*Egypt. J. Rad. Sci. Applic.* **Vol. 34,** No. 1, 2, pp. 105-115 (2021)

**Egyptian Journal of Radiation Sciences and Applications**
**http://ejrsa.journals.ekb.eg/**

EGYPTIAN ATOMIC ENERGY AUTHORITY

# Appraisal of the Protective Role of *Punica granatum* against Biochemical and Cytogenetic Damages Induced By γ-Irradiation in Rats

**Azza M. El-Bahkery, Manal R. Mohammed**[#]
*Department of Radiation Biology, National Center for Radiation Research and Technology; Egyptian Atomic Energy Authority, Cairo, Egypt.*

*PUNICA granatum* (Pg) is an edible fruit known as pomegranate, which is a rich source of polyphenolic compounds and has antioxidant activity. Many studies have been conducted on extracts of various parts of Pg which may have genotoxic effects. However, the purpose of the present study is to assess, for the first time, the radio-protective effect of Pg juice, the safest part of the fruit, against γ-irradiation-induced biochemical and cytogenetic damage in rats by modulating the lipid profile, and the esterases (acetylcholinesterase AchE, carboxylesterase CarE and paraoxonase PON) activity as biomarkers that provide information on the nervous system's integrity, metabolism, and anti-oxidative defenses respectively. Male rats were divided into six groups, group I is the control, group II received Pg juice (400 mg/kg/day) divided into two equal doses twice daily (with 12 h interval) for 30 days, group III and V received 4 Gy and 8 Gy of γ-radiation respectively, group IV and VI received Pg juice for 30 days before being irradiated with 4 and 8Gy of γ-radiation respectively. Radiation altered the levels of CarE and PON activity in plasma and liver, as well as plasma total cholesterol, triglycerides, high-density lipoprotein HDL, low-density lipoprotein LDL, and very low-density lipoprotein VLDL. In comparison to the control group, Pretreatment with Pg juice significantly improves these values and reduced DNA fragmentation significantly. It is possible to conclude that Pg juice protects against ionizing irradiation, which causes biochemical and cytogenetic damage, by modulating anti-oxidative defenses and metabolism.

**Keywords:** DNA fragmentation, Esterases enzymes, Ionizing radiation, Lipid profile, Pomegranate, Radioprotector.

## Introduction

Exposure to ionizing radiation from both artificial and natural sources can harm human health in different ways and lead to a broad spectrum of acute, subchronic, or chronic adverse effects, even biochemically or genetically (Zhao et al., 2012). DNA is the most susceptible molecule to the damaging effects of ionizing radiation. Many studies have established that ionizing radiation not only attacks DNA, inducing single-strand breaks, double-strand breaks, base lesions, clustered damage, cross links, and oxidative base modification (Zhao et al., 2020), but also reacts with free radicals to produce reactive oxygen species, resulting in initiation and enhancement of bio-membrane lipid peroxidation (Tan et al., 2022). Accordingly, there is a growing interest in using natural products as natural anti-radiation substances (Yi et al., 2021), particularly those with anti-inflammatory and antioxidant properties, they are among the promising agents being used as radiotherapy adjuvants to reduce radiation damage (Khayyal et al., 2019).

Pg is an edible fruit cultivated in Mediterranean countries, Asian countries and some regions of the United States. Pg has been widely used as a folk medicine by many cultures (Benchagra et al., 2021). Pg is considered a pharmacy (Rathod et al., 2012). Pg fruit is a rich source of two types of polyphenolic compounds: anthocyanins

[#]Corresponding author email: manalramadan2016@gmail.com
Received 11/01/2022; Accepted 28/03/2022
DOI: 10.21608/EJRSA.2022.115794.1128

