, kidneys, and heart, were subjected to macroscopic evaluation. The organs were subsequently excised and fixed in 10% formalin for histopathological examination.

Histopathology

The excised organs were fixed in 10% formalin. A sample from each organ was dehydrated through a series of isopropanol dilutions (up to 100%), then cleared in xylene. The tissues were infiltrated with molten paraffin wax, then embedded and allowed to solidify on the cooling plate. The embedded blocks were sectioned at a thickness of 2 µm using a microtome. The tissue sections were stained with hematoxylin and eosin (H&E) following standard laboratory procedures (Fischer et al., 2008). The prepared slides were subsequently examined using a light microscope (Optika, Italy) fitted with a SwiftCam digital camera (Model SC-2003, Swift Optical Instruments, USA) at magnifications of 100x and 400x.

Hematological investigation

Whole blood samples (0.2-0.4 mL) were collected into micro EDTA tubes to prevent coagulation (Lippi et al., 2005) and analyzed using a Mindray hematology analyzer (BC-10, China). With 50 µL of blood sample, red blood cell count (RBC), hemoglobin concentration (HGB), red cell distribution width coefficient of variation, mean corpuscular hemoglobin (MCH), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width (coefficient of variation), and red cell distribution width standard deviation were assessed. The white blood cell (WBC) and platelet (PLT) parameters analyzed included total WBC count, lymphocyte count (LYMP), granulocyte count (GRAN), mid-sized cell count (MID), the respective percentages of LYMP%, MID%, and GRAN%, platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV), and PCT. Reference ranges for interpreting hematological parameters were set by the Mindray BC-10 hematology analyzer (Mindray, China) and established during factory calibration for laboratory mice.

Biochemical and ELISA analyses

The blood (1.5 mL) was collected into plain tubes, left to clot at room temperature for 30 minutes, and thereafter centrifuged at 3,500 rpm for 10 minutes to obtain serum (Diehl et al., 2001; Parasuraman et al., 2010). The sera were used for subacute toxicity, biochemical, and immunohistochemical assays and were analyzed using the Architect biochemistry automated analyzer (model c16000, Abbott, USA). The levels of albumin, alanine aminotransferase (ALT), potassium, bilirubin (total), gamma glutamyl transferase, protein (total, albumin, and globulin), direct bilirubin, chloride ion, Alkaline phosphatase, sodium, aspartate aminotransferase (AST), calcium, creatinine (serum, fluid), blood urea nitrogen, for liver and kidney function were evaluated. The ELISA was used to assess tumor necrosis factor-alpha (TNF-α) levels using the Mouse TNF-α Sandwich ELISA kit (Proteintech, USA; catalog no: KE10002), and interleukin-10 (IL-10) levels were assessed using the Mouse IL-10 Sandwich ELISA kit (Proteintech, USA; catalog no: KE10103). Reference ranges for interpreting serum biochemical parameters were predefined by the Abbott Architect c16000 chemistry analyzer (Abbott Laboratories, Abbott Park, USA), established through factory calibration for laboratory mice.

Statistical analysis

Statistical analysis of the collected data was performed using GraphPad Prism software version 8.02. (263). All data collected, including hematological analysis, biochemistry, ELISA, body weights, and food and water consumption, were analyzed as mean ± standard error (SEM). Analysis of variance (ANOVA) was used, followed by Dunnett's multiple comparisons test, to determine statistically significant differences between the control and treatment groups. The significance level was set at a p-value less than 5% ($p < 0.05$).

RESULTS

Water and feed intake

There were no significant differences in water and feed consumption between the treated groups and the control group ($p > 0.05$; Figure 1).

Body weight gain

All experimental groups exhibited a normal, gradual increase in body weight over the 28-day study period, with no statistically significant differences observed between any treatment group and the negative control group ($p > 0.05$; Figure 2). At baseline, the mean body weights were 22.8 g, 22.5 g, 22.7 g, 22.3 g, and 22.2 g for the negative control, 50 mg/kg, 100 mg/kg, 200 mg/kg, and positive control groups, respectively. By day 28, the mean body weights were 25.4 g, 25.9 g, 25.5 g, 24.9 g, and 25.0 g for the negative control, 50 mg/kg, 100 mg/kg, 200 mg/kg, and positive control groups, respectively.

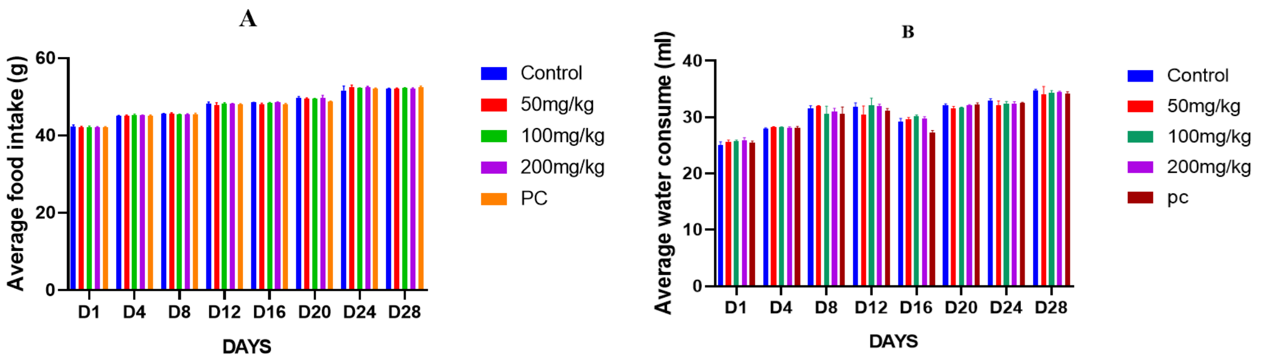

Figure 1. Effects of pomegranate rind extract on feed and water intake during 28 days under a subacute condition. There was no significant difference between the negative control and all groups ($p < 0.05$). **A:** Effects of pomegranate rind extract on the average feed intake. **B:** Effects of Pomegranate rind extract on the average water intake. Control: Negative control group, experimental groups: 50 mg/kg, 100 mg/kg, and 200 mg/kg of the pomegranate rind extract, PC: Positive control group.

