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Radioprotective potential of pomegranate peel extract against gamma irradiation-induced hazards

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Abstract

Background While gamma irradiation's damaging biological effects are well-established, the natural radioprotective agents from agricultural waste remain an underexplored area of significant potential.

Aim of study This study was to investigate the novel use of pomegranate peel ethanol extract (PE) as a radioprotective agent against gamma radiation damage.

Methods We pretreated Wistar rats with PE (100 mg/kg) for 14 days prior to 6 Gy gamma irradiation. We analyzed blood biochemicals, oxidative stress, and inflammatory markers. These included tests of red cell membrane integrity, lipid and protein oxidation, antioxidant enzyme levels, and cytokine profiles.

Results The study showed that PE demonstrated remarkable radioprotective effects across multiple parameters. Antioxidants were significantly enhanced, as evidenced by increased glutathione peroxidase activity (87.00 ± 6.11 mg/ml in PE-treated irradiated rats compared to 26.40 ± 1.21 mg/ml in irradiated controls). Oxidative damage was markedly reduced, with MDA levels dropping from 9.59 ± 0.24 nmol/ml in irradiated controls to near-control levels in PE-treated rats. Notably, PE treatment resulted in unprecedented maintenance of red blood cell membrane integrity post-irradiation. Furthermore, PE exhibited novel modulation of inflammatory cytokines, effectively reducing pro-inflammatory markers IL-6 and TNF-α while simultaneously boosting anti-inflammatory IL-4 and IL-10 levels. These multifaceted protective effects highlight PE's potential as a comprehensive radioprotective agent.

Conclusion This study presents PE as an effective new natural radioprotective agent. Its protective effect is due to its high polyphenol content, which enhances antioxidant defenses, reduces oxidative damage, and prevents inflammation. The findings open new avenues for sustainable, cost-effective radioprotection strategies and demonstrate the potential for repurposing agricultural byproducts for critical health applications.

Keywords Radioprotection, Gamma irradiation, Antioxidants, Agricultural waste

Background

Radiation therapy, a vital component of modern medical and industrial applications, has substantially improved the diagnosis and treatment of numerous diseases and processes (Huynh et al. 2020; Luharia et al. 2022). However, the positive impact of ionizing radiation is inevitably accompanied by potential harm to living organisms and the environment, emphasizing the need for strategies to minimize its deleterious effects (Gudkov et al. 2019). In the midst of this challenge, the exploration of natural

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compounds with radioprotective potential stands as a promising avenue to mitigate radiation-induced damage and advance the field of radioprotective research.

According to Mansour et al. (2008) and Nilsson and Liu (2020), radiotherapy induces DNA damage and also stimulates the generation of free radicals via indirect pathways. During the process of radiotherapy, radiation causes the disintegration of water molecules inside the human body, leading to the formation of free oxygen radicals, namely hydroxyl radicals ($OH^{\cdot}$) and superoxide ($O\ 2^{\cdot-}$). Furthermore, it has been shown that this phenomenon also results in an alteration of the equilibrium between oxidants and antioxidants inside cellular systems (Clement et al. 2020; Akhila et al. 2021). Reactive oxygen species (ROS) have a significant role in the development of detrimental conditions such as carcinogenesis, mutagenesis, aging, and atherosclerosis (Juan et al. 2021; Nitti et al. 2022). The use of antioxidants in cancer therapy and supplementary or preventative medicine is of significant importance, however subject to controversy within this domain (Griñan-Lison et al. 2021).

Studies have shown that extracts from several plants can be beneficial for human disorders caused by free radicals. Reactive oxygen species (ROS) and reactive nitrogen species can cause damage, and these extracts contain chemicals with antioxidant properties (Engwa 2018). Nature contains a diverse range of chemicals known as polyphenols (Singla et al. 2019). Pomegranate peel, an agricultural by-product, is rich in bioactive compounds, including polyphenols and flavonoids (Vučić et al. 2019; Zhao et al. 2022).

Studies have demonstrated that pomegranate peel extracts (PE) containing a significant amount of polyphenols and tannins have strong antioxidant activity (Zhao et al. 2022; Saparbekova et al. 2023). Researchers have found several biological activities, including anti-tumor, antibacterial, and anti-diabetic properties, in extracts from several sections of *P. granatum* (Rahmani et al. 2017). Recent studies have shown evidence of the hepatoprotective qualities of pomegranate. These features make it a valuable therapeutic agent for treating hepatic fibrosis and oxidative damage (Husain et al. 2018; Zamanian et al. 2023). Nevertheless, the high concentration of chemicals present in pomegranate peels necessitates further investigation into the specific molecule responsible for its radical scavenging action (Russo et al. 2018; Xiang et al. 2022). Furthermore, it is important to conduct toxicity tests in order to ascertain the safety threshold of pomegranate peel extract.