(such as delphinidin, cyanidin pelargonidin) and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallagic and ellagic acid esters of glucose), which are responsible for 92% of the antioxidant activity of the whole fruit (Eghbali et al., 2021). The plant possesses an enormous therapeutic value. Many biological activities such as antitumour (Seifabadi et al., 2019), antibacterial (Jam et al., 2022), antifungal (Mendonça et al., 2022), and antiulcer (Gupte et al., 2022) have been screened with various extracts/constituents of different parts of the plant. Pg has a great importance due to its effective antioxidant activity. Some potent antioxidants have been isolated from the fruit juice and have been found to be bioavailable, effective and safe (Licciardello et al., 2018). Pg extract inhibits lipid peroxidation at lower concentrations than vitamin E (Rosenblat et al., 2003). Pg fruit constituents prevent the transcription factor that is activated by reactive oxygen species (ROS) and hence is concerned with the pathophysiology of numerous diseases (Afaq et al., 2005). Pg extract has no side effects and no known drug interactions and prevents liver fibrosis (Thresiamma & Kuttan, 1996).

Many studies have been conducted on the different parts of the Pg such as peel extract, seeds, and bagasse, although they have genotoxic effects as stated in the works of Sánchez-Lamar et al. (2008) and Hussien et al. (2015). So the curent study aims at evaluating the protective effect of the Pg juice, which must be the safest part of the whole fruit, even though it is the edible part (Hussien et al., 2015). Pg juice contains Anthocyanins, which are powerful antioxidant flavonoids, responsible for the dazzling color of the juice. In addition to lignans, several organic acids, fatty acids, alkaloids, triterpenoids and phytosterols (Wu & Tian, 2017). The Pg juice and seeds contain minerals such as Fe, which is relatively dominant, and Ca, Ce, Cl, Co, Cr, Cs, Cu, K, Mg, Mn, Mo, Na, Rb, Sc, Se, Sn, Sr, and Zn (Eghbali et al., 2021).

## Material and Methods

*Animals*

Healthy adult male rats with an average body weight of 170 ± 20 g were obtained from the animal house of the National Center of Radiation Research & Technology (NCRRT). The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with a controlled temperature (24±3°C) during the experimental period. The rats were provided a free standard pellet diet and water *ad libitum*. All the study's protocols and the animal care and handling were in accordance with the guidelines set by the Research Ethics Committee (REC-NCRRT), approval no. (20A/19).

*γ- Irradiation*

The source of radiation was a gamma cell-40 for biological irradiation (Cesium-137) installed at the (NCRRT), Nasr city, Cairo, Egypt, giving a dose rate of 0.43Gy/min during the experimentation time period. The unit provides a uniform γ-irradiation of animals while providing a complete protection for the operating personnel.

*Juice of pomegranate*

Pomegranate juice was produced from pomegranates harvested in Egypt during October 2016. The fresh fruits were washed. The arils were separated after manual peeling, then were manually squeezed by hand press juicer and filtered. To find the effective juice volume of the referenced dose 400mg/Kg b.wt/day (Shah et al., 2016), 5cc of juice was kept at 37 ˚C for 2 days (Targhi et al., 2017). The effective volume was 0.017 gm/ml. For doses of 200 mg/kg of juice, 2 ml of juice were orally administered to animals twice daily with 12h interval (Singh et al., 2011). The division of the dose is to avoid the effect of the circadian rhythms on different physiological functions and variables involved in the absorption, distribution and effect of phenolic compounds metabolism and their bioactivity (Ávila-Román et al., 2021).

*Experimental design*

After one week of acclimation, the animals were divided into six groups, 6 rats each and were treated as follows: Group I: Controls received distilled water (2 ml twice daily). Group II (Pg): Received Pg juice twice daily orally at a dose of 200 mg/kg for 30 days. Group III (4 Gy): exposed to 4 Gy -rays. Group IV (Pg+4Gy): Received Pg juice twice daily orally for 30 days prior to 4 Gy irradiation at a dose of 200 mg/kg. Group V (8 Gy): exposed to 8Gy -rays. Group VI (Pg+8 Gy): Received Pg juice twice daily orally for 30 days prior to 8 Gy irradiation at a dose of 200 mg/kg. At the end of the experimental period, 24 h after irradiation, no perceived lethality. Whereas the applied dose of γ-irradiation (8 Gy) was

reported as lethal dose 11 days post-irradiation; thus the selected irradiation intensity was suitable for initial screening and evaluation of the radio-protective potential (Nikolova et al., 2022). Blood samples were drawn from the heart under light ether anesthesia into a heparinized tube and centrifuged at 3600 rpm for 15 min, and the plasma was separated and used for measurement of carboxyl esterase, PON, Ach, LDL, HDL, total cholesterol, and triglycerides. After the collection of blood samples, all animals were sacrificed by cervical dislocation; the femur and liver of each animal were dissected. The femur was used for the cytogenetic study, and the liver for measurement of carboxyl esterase, PON, and AchE.