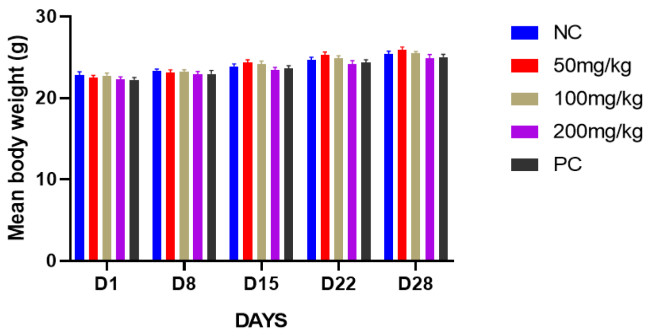

Figure 2. Mean body weight changes of mice subjected to different dosages of the pomegranate rind extract during 28 days under a subacute condition. There was no significant difference between the negative control and all the groups ($p < 0.05$). NC: Negative control group, experimental groups: 50 mg/kg, 100 mg/kg, and 200 mg/kg of the pomegranate rind extract, PC: Positive control group.

Hematological parameters

The results regarding the extract's impact on hematological parameters are presented in Table 1. Daily oral intake of pomegranate rind extract did not result in significant differences in most of the evaluated parameters ($p > 0.05$). However, a significant increase in red cell distribution width standard deviation (RDW-SD) was observed in mice treated with 200 mg/kg of pomegranate rind extract compared with the negative control (0.0384).

Biochemical parameters

Levels of liver and kidney function markers after treatment with 50 mg/kg, 100 mg/kg, and 200 mg/kg of the pomegranate rind extract are presented in Table 2. There were no significant differences between the treatment groups and the negative control group ($p > 0.05$). All hepatic, renal, and electrolyte markers remained within established physiological reference ranges for laboratory mice.

Table 1. The hematological results of mice treated with pomegranate rind extract for 28 days

Parameters	Control	50 mg/kg	100 mg/kg	200 mg/kg	PC	P-value
White blood cell count (10^9/L)	6.56 ± 1.22[a]	6.73 ± 0.52[a]	7.46 ± 0.68[a]	6.63 ± 0.42[a]	8.20 ± 0.74[a]	0.2392
Lymphocyte count (10^9/L)	2.96 ± 0.77[a]	3.30 ± 1.20[a]	1.43 ± 0.49[a]	2.20 ± 0.41[a]	2.20 ± 0.24[a]	0.1540
Absolute mid-range cell count (10^9/L)	0.73 ± 0.24[a]	0.40 ± 0.08[a]	0.56 ± 0.23[a]	0.40 ± 0.28[a]	0.43 ± 0.16[a]	0.5169
Absolute granulocyte count (10^9/L)	6.00 ± 0.35[a]	3.86 ± 1.24[a]	3.36 ± 0.97[a]	3.23 ± 0.69[a]	5.80 ± 0.73[a]	0.0974
Lymphocyte percentage (%)	36.80 ± 1.76[a]	34.33 ± 1.24[a]	43.66 ± 2.05[a]	43.66 ± 2.04[a]	35.66 ± 6.23[a]	0.1404
Mid-range cells (%)	8.46 ± 0.94[a]	7.50 ± 1.39[a]	6.80 ± 1.33[a]	7.03 ± 1.81[a]	7.10 ± 0.85[a]	0.7251
Granulocytes (%)	52.80 ± 2.56[a]	55.30 ± 3.85[a]	52.56 ± 3.36[a]	62.03 ± 3.95[a]	61.96 ± 2.04[a]	0.0575
Red blood cell count (10^{12}/L)	3.83 ± 0.35[a]	4.53 ± 0.73[a]	4.83 ± 0.24[a]	4.53 ± 0.57[a]	2.36 ± 0.16[b]	0.0023
Hemoglobin (g/dL)	16.83 ± 0.44[a]	15.10 ± 0.61[a]	14.86 ± 1.12[a]	16.60 ± 0.22[a]	13.26 ± 1.15[b]	0.0007
Hematocrit (%)	46.00 ± 4.06[a]	39.90 ± 0.71[a]	41.00 ± 2.40[a]	45.03 ± 0.87[a]	41.90 ± 1.68[a]	0.1012
Mean corpuscular volume (fl)	81.26 ± 0.89[a]	83.36 ± 0.79[a]	84.46 ± 3.24[a]	80.53 ± 2.12[a]	68.83 ± 1.27[b]	< 0.0001
Mean corpuscular hemoglobin (pg)	26.76 ± 0.59[a]	27.83 ± 0.41[a]	28.40 ± 0.10[a]	28.43 ± 0.43[a]	26.36 ± 2.17[a]	0.2363
MCHC (g/dL)	35.16 ± 0.71[a]	36.73 ± 0.85[a]	36.13 ± 0.63[a]	36.26 ± 0.72[a]	36.50 ± 0.35[a]	0.2770
RDW-CV (%)	12.46 ± 0.89[a]	14.10 ± 0.66[a]	13.66 ± 0.41[a]	12.33 ± 1.20[a]	13.73 ± 0.41[a]	0.1391
RDW-SD (fl)	40.73 ± 0.97[a]	42.96 ± 0.94[a]	44.26 ± 0.82[a]	45.53 ± 2.06[b]	45.10 ± 1.79[b]	0.0384
Platelet count (10^9/L)	400.66 ± 1.69[a]	410.66 ± 3.68[a]	415.00 ± 4.08[a]	396.00 ± 3.00[a]	432.66 ± 13.91[a]	0.0035
Mean platelet volume (fl)	8.76 ± 0.41[a]	8.93 ± 0.33[a]	10.30 ± 0.45[a]	9.86 ± 0.25[a]	7.60 ± 1.77[a]	0.5427
Platelet distribution width (fl)	16.90 ± 1.65[a]	15.43 ± 0.32[a]	15.30 ± 0.43[a]	16.00 ± 0.77[a]	15.53 ± 0.32[a]	0.3681
Platelet large cell ratio (%)	32.60 ± 2.57[a]	30.36 ± 2.02[a]	30.96 ± 1.56[a]	34.53 ± 0.57[a]	31.43 ± 1.96[a]	0.2576

MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width (coefficient of variation), RDW-SD: Red cell distribution width (standard deviation). PC: Positive control. Values are expressed as mean ± SEM. [ab] Different superscript letters in a row indicate significant differences ($p < 0.05$).