In both planned and unplanned accidental radiation exposures, protecting biological systems from ionizing radiation is important (Singh and Seed 2020). Hemoglobin and polyunsaturated lipids, which are abundant in RBC membranes, react with free radicals to cause lipid peroxidation (Supawat et al. 2023). Red blood cells' (RBCs') ability to resist lysis from a drop in NaCl concentration forms the basis of the osmotic fragility test. Red blood cells (RBCs) exposed to ozone experienced an elevated osmotic fragility due to the peroxidation of lipids and proteins (Selim et al. 2009a, b). Inadequate osmotic resistance can result in intravascular hemolysis, leading to a decrease in the lifespan of red blood cells. Moreover, red blood cell destruction may result in inadequate oxygen consumption in tissue or organs, increasing the possibility of adverse effects on the human body (Hemida et al. 2012; Gwozdzinski et al. 2021). The objective of this study is to analyze and measure specific polyphenol compounds in pomegranate peel extract. Additionally, the study aims to determine the extract's LC50 and evaluate its ability to protect albino rats from the negative effects of gamma radiation, such as reduced levels of tissue antioxidants and damage to red blood cell membranes. Agricultural waste, a byproduct of the agro-industry, presents both an environmental challenge and an untapped resource for potential solutions (Quintero-Herrera et al. 2023). Pomegranate (Punica granatum) has gained considerable attention in recent years owing to its multifaceted health-promoting properties. Notably, pomegranate peel, a byproduct of the pomegranate fruit processing industry, has emerged as a rich source of bioactive compounds with antioxidant, anti-inflammatory, and tissue-protective attributes (Marra et al. 2022). These attributes have prompted the investigation of pomegranate peel extract (PE) as a potential radioprotector. While pomegranate peel extracts (PE) have been studied for their antioxidant properties in different settings, this is the first time that PE has been used to protect against damage caused by gamma irradiation. This study represents one of the first comprehensive investigations into the radioprotective potential of pomegranate peel extract (PE), offering a sustainable solution by repurposing agricultural waste. Such an approach not only mitigates radiation-induced damage, but it also contributes to environmental sustainability by valorizing agro-industrial by-products.

This study aims to investigate whether pomegranate peel ethanol extract (PE) can shield cells from gamma radiation damage. Specifically, we investigate its effects on oxidative stress, antioxidant defense mechanisms, red blood cell membrane integrity, and inflammatory responses in rats exposed to 6 Gy gamma radiation. We aim to investigate the viability of using agricultural waste, such as pomegranate peel, as a natural radioprotective agent with potential applications in mitigating radiation-related hazards.

Methods

In vitro study

Preparation of pomegranate peel extract

We carefully collected the pomegranate peels, dried them thoroughly, and ground them into a fine powder using a grinder (580 V, Germany). Then mixed the powdered plant materials with 70% ethanol and let them macerate for 24 h. We separated the ethanol extracts from the solid residue by filtration after the maceration period. We then concentrated the ethanol extracts using a vacuum rotary evaporator to obtain the concentrated extracts.

HPLC analysis

Agilent 1260 series HPLC equipment carried out the analysis. We used an Eclipse C18 column (4.6 mm × 250 mm i.d., 5 m) for the separation process. We programmed the mobile phase in a linear gradient as follows: 0–5 min (80% A), 5–12 min (60% A), and 12–20 min (82% A). We set the multi-wavelength detector to monitor absorbance at 280 nm. For each sample solution, the injection volume was 5 µl. We maintained the column temperature at 40 °C.

Hemolysis

To determine the degree of hemolysis, the amount of hemoglobin released from normal red blood cells was compared to the total amount of hemoglobin within the cells in order to determine the degree of hemolysis. We incubated 10 l of freshly collected whole blood in 5 mL of normal saline for 30 min. We used spectrophotometry to determine the 540 nm wavelength of the supernatant after centrifuging the samples for 10 min at 3000 rpm. We compared the percent of hemolysis to the total hemolysis of the blood (Hemida et al. 2012).

$$\text{Hemolysis } \% = \left[A_{\text{sample}}/A_{100\%\ \text{lysis}}\right] \times 100 \qquad (1)$$

The letters A sample and A 100% lysis indicate the absorbance of the hemoglobin released from red blood cells (RBCs) in distilled water and normal saline, respectively, after complete hemolysis.

The osmotic fragility (OF) test

The osmotic fragility test provides a quantitative assessment of the degree of hemolysis (Selim et al. 2009a, b). The ratio of whole blood samples to the hypotonic buffer saline was 1:100, respectively. We used saline with different concentrations, ranging from 0 to 0.9%.

To precipitate the nonhemolyzed red blood cells, the samples were centrifuged for 5 min at 3000 rpm after incubating for 30 min at 37 °C. Hemoglobin release into the extracellular fluid indicates red blood cell osmotic lysis. Colorimetric method was used to quantify the amount of hemoglobin present in the media (Hemida et al. 2012). The fragility curve can be evaluated by calculating the average osmotic fragility (H50), which is the concentration of NaCl that results in 50% hemolysis. The fragility curve (the rate of hemolysis, dH/dC vs NaCl concentration) can be differentiated to get additional parameters. These parameters are height, width, and position. The average osmotic fragility (H_{50}) is similar to the point on the x-axis. The hemolysis process's dispersion is reflected in the width at half maximum; a lower dispersion than usual indicates a sudden rupture of the red blood cells. The height of the peak indicates the sample's maximum rate of hemolysis (dH/dC max).

Blood film preparation and staining

Leishman stain was applied to the films and allowed to dry for 5 min. After that, the slides were rinsed with phosphate buffer solution until a pink color was observed.

The blood film was then seen under a microscope with a magnification of × 100 using the CX41 Olympus Microscope, Olympus Corporation, Japan.

In vivo experimental design

Animals Thirty-two male Wistar rats, weighing 150 ± 10 g, were procured from the animal house at the National Research Centre, Dokki, Giza, Egypt. They were placed in a ventilated temperature-controlled room (22 ± 2 °C) in standard cages (polycarbonate) under 12/12 h light/dark cycles. We provided the animals with clean drinking water and a standard diet ad libitum.

The animals were divided into 4 groups (n = 8):

Group I Rats were administered a placebo control, consisting of a daily oral dose of 0.5 ml of normal saline as a vehicle, for a duration of 14 days.

Group II: Extracts group (PE) Rats received oral gavage of pomegranate peel extract (100 mg/kg body weight/day) for 14 days.

Group III: PE + IR group Rats were treated with the extracts for 14 days, and 1 h after the last extract dosage, they were exposed to 6 Gy gamma irradiation.

Group IV: Irradiated (IR) group Rats were administered the vehicle for 14 days and then exposed to whole-body gamma irradiation (6 Gy).