*Biochemical study*

For the determination of the enzyme activity in tissue, the liver was homogenized (1:10 w/v) in pre-cooled 0.038 M Tris-HCl buffer (pH 7.6) using a potter-type homogenizer with a Teflon piston. Homogenate was centrifuged at 10000 r.p.m. at 4$^{o}$C for 20 min. The resulting supernatant was used in the biochemical assay, 50µl for sample. However, for the determination of the enzyme activity in the plasma, 20µl was used for each sample.

*Determination of carboxylesterase activity*

Carboxylesterase activity was measured spectrophotometrically using p-nitrophenil acetate as a substrate according to the method of Clement & Ehardt (1990). The development of a yellow color that resulted from Ƥ-nitrophenol hydrolysis was monitored at 412 nm for 3 min. The unit of the enzyme activity was expressed as 1 µMole of Ƥ-nitrophenol released per minute at room temperature.

*Determination of PON activity*

PON activity was measured spectrophotometrically according to the method described by Sampson et al. (2005).The enzyme activity was assayed using 1.2 mM paraoxan (O,O-diethyle Ƥ-nitrphanyl phosphate) in 50 mM Tris/HCl buffer (pH,6.8) containing 1.0 mM CaCl2 as substrate by increasing the yellow color developed through Ƥ-nitrophenol release. The unit of the enzyme activity was expressed as 1 µMole of Ƥ-nitrophenol released per minute at room temperature.

*Determination of AchE activity*

The assay was undertaken using the method of Ellman et al. (1961) with the modification proposed by Bisso et al. (1991). One unit of the enzyme activity was expressed as 1 µMole of 2-nitro-5-mercapto-benzoate released per minute at room temperature.

*Plasma lipid profile*

For the determination of cholesterol (CHOLESTEROL liquicolor), kit was purchased from Human Gesellschaft für Biochemica und Diagnostica mbH–Germany [CHOD-PAP-Method]. In addition, a triglyceride determination kit (TRIGLYCERIDES liquicolor) was purchased from Human Gesellschaft für Biochemica und Diagnostica mbH – Germany [GPO-PAP-Method].

*Cytogenetic study*

*Comet assay*

Preparation of single cell suspensions: Bone marrow was collected from one femur of each rat, then washed three times with phosphate buffer solution (PBS: NaCl 8.0 g, KCl 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 2.8 g, $KH_2PO_4$ 0.2 g, pH 7.4), homogenized and resuspended with the appropriate amount of PBS. The comet assay was performed according to Singh et al. (1988). At least 1000 cells per group were analyzed (original magnification ×200) under a fluorescent microscope (BX51, Olympus) equipped with a green light excitation and a 590-nm barrier filter. Comets form as the broken ends of a negatively charged DNA molecule become free to migrate in the electric field toward the anode. The comet parameters were calculated and photographed by Tri Tek Comet Score v1.5 software.

*Micronucleus test*

Bone marrow samples were collected from rat's femur at the sacrificing time, according to Schmid (1975), three sample slides were prepared for each animal for the micronucleus assay. The slides were stained with 5% (v/v) Giemsa stain diluted in phosphate buffer (Na2HPO4 0.06 M and KH2PO4 0.06 M, pH 6.8). For each animal, 1500 polychromatic erythrocytes (PCEs) were counted under oil immersion using a LeitzWetzlar – Orthomat binocular optical microscope with a magnification of 1000x to determine the number of the micronucleated polychromatic erythrocytes (MNPCEs) and the micronucleated normochromatic erythrocytes (MNNCEs). In addition, the percentage of polychromatic erythrocytes PCEs was calculated on the basis of

the ratio of PCEs to normochromatic erythrocutes NCEs. The slides were scored blindly according to Titenko-Holland et al. (1997).