To cite this paper: Jonas TNK, Waihenya RW, and Kariuki DW (2026). Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum*) Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice. *World Vet. J.,* 16(2): 312-323. DOI: https://dx.doi.org/10.54203/scil.2026.wvj30

Table 2. Biochemical results of the mice treated with pomegranate rind extract for 28 days

Parameters	Control	50mg/kg	100mg/kg	200mg/kg	PC	P-value
Alkaline phosphatase (U/L)	70.33 ± 1.69^a	73.66 ± 1.69^a	73.66 ± 1.69^a	65.33 ± 2.05^a	72.00 ± 2.80^b	0.0002
Alanine aminotransferase (U/L)	43.33 ± 1.24^a	41.66 ± 1.69^a	41.23 ± 0.89^a	42.33 ± 1.69^a	40.66 ± 5.90^b	0.0276
AST (U/L)	35.66 ± 0.94^a	32.66 ± 1.24^a	33.00 ± 0.81^a	33.66 ± 1.25^a	35.00 ± 0.81^b	0.0011
GGT (U/L)	48.00 ± 0.81^a	45.66 ± 2.49^a	51.00 ± 0.81^a	49.66 ± 1.69^a	46.00 ± 0.81^a	0.0206
Albumin (g/L)	42.40 ± 1.42^a	42.33 ± 1.24^a	43.66 ± 1.69^a	42.93 ± 1.35^a	43.23 ± 1.67^a	0.8805
Total protein (g/L)	77.56 ± 1.23^a	79.80 ± 3.82^a	74.56 ± 1.03^a	76.23 ± 0.65^a	76.46 ± 1.13^a	0.1757
Urea (mmol/L)	7.10 ± 0.71^a	7.70 ± 0.57^a	7.40 ± 0.69^a	7.26 ± 0.20^a	7.13 ± 0.16^a	0.7870
Creatinine (mmol/L)	100.00 ± 2.16^a	99.66 ± 2.49^a	101.00 ± 2.16^a	101.00 ± 1.63^a	103.00 ± 0.81^a	0.5024
Sodium (mmol/L)	150.33 ± 1.24^a	147.33 ± 1.69^a	147.00 ± 0.81^a	147.00 ± 0.81^a	148.33 ± 0.94^a	0.0723
Potassium (mmol/L)	3.90 ± 0.08^a	3.90 ± 0.08^a	3.86 ± 0.12^a	3.83 ± 0.12^a	4.06 ± 0.26^a	0.6001
Chloride (mmol/L)	103.33 ± 8.99^a	101.33 ± 6.18^a	98.33 ± 2.86^a	99.66 ± 2.49^a	129.66 ± 3.29^b	0.0009
Total bilirubin (mmol/L)	17.53 ± 0.49^a	17.30 ± 0.37^a	17.73 ± 0.46^a	18.10 ± 0.16^a	17.53 ± 0.44^a	0.4146
BILD (mmol/L)	< 1.80	< 1.80	< 1.80	< 1.80	< 1.80	

AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, BILD: Direct bilirubin (conjugated bilirubin), PC: Positive control. The values are expressed as mean ± SEM (n = 6). ^{ab}Different superscript letters in a row indicate significant differences from the negative control ($p < 0.05$).

Paw edema reduction

Carrageenan injection produced a marked increase in paw thickness across all groups at 4 hours post-injection. Following treatment administration, paw thickness at 4 hours post-treatment was presented as a percentage of edema reduction relative to the negative control (Table 3).

Tumor necrosis factor alpha levels

Serum TNF-α concentrations differed significantly across the experimental groups ($p < 0.0001$; Figure 3). The negative control group recorded the highest concentration of TNF-α at 410 pg/ml. The treatment group administered 50 mg/kg of pomegranate rind extract recorded a TNF-α level of 327.3 pg/mL, whereas the 100 and 200 mg/kg groups recorded 132.3 and 235.7 pg/mL, respectively. In addition, the level of TNF-α in the positive control group was 139.7 pg/mL. All the treatment groups and the positive control group exhibited significantly lower levels of TNF-α than the negative control ($p < 0.05$; Figure 3).

Interleukin-10 levels

All extract-treated groups demonstrated significantly higher IL-10 levels than the negative control group ($p < 0.05$), with the 50 mg/kg group at 134.7 pg/mL, the 100 mg/kg group at 258.0 pg/mL, and the 200 mg/kg group at 367.3 pg/mL. Furthermore, the IL-10 level in the positive control group was measured at 120.0 pg/mL (Figure 4).

Macroscopic evaluation

Macroscopic evaluation revealed that organs from the extract-treated groups and the positive control group exhibited no structural alteration. No observable differences among organs were detected in mice receiving 50 mg/kg, 100 mg/kg, or 200 mg/kg of pomegranate rind extract compared with the negative control group.

Histopathological parameters

Histological examination of the spleen, kidneys, heart, lungs, and liver revealed preserved structural organization in the negative control and extract-treated groups, with no pathological alterations (Figure 5). The positive control group revealed mild centrilobular hepatocyte vacuolation in the liver and mild tubular epithelial cell vacuolation in the kidneys.