Euthanasia and samples collection At 24 h after the final administration, after an overnight fast, the rats were anesthetized with pentobarbital sodium (50 mg/kg) and subsequently euthanized. Blood samples were collected. The vena cava blood was obtained and divided into two parts. The first part was centrifuged to extract serum, and the second part was treated with heparin as an anticoagulant for analysis of Hb. Serum samples were stored at − 80 °C for future analysis.

Toxicology testing in animals

Acute oral toxicity studies and dose determination

Administration of ethanolic extracts of PE was given at various doses ranging from 100 to 6000 mg/kg body weight were administered. The LD50 was calculated according to the equation suggested by (Kokoski et al. 1990).

Measurement of biochemical parameters

Lipid peroxidation and NO Lipid peroxidation (MDA) assay was conducted following the method of Moore and Roberts (1998). We performed the quantification of nitric oxide (NO) levels by incubating the sample with the Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) for 10 min at room temperature, while protecting it from light, and then measuring the absorbance at 540 nm, following the method outlined by Green et al. (1982).

Detection of antioxidants Levels of reduced glutathione peroxidase (GSH-Px) was determined using commercial kits following the manufacturer's instructions.

Total antioxidant capacity (TAC) in serum was measured according to the method of Benzie and Strain (1999), using the ferric reducing ability (FRAP) assay. CAT (mg/ml), and GPX (mg/ml) were determined using commercial kits following the manufacturer's instructions according to the method of Aebi (1984), and Paglia and Valentine (1967), respectively using Bio-Diagonstics kits (Giza, Egypt).

Radiation facility Whole-body gamma irradiation of rats was conducted using a Canadian gamma cell-40 (137Cs) at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Placed in a plastic sample dish, unstimulated rats were subjected to radiation at a dosage of 6 Gy, at a rate of 0.622 rad/s.

Study was taken up after the approval of Medical Research Ethics Committee, Federal Wide Assurance (FWA) 00014747 RHDIRB 2017103002 (Approval No 13010419-2) in 6-2-2023.

Statistical analyses

The data is presented as mean values ± standard errors. Statistical comparisons among the different groups were conducted utilizing a one-way analysis of variance (ANOVA), followed by Tukey's test for post hoc analysis by using Graph Pad Prism Software (V. 10.2.0, GraphPad Software Inc., San Diego, CA, USA). A significance level of $p < 0.05$ was established for determining the significance of the observed differences between groups.

Results

High performance liquid chromatography (HPLC)

HPLC was used for the standardization of phenolic and flavonoid compounds. Currently, HPLC represents the most reliable analytical technique for the characterization of polyphenolic compounds. Therefore, flavonoids and phenols were quantified in the extract of pomegranate peel using this technique. The extract was examined using HPLC along with available polyphenol standards at a wavelength of 280 nm to investigate the presence of polyphenolic compounds and determine their concentrations. The results were summarized in Table 1.

According to Table 1, HPLC identified ten and fifteen polyphenols in pomegranate peel extract, respectively. These polyphenols are the main phenolic compounds responsible for the antioxidant activity. In pomegranate peel, the most abundant phenolic compound is gallic acid, followed by catechin, chlorogenic acid, and ellagic acid, with concentrations of 13,442.14 µg/g, 5708.96 µg/g, 2849.81 µg/g, and 2260.66 µg/g, respectively. On the other hand, vanillin has the lowest concentration, 46.48 µg/g. For the flavonoids, the result represented their absence except naringenin, which was detected in a small amount.

Table 1 The concentration of the different polyphenol detected in pomegranate peel extract

Pomegranate peel extract			
	Area	**Conc. (µg/ml = 20 mg/ml)**	**Conc. (µg/g)**
Gallic acid	3113.34	268.84	13,442.14
Chlorogenic acid	416.21	57.00	2849.81
Catechin	461.07	114.18	5708.96
Methyl gallate	81.13	4.43	221.42
Coffeic acid	0.00	0.00	0.00
Syringic acid	27.86	1.89	94.47
Pyro catechol	0.00	0.00	0.00
Rutin	0.00	0.00	0.00
Ellagic acid	243.90	45.21	2260.66
Coumaric acid	0.00	0.00	0.00
Vanillin	21.24	0.93	46.48
Ferulic acid	18.57	1.27	63.44
Naringenin	42.83	5.17	258.26
Daidzein	0.00	0.00	0.00
Querectin	0.00	0.00	0.00
Cinnamic acid	0.00	0.00	0.00
Apigenin	0.00	0.00	0.00
Kaempferol	0.00	0.00	0.00
Hesperetin	0.00	0.00	0.00

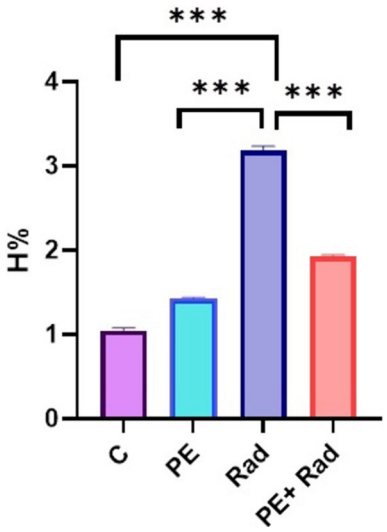

Fig. 1 Hemolysis % of red blood cells of different groups

Hemolysis

The release of hemoglobin from red blood cells incubated in an isotonic saline solution provides a rough indication of membrane damage. We measured the percentage (%) of hemolysis for all groups, as shown in Fig. 1. The percentage of hemolysis in the control groups, C and P, was 1.048% and 1.428%, respectively. In the irradiated group (R), the percentage of hemolysis increased to 3.187%. However, the group that received pomegranate peel extract pretreatment before irradiation (PR) saw a reduction in hemolysis to 1.931%. These results indicate a significant increase in hemolysis in the irradiated group compared to both the control (C) and P groups. On the other hand, pretreatment with pomegranate peel extract significantly reduced the percentage of hemolysis compared to the irradiated group.