*Statistical analysis*

The obtained data were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant (Festing & Altman, 2002).

## Rasults

*Biochemical study*

*Carboxylesterase, PON, and AchE activity in tissue and plasma*

Table 1 shows the effect of Pg juice and/or ionizing radiation (4 and 8 Gy) on esterases activity (AchE, CarE and PON) in plasma and liver after 30 days of treatment with Pg juice. The administration of Pg juice had no significant effect on the AchE and CarE activity in both plasma and liver. However, when compared to the control group, it significantly increased PON activity in both plasma and liver. On the other hand, irradiation with 4GY and 8Gy caused a significant reduction of the CarE & PON activity in both plasma and liver when compared with the control group. Meanwhile, in plasma, AchE activity did not change significantly after 4Gy or 8Gy with or without Pg juice administration, but the dose of 8Gy caused a significant increase in the AchE activity in the liver and Pg juice administration did not show any significant effect on it at P= 0.005. Oral administration of Pg juice prior to 4 Gy γ-irradiation increased the CarE & PON activity in both plasma and liver and counteracted the inhibitory effect of irradiation on plasma and liver CarE & PON activity, completely restoring normal levels but not for PON activity in plasma at P= 0.005. Although Pg juice treatment prior to 8 Gy γ-irradiation improved the inhibitory effect of ionizing radiation in the plasma and liver CarE & PON activity compared to the 8 Gy exposed animals, the activity of these enzymes was still significantly different when compared with the control group at P= 0.005.

*Lipid profile*

Treatment with Pg juice did not cause a significant increase in the plasma lipid profile (cholesterol, triglyceride, HDL, and LDL), except for VLDL, which became significantly lower than in the control group, as shown in Table 2. Exposure to 4 Gy and 8Gy produced a significant increase in the lipid profile parameters except for plasma HDL levels, which were found to be insignificant in all the treated groups when compared with control. Whereas Pg juice administration prior to 4 Gy and 8 Gy irradiation resulted in a reduction in cholesterol, triglycerides, and VLDL, this was still significantly higher when compared to the control group. Pg juice restored LDL to its normal level, when administered before 4Gy γ-irradiation and improved LDL/HDL and total choleterol/HDL ratios.

**TABLE 1. The effects of Ionizing radiation, Pg juice and their combination on plasma and liver AchE, CarE and PON**

| Groups | AchE activity | | CarE activity | | PON activity | |
|---|---|---|---|---|---|---|
| | Plasma$^{\upsilon}$ | Liver $^{\beta}$ | Plasma$^{\upsilon}$ | Liver $^{\beta}$ | Plasma$^{\upsilon}$ | Liver $^{\beta}$ |
| Control | 185.44 ± 4.697 | 254.44 ± 9.560 | 135.37 ± 2.413 | 1551.8 ± 45.35 | 471.89 ± 11.332 | 2592.9 ± 107.46 |
| Pg | 180.82 ± 8.157 | 250.18 ± 6.102 | 182.80 ± 2.703 | 1815.1 ± 37.59 | 534.82*** ± 10.193 | 2920.5*** ± 49.57 |
| 4 Gy | 194.126 ± 6.245 | 261.53 ± 12.353 | 86.074*** ± 3.435 | 1165.2*** ± 30.71 | 236.24*** ± 9.321 | 1962.8*** ± 61.04 |
| Pg+4 Gy | 190.08 ± 4.902 | 255.53 ± 12.353 | 140.92 ± 4.704 | 1532.9 ± 49.77 | 361.62*** ± 8.050 | 2561.8 ± 91.80 |
| 8 Gy | 200.89 ± 3.602 | 286.57** ± 6.604 | 73.06*** ± 4.798 | 985.56*** ± 73.16 | 210.57*** ± 4.436 | 1507.0*** ± 50.88 |
| Pg+8 Gy | 199.19 ± 6.912 | 283.58** ± 2.565 | 111.70*** ± 4.529 | 1006.6*** ± 17.13 | 297.84*** ± 5.456 | 2035.1*** ± 29.29 |

(ʊ), Activity expressed as µm substrate hydrolyzed /min/ml plasma.