Table 3. Percentage edema reduction at 4 hours post-treatment during four weeks of weekly carrageenan induction in mice treated with *Punica granatum* rind extract

Treatment group	Week 1	Week 2	Week 3	Week 4
Negative control	0.0%	0.0%	0.0%	0.0%
PRE 50 mg/kg	25.3%	37.8%	40.2%	42.5%
PRE 100 mg/kg	51.9%	58.5%	61.0%	67.5%
PRE 200 mg/kg	73.4%	75.6%	84.1%	86.2%
PC	84.8%	89.0%	90.2%	98.8

PRE: *Punica granatum* rind extract, PC: Positive control

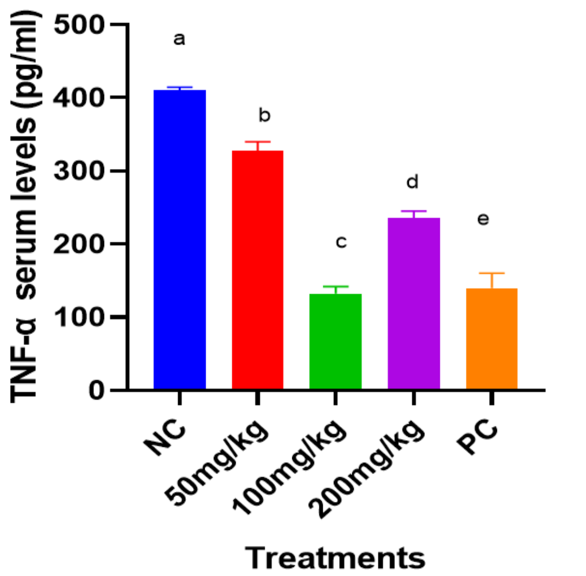

Figure 3. Serum TNF-α concentrations in mice following 28 days of oral administration of *Punica granatum* rind extract. Values are expressed as mean ± SEM (n = 6). [abc] Different superscript letters above bars indicate statistically significant differences from the negative control ($p < 0.0001$). NC: Negative control, PC: Positive control.

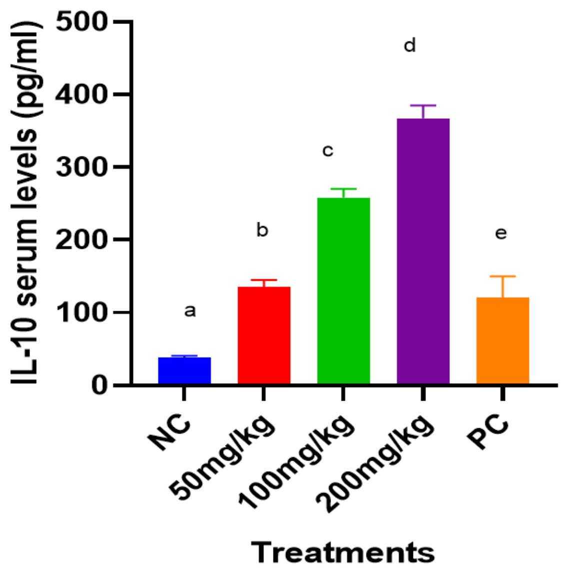

Figure 4. Serum IL-10 concentrations in mice following 28 days of oral administration of *Punica granatum* rind extract. Values are expressed as mean ± SEM (n = 6). [abcd] Different superscript letters above bars indicate statistically significant differences from the negative control ($p < 0.0001$). NC: Negative control, PC: Positive control.

To cite this paper: Jonas TNK, Waihenya RW, and Kariuki DW (2026). Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum*) Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice. *World Vet. J.*, 16(2): 312-323. DOI: https://dx.doi.org/10.54203/scil.2026.wvj30