Osmotic fragility

Figure 2a presents the osmotic fragility curve based on experimental data, which shows the percentage of hemolysis plotted against NaCl concentration. Figure 2b represents the differentiated osmotic fragility curve, showing the rate of hemolysis (dH/dC) versus NaCl concentration. In Fig. 2a, the hemolysis curve shifts to the right, indicating that the irradiated group had a higher NaCl concentration than both control groups, C and PE. This shift reflects an increase in the average osmotic fragility (H50), as shown in Table 2. Furthermore, administering pomegranate peel extract reversed this shift, bringing it closer to control levels.

Figure 2b demonstrates that the irradiated group (R) exhibited less spread-out hemolysis (S) and a higher peak rate of hemolysis (dH/dC) max. Additionally, the center of the peaks (H50) shifted toward higher NaCl

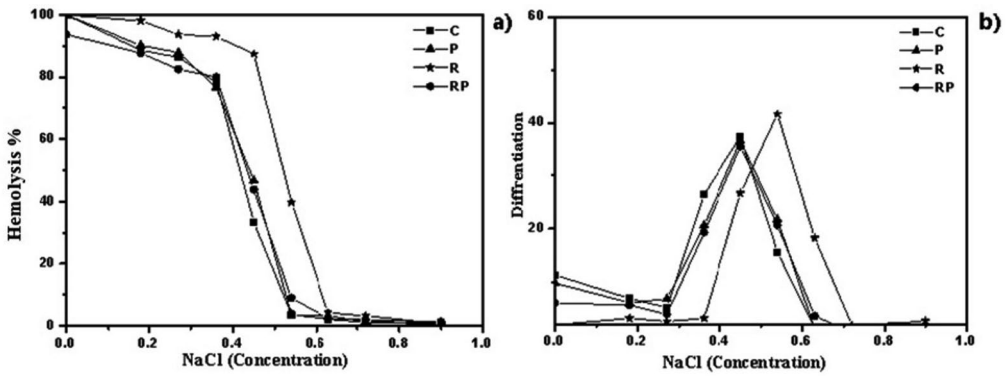

Fig. 2 The osmotic fragility curve obtained from experimental data

Table 2 The width (S), the height (H) and center position (P)

Group	C	PE	Rad	PE + Rad
$H_{50\%}$	0.4172 ± 0.003	0.4393 ± 0.006*	0.521 ± 0.006***	0.4364 ± 0.003#
S	0.2026 ± 0.002	0.2125 ± 0.002#	0.1941 ± 0.003#	0.214 ± 0.002*
[dH/dC] H	37.47 ± 0.04	36.28 ± 0.04***	41.95 ± 0.03***	35.62 ± 0.02***
P	0.449 ± 0.002	0.4525 ± 0.002#	0.5378 ± 0.003***	0.45 ± 0.003#

Results are presented as the mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was performed for 3 replicates

***$p < 0.0001$; *$p < 0.05$; #$p > 0.05$ (non-significant) versus C

concentrations in the red blood cells compared to the control (C) and PE groups. The H50 and (dH/dC) max values went down after pretreatment with pomegranate peel extract, while the spread of hemolysis (S) went up compared to the irradiated group (R), as shown in Table 2.

Blood film

Figure 3a–d illustrated blood film images for the red blood cells collected from animals of different groups, the results showed that the RBCs appeared with normal structures have smooth surface in control groups C and P (Fig. 3a, b). Instead, Irradiation transformed RBCs to echinocytes were characterized by an irregular surface with Spicules of uniform size (12). Oral administration of pomegranate peel extract diminishes the formation of echinocytes red blood cells, reducing the radiation induced damage (Fig. 3d).

LD50 of PE in rats

Pomegranate peel extract (PE) had an oral LD50 of more than 5000 mg/kg body weight in Wistar rats, as shown in the acute toxicity experiments. There were no negative effects seen during the 14-day observation period.

Oxidative stress and enhancement of antioxidant defenses

We thoroughly assessed oxidative stress parameters to evaluate the impact of PE on the antioxidant defense system in response to gamma irradiation. The control group had 82.33 ± 6.45 mg/ml of GSH-PX activity, which means that the antioxidant enzyme was working normally. The IR group showed a significant decrease in GSH-PX activity, which dropped to 26.40 ± 1.21 mg/ml. The PE + IR group, on the other hand, had a significant rise in GSH-PX activity, reaching 87.00 ± 6.11 mg/ml. This suggests that PE improves antioxidant enzyme defense. The F-value for this parameter was F (3, 20) = 216.01, with a highly significant p value of $p < 0.001$ (Fig. 4a).

Total antioxidant levels were also significantly affected by the treatments ($p < 0.05$). The control group showed a mean total antioxidant level of 1.72 ± 0.01. The PE + R group had a mean level of 1.01 ± 0.003, while the R group had a significantly lower level of 0.35 ± 0.02. This difference was statistically significant, with an F-value of 24.427 (Fig. 4b).