(β), Activity expressed as µm substrate hydrolyzed /min/gm tissue

(*), Significant at P = 0.05; (**), significant at P = 0.01; (***), significant at P = 0.005, ANOVA One way test.

**TABLE 2.** The effects of Ionizing radiation, Pg juice and their combination on plasma Total cholesterol, Triglyceride, HDL, LDL, and VLDL. The results are presented as means ± SD (values expressed as mg of substance/dl)

| Groups | Total Cholesterol | Triglyceride | HDL | LDL | VLDL |
|---|---|---|---|---|---|
| Control | 65.5 ± 1.86 | 75.4 ± 4.853 | 30.0 ± 1.224 | 13.7 ± 0.643 | 20.6 ± 0.867 |
| Pg | 61.8 ± 0.583 | 67.2 ± 1.827 | 39.4 ± 0.509 | 8.4 ± 0.429 | 14.00** ± 0.426 |
| 4 Gy | 80.2*** ± 3.215 | 127.4*** ± 3.458 | 30.6 ± 0.979 | 23.2*** ± 0.831 | 25.5*** ± 1.088 |
| Pg+4 Gy | 73.4*** ± 1.964 | 108.2*** ± 5.238 | 32.9 ± 0.823 | 17.7 ± 0.928 | 21.3*** ± 1.301 |
| 8 Gy | 100.6*** ± 4.445 | 179.6*** ± 2.610 | 30.8 ± 2.457 | 33.1*** ± 1.324 | 36.1*** ± 0.536 |
| Pg+8 Gy | 83.0*** ± 2.569 | 108.6*** ± 3.544 | 33.8 ± 0.969 | 27.8*** ± 1.480 | 21.6*** ± 0.909 |

(*), Significant at P= 0.05; (**), significant at P= 0.01; (***), significant at P= 0.005.

*Cytogenetic study*

*Comet assay*

The chosen comet parameters to be recorded are: the percentage of DNA in the comet tail DNA%T (ratio between the intensity of the tail and the intensity of the comet), tail length TL, tail moment TM (product of the TL and DNA%T) and olive moment OM (product of DNA%T and the distance between the intensity centroids of the head and the tail along the horizontal axis of the comet). These comet parameters are used to assess the DNA damage. As illustrated in Fig 1 and Table 3 γ-irradiation in both doses of 4 Gy and 8 Gy caused a significant increase in the recorded comet parameters that designated the DNA damage induced by γ-irradiation when compared with the control group. On the other hand, oral administration of the Pg juice did not show any significant damage to DNA as compared with the control group. However, administration of Pg juice orally caused a significant reduction in the DNA damage that was induced by 4 Gy and 8 Gy when compared with the respective irradiated groups.

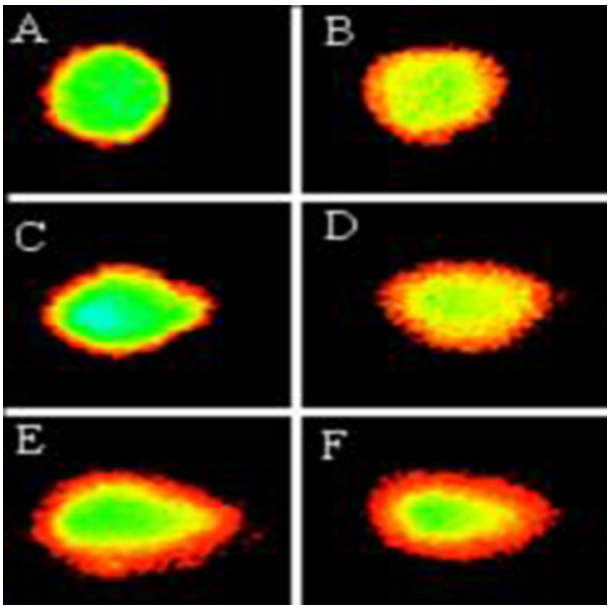


**Fig. 1. Photomicrograph showing, (A): Typical of undamaged cell from the control group. Different degrees of comet that were observed in all different treated groups; where B: From Pg group, C: From 4 Gy, D: From Pg+4 Gy, E: 8 Gy and F: From Pg+8 Gy**