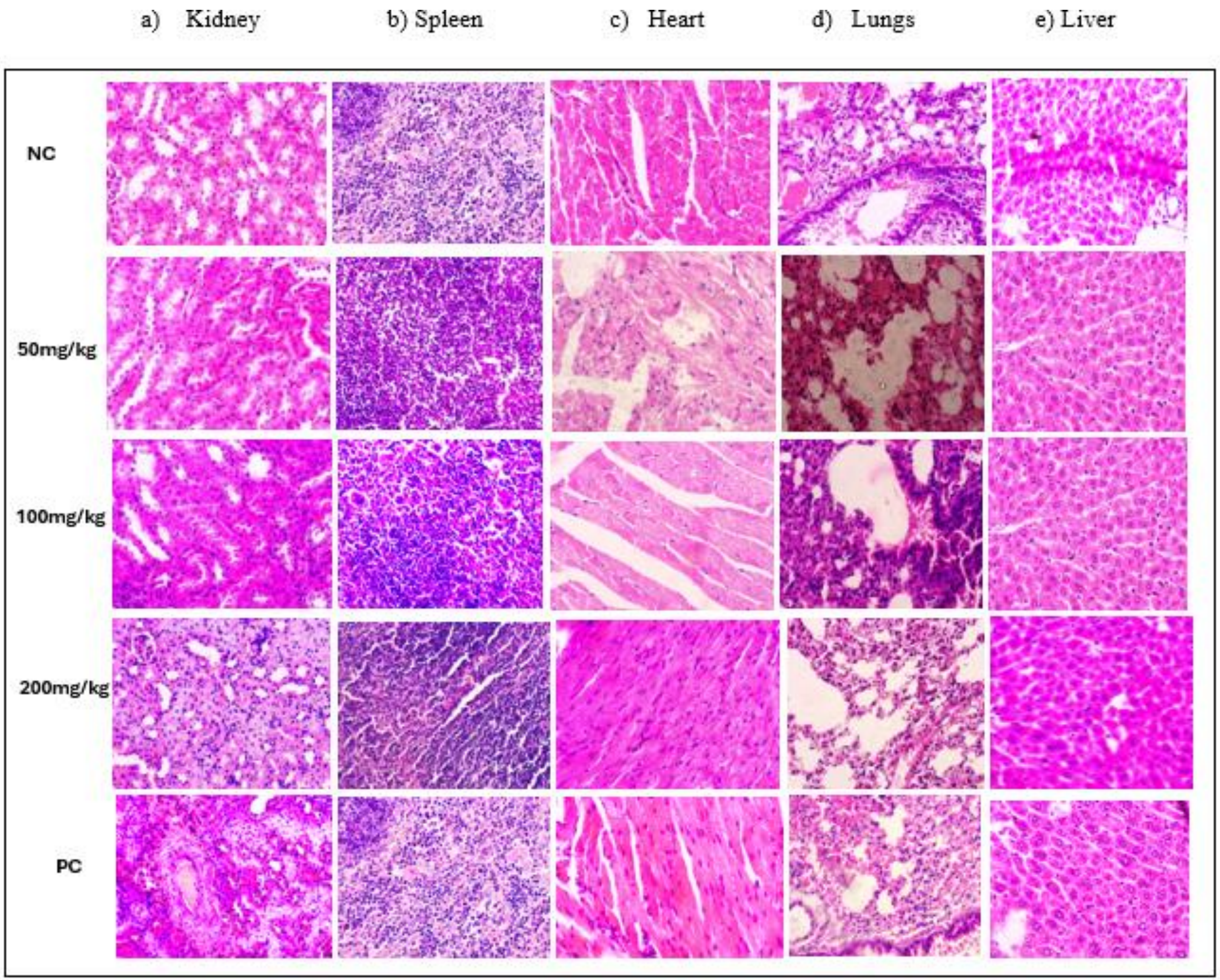

Figure 5. Microscopic evaluation of vital organs in mice that received *Punica granatum* rind extract collected on day 29. **a:** Renal sections from the negative control and extract-treated groups displayed normal cortical and medullary architecture with intact glomerular and tubular structures. The positive control group exhibited mild tubular epithelial cell vacuolation, **b:** Splenic sections across all groups showed preserved follicular architecture with distinct red and white pulp regions and no observable abnormalities, **c:** Cardiac tissue sections across all experimental groups demonstrated intact myocardial fibers with no evidence of cellular disruption or inflammatory infiltration, **d:** Pulmonary sections revealed a well maintained alveolar architecture with no structural compromise observed in any group, **e:** Hepatic sections from the negative control and all extract-treated groups showed normal lobular organization with well-preserved hepatocytes. Mild centrilobular hepatocyte vacuolation was noted in the positive control group (H&E, magnification 400x).

To cite this paper: Jonas TNK, Waihenya RW, and Kariuki DW (2026). Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum*) Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice. *World Vet. J.,* 16(2): 312-323. DOI: https://dx.doi.org/10.54203/scil.2026.wvj30

DISCUSSION

According to the current findings, exposure to *P. granatum* extract did not result in any signs of systemic toxicity, and the extract was well tolerated throughout the study (Widyarini et al., 2023). Posadas et al. (2004) demonstrated that carrageenan injection into the rodent paw produced a biphasic response, characterized by the early release of mediators followed by prolonged cellular infiltration. Multiple administrations of carrageenan prolong the inflammatory response, resulting in a recurrent inflammatory condition that affects physiological functions and tissue integrity in animal models (Komisarska et al., 2024). Evaluating the safety profile of therapeutic agents during chronic inflammatory conditions provides a more clinically relevant assessment than studies conducted on healthy subjects without inflammatory stress. An increase in paw thickness indicated immune cell infiltration along with the buildup of inflammatory exudates at the injection site. The observed increase in the paw thickness following carrageenan administration in the present study was consistent with the characteristic inflammatory response of the carrageenan-induced paw edema model in rodents (Posadas et al., 2004). In the current study, post-carrageenan injection and paw thickness increased progressively across all the groups, confirming inflammation induction. Paw edema reduction was dose-dependent across all four weeks, with each week showing more reduction than the previous week. Peak edema was recorded at 4 hours post-induction, consistent with the cellular phase of the carrageenan inflammatory response, which occurs between the third- and fifth hours post-injection (Widyarini et al., 2023). The progressive improvement in edema reduction suggested a cumulative anti-inflammatory effect with repeated *P. granatum* rind extract administration. The current findings were consistent with those of Karwasra et al. (2019), who also observed a dose-dependent decrease in carrageenan-induced inflammation after pomegranate supplementation. Meloxicam (2 mg/kg) produced the greatest edema reduction, reaching up to 98.8% by week four. Although 200 mg/kg of pomegranate rind extract did not have the efficacy of meloxicam, its dose-dependent cumulative anti-inflammatory effects, along with its favorable safety profile, indicated that pomegranate rind extract could be a potential alternative therapy. The mild anti-inflammatory effects observed at lower doses align with the bioavailability of the polyphenols, indicating that higher concentrations are necessary for greater inflammation suppression (Singh et al., 2023).

Daily administration of pomegranate rind extract exhibited no fatalities or morbidity related to toxicity during the entire study period. To assess the extract's toxicity, changes in physical appearance, feeding behavior, behavioral shifts such as lethargy or aggression, and gastrointestinal disturbances should be observed (Parasuraman, 2011); none of the signs were observed in any groups treated with pomegranate extract during the present study. The current findings indicated that daily administration of the pomegranate ethanol extract did not affect appetite or cause weight loss. The results of the current study are consistent with previous subacute toxicity studies of *P. granatum* peel extract in Swiss albino mice (Bassiri Jahromi et al., 2015) and with the study by Vale et al. (2020) in mice receiving hydroethanolic peel extract at doses up to 2000 mg/kg. The lack of notable differences in feed and water intake in the group that received meloxicam at 2 mg/kg aligns with the well-documented minimal dose-dependent gastrointestinal effects of NSAIDs at the specified dose of meloxicam (Botta et al., 2001; Richardson et al., 2022). Meloxicam is known to be associated with gastrointestinal disturbances at high doses (Botta et al., 2001; Richardson et al., 2022). Under subacute conditions in the present study, there were no substantial changes in feeding habits. Gastrointestinal toxicity associated with meloxicam administration in mice, including intestinal lesions and gastric ulcerations, has been reported at higher doses of 15-20 mg/kg (Botta et al., 2001; Richardson et al., 2022). The meloxicam dose administered in the present study was insufficient to induce gastrointestinal disturbances under subacute conditions. The treated mice maintained their normal feeding behaviors, and their body weights increased consistently, comparable to the control group. The findings of the current study suggested that prolonged exposure to pomegranate rind extract did not adversely affect normal growth, alter feeding behavior, or induce physiological changes that could compromise the overall health of Balb/c mice. Furthermore, macroscopic examination of the liver, spleen, heart, lungs, and kidneys from extract-treated mice demonstrated no visible abnormalities compared to the control group.