Catalase (CAT) activity, a key enzyme for breaking down hydrogen peroxide, was 5.92 ± 0.29 in the control group. Administration of pomegranate extract (PE control) led to a significant increase in CAT activity to 9.00 ± 0.58. CAT activity remained high in the PE + R group at 7.33 ± 0.33, while radiation exposure alone

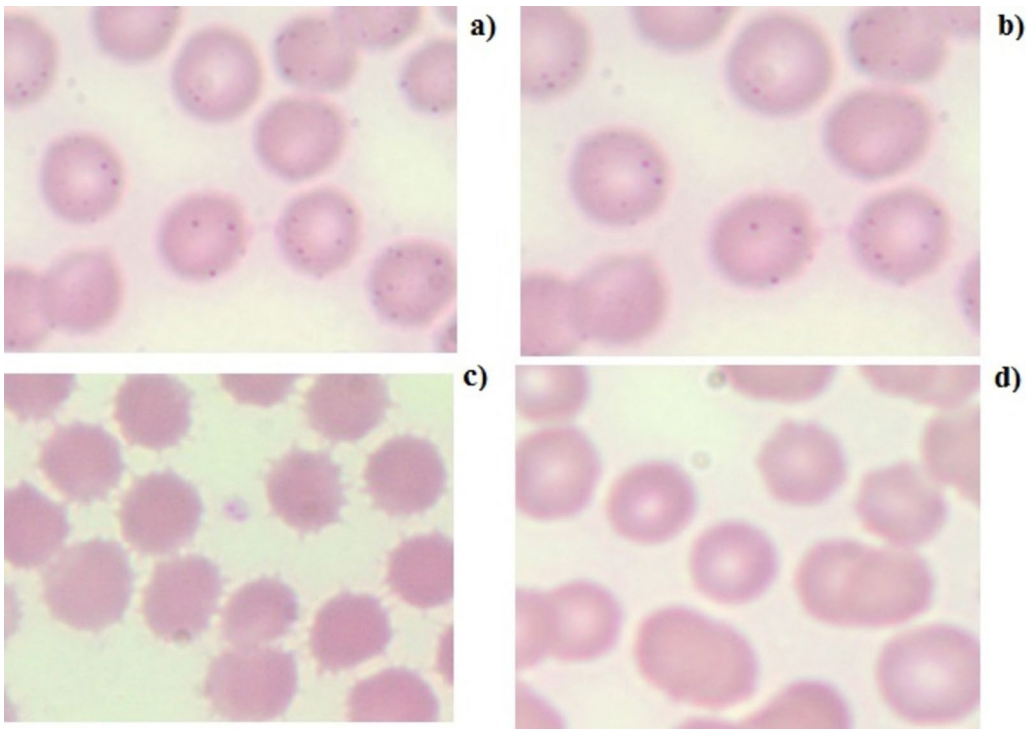

Fig. 3 Blood film microscopic observation for the RBCs collected from animals of different groups with magnification power (100×) and (96 DPI). **a** Control, **b** normal rats + PE, **c** rats exposed to 6 Gy whole body γ rays. **d** irradiated rats + PE

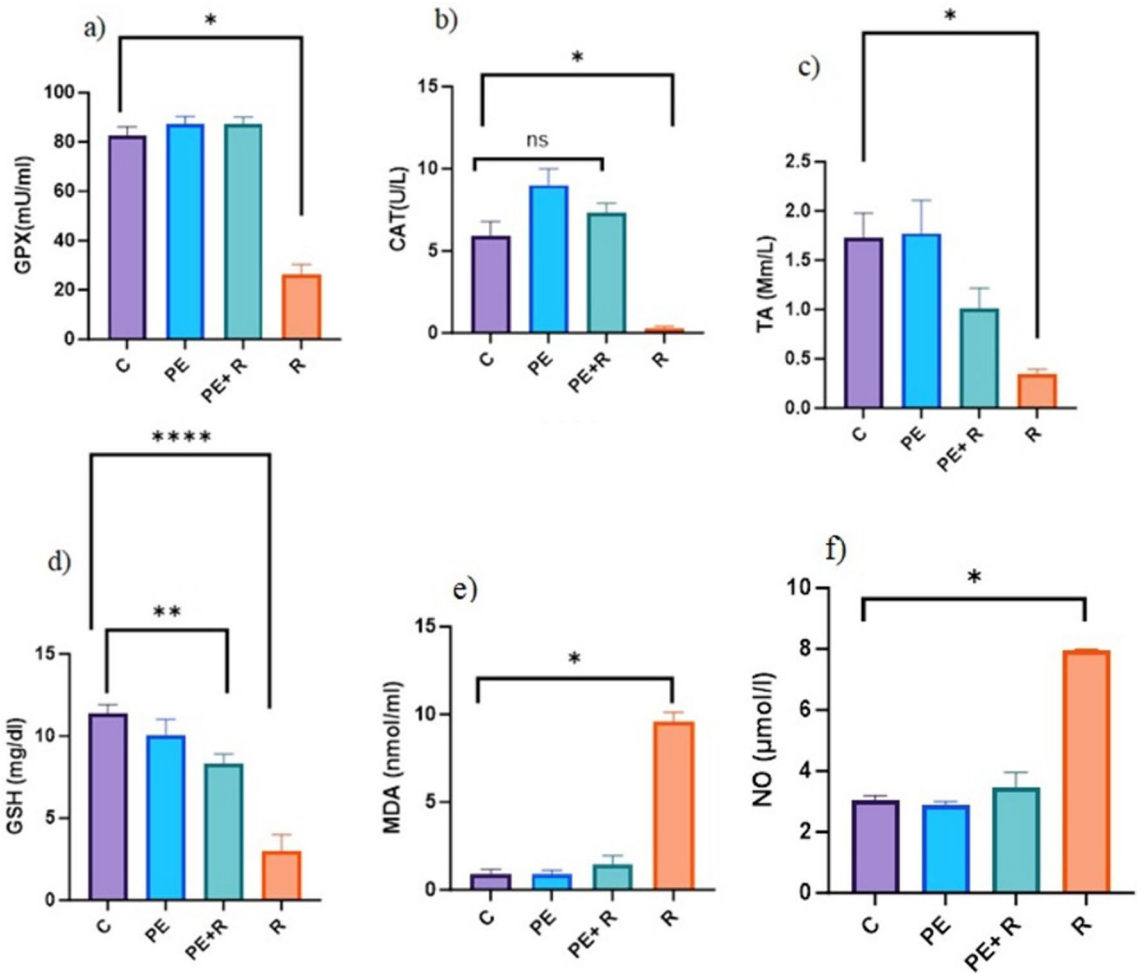

Fig. 4 Effect of PE on **a** GPX, **b** CAT, **c** TA, and **d** GSH, **e** MDA, **f** NO in rats. Results are expressed as mean ± SEM. Statistical significance is indicated at *$p < 0.05$; **$p < 0.001$; ****$p < 0.0001$ (one-way ANOVA followed by Tukey's post hoc comparison, ns (non-significant) differences

caused a dramatic reduction to 0.30 ± 0.02. The F-value for this parameter was F (3, 20) = 80.88, with a p value of $p < 0.05$ (Fig. 4c).