**TABLE 3. The effect of oral administration of Pg juice on the comet parameters (DNA%T, TL, TM, and OM) which indicated the genetic damage induced by (4 Gy and 8 Gy) γ-irradiation in rat bone marrow [The results are presented as means ± SD]**

| Groups | DNA%T | TL | TM | OM |
|---|---|---|---|---|
| Control | 6±1.1 | 3±0.1 | 0.4±0.1 | 0.78±0.2 |
| Pg | 9.6±2.0$^{bc}$ | 4.65±1.9$^{bc}$ | 1.08±0.7$^{bc}$ | 1.43±0.5$^{bc}$ |
| 4 Gy | 47.2±3.0$^{a}$ | 28.82±3.9$^{a}$ | 18.56±4.8$^{ac}$ | 12.4±2.5$^{a}$ |
| Pg+ 4 Gy | 9.3±1.6$^{bc}$ | 5.05±1.8$^{bc}$ | 1.18±0.9$^{bc}$ | 1.53±0.6$^{bc}$ |
| 8 Gy | 52.6±4.0$^{a}$ | 34.28±8.0$^{a}$ | 24.76±6.3$^{ab}$ | 13.76±3.1$^{a}$ |
| Pg+ 8 Gy | 15.4±1.8$^{abc}$ | 9.27±1.7$^{bc}$ | 3.07±0.4$^{bc}$ | 3.1±0.5b$^{c}$ |

a: Compared with control group
b: Compared with 4 Gy group
c: Compared with 8 Gy group
(P< 0.005)

*Micronucleus test*

The micronucleus test was used to detect MNPCEs and MNNCEs that are recognized by an intermittent small nucleus as shown in Fig. 2. The incidence of MNPCE and MNNCE significantly increased and the ratio of PCE/PCE+NCE significantly decreased in the 4 Gy & 8 Gy groups as compared with the control group, as shown in Table 4, due to the genotoxicity which was caused by γ-irradiation. On the other hand, there were no significant changes in the frequency of MNPCE, MNNCE, and the ratio of PCE/PCE+NCE when compared to the control group after the oral administration of Pg juice alone without γ-irradiation. When compared to the 4 Gy group, oral administration of Pg juice prior to 4Gy -irradiation resulted in a significant reduction in the frequency of MNPCE and MNNCE as well as an increase in the ratio of PCE/PCE+NCE. Moreover, oral administration of Pg juice before 8 Gy γ-irradiation reduced the incidence of MNPCE and MNNCE as compared with the respective irradiated group.

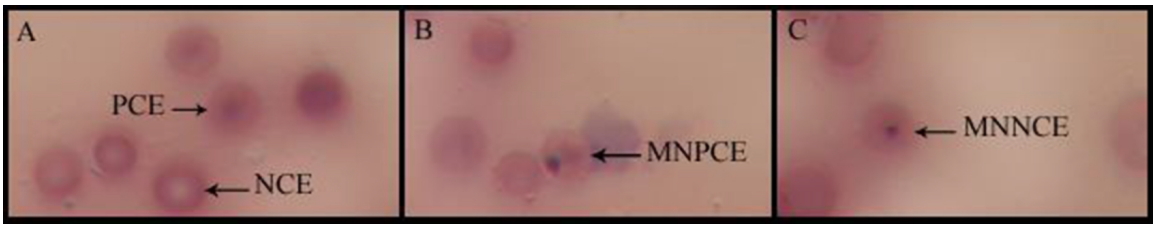


**Fig. 2. Light-microscopic photos of different rat's bone marrow smears showing; (A): Normal PCE and NCE, (B): MNPCE, (C): MNNCE**