Histopathological evaluation supported the safety profile of the pomegranate rind extract, as no structural alterations were observed in the mice's tissues. Normal tissue structure was preserved across all treated groups, indicating that, under the subacute experimental conditions of the present study, the extract did not induce toxicity. The findings of the current study are particularly relevant to sustained inflammatory stress, in which failure to resolve chronic inflammation is often associated with tissue damage and organ injury (Medzhitov, 2008; Komisarska et al., 2024). Mild centrilobular hepatocyte vacuolation, indicating intracellular accumulation, was observed in the liver of the positive control group. In the kidney, mild vacuolation of tubular epithelial cells was observed. The histopathological observations were supported by liver and kidney biochemical markers in the present study.

The kidneys and liver are key organs for detoxifying the body, as they metabolize harmful substances and facilitate their removal (Wurihan et al., 2022). Consequently, assessing serum-based, organ-specific biochemical markers associated with the kidneys is essential for determining potential toxicity (Wurihan et al., 2022). Elevated serum levels of hepatic enzymes, particularly AST and ALT, along with renal waste products such as urea and creatinine, may indicate

To cite this paper: Jonas TNK, Waihenya RW, and Kariuki DW (2026). Anti-inflammatory and Subacute Safety Effects of Pomegranate (*Punica granatum*) Rind Extract in Recurrent Carrageenan-Induced Inflammation in BALB/c Mice. *World Vet. J.*, 16(2): 312-323. DOI: https://dx.doi.org/10.54203/scil.2026.wvj30

hepatic or renal tissue damage or necrosis, resulting in their release into the circulatory system. Therefore, increased levels of hepatic and renal markers are commonly used as indicators of hepatotoxicity or nephrotoxicity (Jegnie et al., 2023; Adeyele et al., 2024). In the present study, serum concentrations of ALP, AST, ALT, GGT, urea, creatinine, and electrolytes remained within established physiological reference ranges across all extract-treated groups, indicating that oral administration of *P. granatum* rind extract did not adversely affect hepatic or renal function in BALB/c mice. The findings of the present study are consistent with previous subacute toxicity investigations of pomegranate peel extract, in which renal biochemical markers remained within physiological reference ranges following oral administration, thereby supporting the safety of the pomegranate-derived extract for hepatic and renal health (Bassiri Jahromi et al., 2015; Vale et al., 2020). The TNF-α is a strong pro-inflammatory cytokine, involved in the recruitment of leucocytes and tissue injury (Mittal et al., 2014). In the present study, TNF-α was notably reduced in all extract-treated groups compared with the negative control group. The strongest suppression in TNF-α level was observed at a dosage of 100 mg/kg of pomegranate rind extract (132.3 pg/mL), which was similar to that of the meloxicam group (139.7 pg/mL). The IL-10, a cytokine involved in anti-inflammatory responses and tissue repair, increased in a dose-dependent manner across all extract-treated groups, surpassing the level observed in the meloxicam group. The contrasting regulation of TNF-α and IL-10 indicated a coordinated immunomodulatory effect of the pomegranate rind extract. Similar cytokine modulation patterns have been reported in mice administered pomegranate peel extract. Manickam et al. (2022) demonstrated that daily oral administration of pomegranate peel extract at 200 mg/kg for 12 weeks in $Apoe^{-/-}$ mice reduced TNF-α and increased IL-10, accompanied by decreased atherosclerotic plaque necrosis and improved collagen content in lesions. Similarly, Karwasra et al. (2019) found that pomegranate supplementation at 50, 100, and 200 mg/kg in experimental rheumatoid arthritis decreased inflammation and improved joint function by inhibiting NF-κB signaling. However, the observed nonlinear dose response in the present study, in which 100 mg/kg of pomegranate rind extract resulted in greater TNF-α suppression than 200 mg/kg, which was contrasted with the dose-dependent reduction reported in previous studies. The variations observed in these studies could be due to differences in the animal model, extract preparation, or the impact of recurrent inflammation on cytokine dynamics. Balanced cytokine regulation is essential for resolving inflammation. In chronic inflammatory models, such as sustained carrageenan exposure, cytokine imbalance can cause progressive tissue damage (Posadas et al., 2004).

Hematological parameters were assessed as part of the subacute toxicity evaluation, as changes in blood indices provide measurable evidence of potential systemic toxicity following repeated compound exposure (Jegnie et al., 2023). In the present study, hematological results demonstrated a notable difference in RDW-SD between the control and 200 mg/kg group. All values remained within established physiological reference ranges with no signs of systemic toxicity, indicating that these differences were not biologically adverse. The positive control group similarly exhibited significant differences in erythrocyte parameters, including RBC and hemoglobin. Carrageenan initiates a strong inflammatory response, resulting in increased pro-inflammatory cytokines and alterations in hematopoiesis (Posadas et al., 2004). Inflammatory stress alone was sufficient to alter blood parameters, including white blood cell counts, platelet counts, and red blood cell indices, without any direct toxic effects of the administered extract. Therefore, the notable differences could suggest an ongoing inflammatory condition rather than a direct toxic effect of the extract.

Toxicological interpretation should consider both the biological context and the observed change, rather than relying solely on statistical significance, when determining adverse effects (Organisation for Economic Co-operation and Development, 2002; EFSA Scientific Committee et al., 2017). Parameters within normal physiological limits are generally regarded as non-adverse, especially when no histopathological or biochemical abnormalities are present (Organization for Economic Co-operation and Development, 2002). As demonstrated in the present study, the absence of tissue damage and maintenance of normal body weight in all extract-treated groups supported that the extract was well tolerated under the subacute conditions.

CONCLUSION

The current findings demonstrated that repeated oral administration of pomegranate rind extract over 28 days produced dose-dependent anti-inflammatory effects. A progressive decrease in paw edema, along with suppression of TNF-α and an increase in IL-10, occurred in a dose-dependent manner. There were no mortalities, clinical signs of toxicity, treatment-associated histopathological changes, or adverse effects on hepatic and renal function, which suggested that the extract was well tolerated throughout the study period. The current findings supported the potential of *P. granatum* rind extract as a safe and effective therapeutic candidate for inflammatory conditions. Although the present study was conducted in male mice over a 28-day period, long-term toxicity studies including both sexes, as well as investigations of the underlying molecular mechanisms, are necessary to establish a comprehensive safety and efficacy profile for broader therapeutic application.