Malondialdehyde (MDA) levels showed that lipid peroxidation went up a lot in the R group, reaching 9.59 ± 0.24 nmol/ml compared to 0.87 ± 0.03 nmol/ml in the control group. The MDA levels dropped significantly in the PE + R group, showing that PE protects against lipid peroxidation caused by radiation. This difference was highly significant, with F (3, 20) = 304.547, $p < 0.001$ (Fig. 4e).

Nitric Oxide (NO) levels were significantly elevated in the R group (7.93 ± 0.31 µmol/l) compared to the control group (3.03 ± 0.04 µmol/l). The NO levels in the PE + R group, on the other hand, dropped sharply to 3.47 ± 0.04 µmol/l, showing that PE can stop too much NO production. This difference was statistically significant, with F (3, 20) = 260.216 (Fig. 4f).

The effect of PE on the cytokines

The impact of pomegranate peel extract (PE) on key cytokines, including IL-4, IL-6, IL-10, and TNF-α, in response to gamma irradiation was evaluated (Fig. 5a–d). We observed significant variations in cytokine levels across the experimental groups.

In the control group, the anti-inflammatory cytokine IL-4 had a mean value of 25.79 ± 1.57. However, radiation exposure greatly decreased this value (F = 17.341, $p < 0.001$). In the PE Control group, IL-4 levels increased slightly to 27.33 ± 2.13, while the PE + R group exhibited a decrease to 21.00 ± 1.03. In contrast, the irradiated group (R) showed a significant drop in IL-4 levels to 9.67 ± 0.57, indicating suppression of anti-inflammatory responses after radiation exposure.

For the pro-inflammatory cytokine IL-6 (Fig. 5b), the control group had a mean value of 14.80 ± 0.73, but the irradiated group had a huge rise to 101.30 ± 8.90,

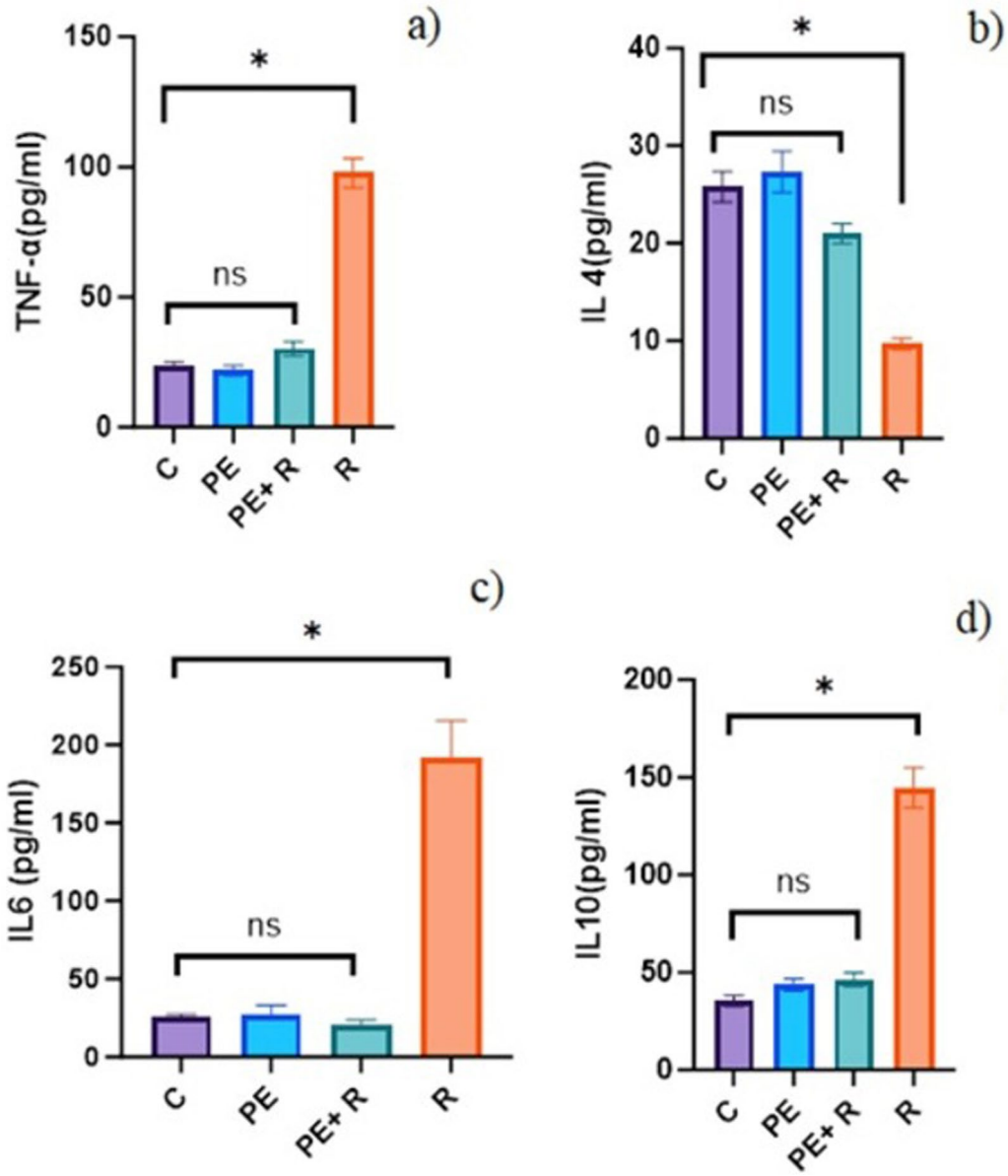

Fig. 5 Effect of PE on cytokines **a** TNF-α, **b** IL-4, **c** IL-6, **d** IL-10 in rats. Results are expressed as mean ± SEM. Statistical significance is indicated at $*p < 0.05$ (one-way ANOVA followed by Tukey's post hoc comparison, ns (non-significant) differences