**TABLE 4. The effect of oral administration of Pg juice on the genotoxicity (indicated by MNPCE, MNNCE and PCE ratio) induced by 4 Gy and 8 Gy γ-irradiation in rat bone marrow [The results are presented as means ± SD]**

| Groups | MNPCE/1000PCE | MNNCE/1000NCE | PCE/PCE+NCE% |
|---|---|---|---|
| Control | 1.8±1.3 | 0.4±0.5 | 48.6±2.3 |
| Pg | 2.2±0.8$^{bc}$ | 1.8±0.4$^{bc}$ | 45.4±4.0$^{bc}$ |
| 4 Gy | 15.6±2.2$^{ac}$ | 7.2±0.8$^{a}$ | 34±3.8$^{a}$ |
| Pg+4 Gy | 5.8±0.8$^{abc}$ | 4.4±1.1$^{abc}$ | 43.6±3.8$^{b}$ |
| 8 Gy | 25.4±5.2$^{ab}$ | 8.6±2.3$^{a}$ | 37±9.5$^{a}$ |
| Pg+8 Gy | 10.2±1.3$^{abc}$ | 6±1.6$^{ac}$ | 42.6±4.5$^{b}$ |

a: Compared with control group
b: Compared with 4Gy group
c: Compared with 8Gy group
(P<0.005)

## Discussion

Radiation can be utilized as adjuvant therapy in cancer, but it can also impact tissues with higher levels of cellular division, resulting in inflammation and necrosis as end point hallmarks of high dose radiation injury. Multiple physiological processes may cause cellular damage when exposed to radiation; however the formation of oxygen free radicals followed by lipid peroxidation may be one of the most important components in this chain of events (Ceron et al., 2014). ROS interact with biological components such as DNA, lipids, and proteins. In radiation-exposed rats, liver damage causes a disruption in the oxidant/antioxidant ratio as well as an inflammatory response including DNA breakage and the generation of inflammatory cytokines (Sisakht et al., 2020). The present work focuses on the DNA damage and esterases enzyme family in the plasma and the liver tissues, which is known as an active role in detoxification (Fukami & Yokoi, 2012; Hatfield et al., 2016), oxidative stress (Li et al., 2013), and atherosclerosis (Khosravi et al., 2019).

The current study's findings revealed that whole-body γ-irradiation (4 & 8 Gy) of rats resulted in a substantial decrease in the activity of CarE and PON in plasma, and liver tissues as compared to their equivalent values in the control group of rats. The considerable drop in PON activity may be due to an increase in its use to neutralize the excess of free radicals produced in the various tissues after γ-irradiation; these findings are corroborated by Kimak et al. (2011). CarE is reduced by γ-irradiation but in a way that is difficult to interpret from a biological standpoint. CarE appears to be highly sensitive to pollutants or radiation in insects, mollusks and rodents, with responses that are quite precise since they are not coupled with a change of tissue protein concentration. This susceptibility to environmental stresses is expected given that these enzymes are involved in a variety of metabolic activities, including hormone metabolism, reproduction and development, nervous system development, and cell signaling. Therefore gamma rays may have impacts on general metabolism (Gagnaire et al., 2019). Another possible explanation for the decrease in the enzyme activity caused by irradiation is that enzymes are protein molecules, and thus oxidative modification of proteins could contribute to enzyme inactivation. Furthermore, it is hypothesized that the enzyme-inactivating action of ROS or lipid peroxides induced by irradiation can overwhelm enzyme synthesis capability (Yang et al., 2011). The administration of Pg juice prior to γ-irradiation had a modulatory impact on the activity of CarE and PON in plasma, and liver tissues. This may be attributed to the high polyphenolic content of Pg, which contributes to its antioxidant properties (Seeram et al., 2005; Kaur et al., 2006).

AChE, the enzyme that swiftly divides acetylcholine into acetate and choline, has non-cholinergic actions that may contribute in the regulation of cell growth and death (Xiang et al., 2008). The current study's AchE activity results showed that it is not affected dramatically by irradiation and this is in accordance with the study of Gagnaire et al. (2019) who suggested that AchE does not appear to be a meaningful long-term biomarker for the effect of γ-irradiation.