DECLARATIONS

Acknowledgements

Appreciation is extended to PAUSTI for providing the laboratory materials to facilitate the research and to Mr. Mark Mogaka Nyandege for his assistance at SAFARI.

Authors' contributions

Twahafifwa Nghuuluka Kanemo Jonas was responsible for developing the study methodology, overseeing data curation, and preparing the original manuscript draft. Rebecca Wanjiku Waihenya and Daniel Wainaina Kariuki contributed to the critical revision and editing of the manuscript. All authors have read and approved the final edition of the manuscript before publication in the present journal.

Availability of data and materials

All data produced and examined in this study are included in the manuscript, with no supplementary files. Requests for additional data generated in this study should be directed to the corresponding author.

Competing interests

The authors declared no conflicts of interest.

Ethical considerations

The authors confirmed that the present study is original, has not been previously submitted for publication or under review elsewhere, and adheres to ethical standards regarding data integrity and the avoidance of plagiarism. The authors also affirmed that no artificial intelligence tools were used in the writing and preparation of the manuscript.

Funding

The present study was conducted with financial support from the African Union under the PAUSTI scholarship program.

REFERENCES

Adeyele EI, Ayanyemi EO, Akomolafe RO, Sesan OO, Aladesanmi OT, and Adetutu AO (2024). Assessment of the toxic influence of locally formulated pesticides on hepatic and renal biomarkers in male Wistar rats. Toxicology Research, 13(5): tfae157. DOI: https://www.doi.org/10.1093/toxres/tfae157

Aviram M and Rosenblat M (2012). Pomegranate protection against cardiovascular diseases. Evidence-Based Complementary and Alternative Medicine, 2012: 382763. DOI: https://www.doi.org/10.1155/2012/382763

Bassiri Jahromi S, Pourshafie MR, Mirabzadeh E, Tavasoli A, Katiraee F, Mostafavi E, and Abbasian S (2015). Toxicity of *Punica granatum* peel extract in mice. Jundishapur Journal of Natural Pharmaceutical Products, 10(4): e23770. DOI: https://www.doi.org/10.17795/jjnpp-23770

Bhandary BSK, Sharmila KP, Kumari NS, and Bhat SV (2013). Acute and subacute toxicity study of the ethanol extracts of *Punica granatum* (Linn.) whole fruit and seeds and synthetic ellagic acid in Swiss albino mice. Nitte University Journal of Health Science, 3(2): 38-43. Available at: https://nitte.edu.in/journal/

Bindu S, Mazumder S, and Bandyopadhyay U (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochemical Pharmacology, 180: 114147. DOI: https://www.doi.org/10.1016/j.bcp.2020.114147

Botta L, Perazzo MG, Longo G, and Izzo AA (2001). Effects of food intake and oxidative stress on intestinal lesions caused by meloxicam and piroxicam in rats. European Journal of Pharmacology, 414(2-3): 185-191. DOI: https://www.doi.org/10.1016/s0014-2999(01)00806-2

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, and Zhao L (2018). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6): 7204-7218. DOI: https://www.doi.org/10.18632/oncotarget.23208

Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, and van de Vorstenbosch C (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology, 21(1): 15-23. DOI: https://www.doi.org/10.1002/jat.727

Dudhgaonkar SP, Tandan SK, Bhat AS, Jadhav SH, and Kumar D (2006). Synergistic anti-inflammatory interaction between meloxicam and aminoguanidine hydrochloride in carrageenan-induced acute inflammation in rats. Life Sciences, 78(10): 1044-1048. DOI: https://www.doi.org/10.1016/j.lfs.2005.06.002

EFSA scientific committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C et al. (2017). Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal, 15(8): 4970. DOI: https://www.doi.org/10.2903/j.efsa.2017.4970

Fischer AH, Jacobson KA, Rose J, and Zeller R (2008). Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harbor Protocols, 2008(5): pdb.prot4986. DOI: https://www.doi.org/10.1101/pdb.prot4986

Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, and Li J (2022). Aging and aging-related diseases: From molecular mechanisms to interventions and treatments. Signal Transduction and Targeted Therapy, 7(1): 391. DOI: https://www.doi.org/10.1038/s41392-022-01251-0

Ismail T, Sestili P, and Akhtar S (2012). Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. Journal of Ethnopharmacology, 143(2): 397-405. DOI: https://www.doi.org/10.1016/j.jep.2012.07.004

Jegnie M, Abula T, Woldekidan S, Chalchisa D, Asmare Z, and Afework M (2023). Acute and sub-acute toxicity evaluation of the crude methanolic extract of Justicia schimperiana leaf in Wistar albino rats. Journal of Experimental Pharmacology, 15: 467-483. DOI: https://www.doi.org/10.2147/JEP.S441273

Kandylis P and Kokkinomagoulos E (2020). Food applications and potential health benefits of pomegranate and its derivatives. Foods, 9(2): 122. DOI: https://www.doi.org/10.3390/foods9020122

Karwasra R, Singh S, Sharma D, Sharma S, Sharma N, and Khanna K (2019). Pomegranate supplementation attenuates inflammation, joint dysfunction via inhibition of NF-κB signaling pathway in experimental models of rheumatoid arthritis. Journal of Food Biochemistry, 43(8): e12959. DOI: https://www.doi.org/10.1111/jfbc.12959

Komisarska P, Pinyosinwat A, Saleem M, and Szczuko M (2024). Carrageenan as a potential factor of inflammatory bowel diseases. Nutrients, 16(9): 1367. DOI: https://www.doi.org/10.3390/nu16091367

Lansky EP and Newman RA (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnopharmacology, 109(2): 177-206. DOI: https://www.doi.org/10.1016/j.jep.2006.09.006

Larrosa M, González-Sarrías A, Yáñez-Gascón MJ, Selma MV, Azorín-Ortuño M, Toti S, Tomás-Barberán F, Dolara P, and Espín JC (2010). Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. The Journal of Nutritional Biochemistry, 21(8): 717-725. DOI: https://www.doi.org/10.1016/j.jnutbio.2009.04.012