showing that radiation exposure had a significant effect ($F = 476.084$, $p < 0.001$). The PE Control group had a slight elevation to 20.10 ± 0.88, and the PE + R group showed a further increase to 26.00 ± 1.85. This rise in IL-6 levels after radiation exposure underscores the pro-inflammatory effect of ionizing radiation.

IL-10, another anti-inflammatory cytokine, exhibited significant variations among the groups (Fig. 5c). The control group had a mean value of 35.56 ± 0.34, significantly altered by radiation exposure ($F = 241.482$, $p < 0.001$). The PE Control group showed a modest increase to 43.02 ± 3.21, and the PE + R group further increased to 46.35 ± 3.59. However, the irradiated group showed a substantial decrease in IL-10 levels, dropping to 14.59 ± 1.03, highlighting the suppression of anti-inflammatory responses.

Finally, TNF-α, a pro-inflammatory cytokine (Fig. 5d), demonstrated consistent trends with other pro-inflammatory markers. The control group had a mean value of 23.80 ± 1.37, and the PE control group exhibited a slight decrease to 21.85 ± 1.91. In the PE + R group, TNF-α levels moderately increased to 30.30 ± 2.82. The irradiated

group, however, displayed a significant surge in TNF-α levels to 97.78 ± 5.69 (F = 193.840, $p < 0.001$), emphasizing the pro-inflammatory effects of radiation exposure.

Discussion

The main goal of this study was to find out how pomegranate peel extract (PE) affects the flexibility of red blood cell membranes and oxidative stress, especially when exposed to gamma irradiation. Gallic acid, the characteristic phenolic compound in PE, was identified as a key factor due to its anti-inflammatory, antioxidant, free radical scavenging, and radioprotective properties (Xu et al. 2021; Mo et al. 2022). Additionally, it has been shown to have hepatoprotective benefits (Sayed et al. 2022). According to the findings, the antioxidant activity of ellagic acid is three times greater than that of vitamin C or vitamin E (Jafari et al. 2020). The findings indicate that the animals' exposure to ionizing radiation reduced the flexibility of the RBCs' membrane (Hemida et al. 2012; Elnasharty and Elwan 2023). This study also showed a significant alteration in the shape of erythrocytes following exposure to echinocytes. Previous studies diagnosed with hemolytic anemia typically exhibit these cells (Walski et al. 2014; Bogdanova et al. 2020). Based on observed structural changes, we deduced the harm irradiation causes to red blood cells. Exposure to γ-ray radiation can result in several detrimental effects on erythrocytes, including protein structural modifications, deformability alterations, permeability changes, and cell membrane rupture. These effects are visually apparent as morphological abnormalities. Finally, it can be considered that the Pomegranate peel extract pretreatment offer excellent protection damages cell membrane of red blood cell caused by gamma radiation. The role of polyphenol hydroxyl groups in limiting the production of free radicals during irradiation is clear because they are antioxidants and can lower the amount of free radicals in the body (Singha and Das 2016; Martemucci et al. 2022). The findings of this study underscore the novel application of pomegranate peel extract (PE) as a radioprotective agent. While natural antioxidants are well-established for their free radical scavenging activities, this study pioneers the use of pomegranate peel, an agricultural by-product, in mitigating gamma radiation-induced oxidative stress and cellular damage. The results demonstrate that PE not only enhances antioxidant defenses but also protects red blood cell membrane integrity, a crucial factor in minimizing radiation-induced hematological damage. Furthermore, by exploring the radioprotective properties of a readily available, cost-effective by-product, this research opens new pathways for utilizing agricultural waste in biomedical applications. The repurposing of such materials reflects an innovative approach to addressing the dual challenges of radiation exposure and environmental sustainability. Researchers have found that these compounds provide in vivo protection against gamma radiation (Singha and Das 2016; Wong et al. 2021; Chamorro et al. 2022). Ellagic acid has shown the ability to counteract the damage induced by gamma radiation (Salem et al. 2016; Xue et al. 2022).

The effects of ionizing radiation on blood cells and its relationship to crucial biomarkers such as malondialdehyde (MDA), nitric oxide (NO), and glutathione peroxidase (GSH-PX) have been of paramount interest in radiation biology and radioprotection research. Understanding the impact of radiation on these biomarkers is essential for elucidating the mechanisms underlying radiation-induced cellular damage and for identifying potential radioprotective agents, such as pomegranate peel extract. Ionizing radiation, such as gamma irradiation, can cause a range of detrimental effects on blood cells, particularly red blood cells (RBCs). One of the most well-documented consequences of radiation exposure is the induction of oxidative stress within cells (Schuermann and Mevissen 2021; Obrador et al. 2022). This oxidative stress leads to the peroxidation of membrane lipids and the production of reactive oxygen species (ROS), ultimately affecting the integrity and function of cellular components, including RBCs.

The alterations in oxidative stress parameters and antioxidant defenses are of particular interest. Gamma irradiation led to a substantial decrease in glutathione peroxidase (GSH-PX). The restoration of these parameters in the Extracts + IR Group emphasizes the potent antioxidant properties of PE. Glutathione peroxidase (GSH-PX) is a vital enzyme that acts as an antioxidant and has a key function in safeguarding cells against oxidative damage. When exposed to radiation, the body's natural antioxidant defences, such as GSH-PX, are put to the test by the increased production of ROS and the oxidative stress that follows. A decrease in GSH-PX activity can impair the cell's ability to neutralize ROS and mitigate oxidative damage.