Lippi G, Salvagno GL, Brocco G, and Guidi GC (2005). Preanalytical variability in laboratory testing: Influence of the blood drawing technique. Clinical Chemistry and Laboratory Medicine, 43(3): 319-325. DOI: https://www.doi.org/10.1515/CCLM.2005.055

Malm Tillgren S, Nieto-Fontarigo JJ, Cerps S, Ramu S, Menzel M, Mahmutovic Persson I, Meissner A, Akbarshahi H, and Uller L (2023). C57Bl/6N mice have an attenuated lung inflammatory response to dsRNA compared to C57Bl/6J and BALB/c mice. Journal of Inflammation, 20(1): 6. DOI: https://www.doi.org/10.1186/s12950-023-00331-4

Manickam V, Dhawan UK, Singh D, Gupta M, and Subramanian M (2022). Pomegranate peel extract decreases plaque necrosis and advanced atherosclerosis progression in Apoe-/- mice. Frontiers in Pharmacology, 13: 888300. DOI: https://www.doi.org/10.3389/fphar.2022.888300

Medzhitov R (2008). Origin and physiological roles of inflammation. Nature, 454(7203): 428-435. DOI: https://www.doi.org/10.1038/nature07201

Mittal M, Siddiqui MR, Tran K, Reddy SP, and Malik AB (2014). Reactive oxygen species in inflammation and tissue injury. Antioxidants and Redox Signaling, 20(7): 1126-1167. DOI: https://www.doi.org/10.1089/ars.2012.5149

Organisation for economic co-operation and development (2002). Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies. OECD Series on Testing and Assessment, No. 35. Organisation for Economic Co-operation and Development, Paris, pp. 1-112. DOI: https://www.doi.org/10.1787/9789264078499-en

Parasuraman S (2011). Toxicological screening. Journal of Pharmacology and Pharmacotherapeutics, 2(2): 74-79. DOI: https://www.doi.org/10.4103/0976-500X.81895

Parasuraman S, Raveendran R, and Kesavan R (2010). Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics, 1(2): 87-93. DOI: https://www.doi.org/10.4103/0976-500X.72350

Patel C, Dadhaniya P, Hingorani L, and Soni MG (2008). Safety assessment of pomegranate fruit extract: Acute and subchronic toxicity studies. Food and Chemical Toxicology, 46(8): 2728-2735. DOI: https://www.doi.org/10.1016/j.fct.2008.04.035

Perrot S, Guilbaud G, and Kayser V (1999). Effects of intraplantar morphine on paw edema and pain-related behaviour in a rat model of repeated acute inflammation. Pain, 83(2): 249-257. DOI: https://www.doi.org/10.1016/S0304-3959(99)00110-4

Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, and Cirino G (2004). Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. British Journal of Pharmacology, 142(2): 331-338. DOI: https://www.doi.org/10.1038/sj.bjp.0705650

Richardson CA, Flecknell PA, and Crabbs T (2022). Toxic effects of high-dose meloxicam and carprofen on female CD1 mice. Journal of the American Association for Laboratory Animal Science, 61(1): 75-82. DOI: https://www.doi.org/10.30802/AALAS-JAALAS-21-000049

Schjerning AM, McGettigan P, and Gislason G (2020). Cardiovascular effects and safety of (non-aspirin) NSAIDs. Nature Reviews Cardiology, 17(9): 574-584. DOI: https://www.doi.org/10.1038/s41569-020-0366-z

Singh J, Kaur HP, Verma A, Chahal AS, Jajoria K, Rasane P, Kaur S, Kaur J, Gunjal M, Ercisli S et al. (2023). Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and its application: A comprehensive review. ACS Omega, 8(39): 35452-35469. DOI: https://www.doi.org/10.1021/acsomega.3c02586

Sohail R, Mathew M, Patel KK, Reddy SA, Haider Z, Naria M, Habib A, Abdin ZU, Razzaq Chaudhry W, and Akbar A (2023). Effects of non-steroidal anti-inflammatory drugs (NSAIDs) and gastroprotective NSAIDs on the gastrointestinal tract: A narrative review. Cureus, 15(4): e37080. DOI: https://www.doi.org/10.7759/cureus.37080

Sultana B, Anwar F, and Ashraf M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14(6): 2167-2180. DOI: https://www.doi.org/10.3390/molecules14062167

Vale EP, Do Rego LR, Pureza DDN, Silva PGDB, De Sousa FFO, and Monteiro Neto MDAB (2020). Cytogenetic and toxicological effects of *Punica granatum* Linnaeus fruit peel hydroethanolic extract in mice. South African Journal of Botany, 130: 465-470. DOI: https://www.doi.org/10.1016/j.sajb.2020.01.041

Viuda-Martos M, Fernández-López J, and Pérez-Álvarez JA (2010). Pomegranate and its many functional components as related to human health: A review. Comprehensive Reviews in Food Science and Food Safety, 9(6): 635-654. DOI: https://www.doi.org/10.1111/j.1541-4337.2010.00131.x

Widyarini S, Soleha S, Akrom AM, and Paryuni AD (2023). Carrageenan-induced acute inflammation on back-skin of mice: Histopathological features, number of inflammatory cells, and expression of COX-2, COX-1, and IL-6. World's Veterinary Journal, 13(4): 520-530. DOI: https://www.doi.org/10.54203/scil.2023.wvj55

Winter CA, Risley EA, and Nuss GW (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Experimental Biology and Medicine, 111(3): 544-547. DOI: https://www.doi.org/10.3181/00379727-111-27849

Woode E, Asante-Kwatia E, Mensah AY, and Barimah KB (2022). Effect of diclofenac and andrographolide combination on carrageenan-induced paw edema and hyperalgesia in rats. Dose Response, 20(2): 15593258221103846. DOI: https://www.doi.org/10.1177/15593258221103846

Wurihan, Aodungerle, Bilige, Lili, Sirguleng, Aduqinfu, and Bai M (2022). Metabonomics study of liver and kidney subacute toxicity induced by garidi-5 in rats. Chinese Herbal Medicines, 14(3): 422-431. DOI: https://www.doi.org/10.1016/j.chmed.2022.05.003

Publisher's note: Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.