Lipid peroxidation, as evidenced by malondialdehyde (MDA) levels, was notably higher in the IR Group. The reduction in MDA levels in PE presence indicates its ability to mitigate radiation-induced lipid peroxidation. The susceptibility of membrane lipids to the adverse impacts of free radicals is higher. MDA is a significant byproduct of the oxidation process that occurs in peroxidized polyunsaturated fatty acids. The presence of elevated levels of MDA serves as a crucial marker for lipid peroxidation, as shown in studies conducted by Yaz et al. (2019) and Ye et al. (2020). Only the groups subjected to irradiation showed an increase in MDA and lipid peroxidation levels. In contrast, PE administration reduced the amounts

of malondialdehyde (MDA) to levels comparable to the control group. This observation suggests that PE has the potential to effectively prevent lipid peroxidation, hence safeguarding membrane lipids from oxidative harm. Malondialdehyde (MDA) is a commonly used biomarker to assess lipid peroxidation, a key event in oxidative stress. Ionizing radiation exposure makes RBC membranes susceptible to lipid peroxidation due to ROS production. This leads to an increase in MDA levels, as MDA is a natural byproduct of lipid peroxidation. Elevated MDA levels in the blood are indicative of oxidative damage and can negatively impact the function and lifespan of RBCs (Tripathi et al. 2021).

Nitric oxide (NO), a signaling molecule with diverse functions, played a significant role in the outcomes observed in this study. The Extracts + IR group reduced the elevated NO levels in the IR group, indicating radiation-induced nitrosative stress and highlighting the potential anti-inflammatory properties of pomegranate peel extract (PE). NO is a multifaceted molecule crucial for various physiological processes, such as regulating vascular tone and blood flow. However, exposure to ionizing radiation often triggers an increase in NO production within the vascular system. This excess NO can contribute to oxidative stress, disrupt vascular homeostasis, and lead to endothelial dysfunction, further amplifying the harmful effects of radiation exposure.

The present study demonstrated that the administration of pomegranate peel extract had a significant impact on key biomarkers. Specifically, the extract effectively reduced MDA levels, attenuating lipid peroxidation induced by radiation. The observed reduction in NO levels suggests that the extract may help restore vascular homeostasis by mitigating excessive NO production. The fact that GSH-PX activity increased also shows that the extract strengthens the antioxidant defense system, which protects cell parts like red blood cells (RBCs).

Pomegranate peel extract reduced pro-inflammatory cytokines like tumor necrosis factor (TNF-) and interleukin-6 (IL-6). At the same time, the extract increased anti-inflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10). These findings suggest that the extract possesses both anti-inflammatory and immunomodulatory properties.

The results of this work underscore the potential radioprotective properties of pomegranate extract. The study showed that pomegranate extract could mitigate some of the hematological changes induced by ionizing radiation. Moreover, the modulation of cytokine levels suggests potential anti-inflammatory and immunomodulatory effects of pomegranate extract.

The utilization of agro waste materials, such as pomegranate peel, in radioprotective strategies is a noteworthy aspect of this study. It not only addresses environmental concerns related to agricultural waste management but also demonstrates the potential for developing sustainable and cost-effective radioprotective agents. PE, as a byproduct of the pomegranate processing industry, exemplifies the value of repurposing agro waste for radioprotection.

Conclusions

This study highlights the significant and novel radioprotective potential of pomegranate peel extract (PE) against gamma irradiation-induced hazards. By demonstrating that an agricultural by-product can effectively mitigate radiation-induced damage, this research provides a sustainable approach to radioprotection. The use of PE not only enhances antioxidant defenses but also preserves red blood cell membrane integrity, showcasing its potential as a cost-effective and accessible radioprotective agent. These findings suggest potential applications of PE in radiation oncology, where it could be developed as a supplement for cancer patients undergoing radiotherapy, helping to reduce side effects and improve treatment outcomes. These findings underscore the importance of further research into the biomedical applications of agricultural waste products.

Abbreviations

PE	Pomegranate peel ethanol extract
TA	Total antioxidant capacity
UV	Ultra violet
FTIR	Fourier Transform Infrared
CAT	Catalase
GSH	Glutathione
GPX	Glutathione peroxidase
MDA	Malondialdehyde
NO	Nitric oxide
Hb	Hemoglobin
ROS	Reactive oxygen species
Rad	Radiation
IR	Irradiation
FRAP	The ferric reducing ability
K	Potassium
Na	Sodium
$OH^{\cdot}$	Hydroxyl radicals
$O\ 2^{\cdot-}$	Superoxide
OF	The osmotic fragility
HPLC	High performance liquid chromatography
Il-4	Interleukin 4
IL-6	Interleukin 6
IL-10	Interleukin 10
TNF-α	Tumor necrosis factor alpha

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Author contributions
SFH Suggested point of the work, prepared PE, collected blood sample, measured and discussed hemolysis, osmolaraty and blood film of red blood cells and contributed to manuscript organization. MS contributed to manuscript organization. All the authors shared in the manuscript revision. KNA, AlH conceptualized and gathered the data with regard to this work, methodology, writing the original draft, review, and editing. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published. The raw data supporting the conclusion of this article will be made available by the authors without undue reservation.

Declarations

Ethics approval and consent to participate
Study was taken up after the approval of Medical Research Ethics Committee, Federal Wide Assurance (FWA) 00014747 RHDIRB 2017103002 (Approval No 13010419–1) in 6–2-2023. This research was done in compliance with the ARRIVE guidelines and regulations (https://arriveguidelines.org).

Consent for publications
Not applicable.

Competing interests
The author declares that they have no competing interests.

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