The results obtained from the present work indicated that Pg juice significantly reduced VLDL, than in the control group. Some previous studies indicated that Pg juice reduces oxidative stress and plasma lipid in healthy individuals (Aviram et al., 2000; Matthaiou et al., 2014). The reason for the lack of knowledge about the action mechanism of Pg could be attributed in part to the variety of fruit components and the regulation of antioxidant and detoxification enzymes, cell signalling, and gene expression, among other cellular effects (Sahebkar et al., 2016). Meanwhile 4 Gy and 8 Gy irradiation elevated cholesterol, triglycerides, LDL, and VLDL levels, Pg juice administration prior to irradiation combated this elevation and improved LDL/HD and total/HDL ratios. Ionizing radiation caused oxidative stress, which could affect hepatic lipid metabolism and serum lipoproteins (El-Missiry et al., 2007). According to Onody et al. (2003), ionizing irradiation is allied with the induction of oxidative stress as well as elevated levels of lipid fractions and LDL. The hyper-lipidemic state observed in the irradiated rats may be due to increased fat mobilization from adipose tissues as a result of radiation-induced cellular bio-membranes injury. Moreover, a decrease in lipoprotein lipase activity (clearance aspect) reduces the lipid uptake by adipose cells. Likewise, the increased total cholesterol level could be a result of increased synthesis as an early reaction required for bio-membranes repair (Saada et al., 2003). Pg juice

contains Tannins, Cumarins, Saponins, Flavonoids and Triterpens (Mustafa & AbdElrahman, 2015). Tannic acid conveyed anti hyper-lipidemic activity in rats (Seeram et al., 2004).

In the present study, cytogenetic results in bone marrow cells showed highly significant frequencies of MNi and comet parameters in irradiated rats as compared to the control group. These results are in-accordance with Song et al. (2007). Free radicals are produced by ionizing irradiation oxidise lipids, producing reactive compounds such as aldehydes, ketones, and hydroxy-acids. The direct interaction of these chemicals with DNA has been shown to cause genetic damage (Harangi et al., 2004). On the other hand, Pg juice exhibits no genotoxicity at used dose and this is in agreement with Hussien et al. (2015). Moreover, The authors found that pretreatment with Pg juice significantly decreased DNA damage induced by gamma irradiation. The flavonoid rich fractions of Pg juice have anti-peroxidative effect as stated in the study of Sudheesh & Vijayalakshmi (2005). Moreover, Guo et al. (2007) reported that, Pg extracts has antioxidant activity and prevent DNA damage. Additionally, De Salvi (2005) revealed that the Pg whole fruit extract could scavenge reactive oxygen species caused by hydrogen peroxide, a mechanism that allows it to protect the DNA against the lesions provoked by it. The antimutagenic effect of the bioactive Pg compounds has been demonstrated by a decrease in the frequency of genotoxicant-induced chromosomal aberrations (Alekperov, 2002). The Pg fruits also exhibited scavenging ability for (O2.), ($H_2O_2$), (OH) and (NO), with its potent free radical quenching capacity and expected to inhibit oxidative damage to biomolecules (Noda et al., 2002). Droge (2002) reported that certain flavonoids and phenols have protective effect due to its antioxidant properties. Antioxidant potential of Pg juice is attributed to its high polyphenolics content (Seeram et al., 2005; Kaur et al., 2006). In addition, the PON inhibits the formation of new oxidative radicals by hydrolyzing lipid peroxides, thereby avoiding DNA oxidative damage. Increased PON activity inhibits lipid peroxidation, which may contribute to the the reduction of oxidative DNA damage observed by the comet test (Harangi et al., 2004).

## Conclusion

Pg juice pretreatment prior to γ-irradiation in rats had a modulatory effect on esterasing enzyme disruption, hyper-lipidimic activity, and cytogenetic damage caused by irradiation. This effect could be attributed to its ability to scavenge free radicals. The Pg juice contains an enormous amount of polyphenolic compounds, which may account for its antioxidant activity.

*Disclosure of interest*: The authors report no conflicts of interests

## References

Afaq, F., Saleem, M., Krueger, C.G., Reed, J.D., Mukhtar, H. (2005) Anthocyanin-and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-κB pathways and inhibits skin tumorigenesis in CD-1 mice. *International Journal of Cancer*, **113**(3), 423-433.

Alekperov, U.K. (2002) Plant antimutagens and their mixtures in inhibition of genotoxic effects of xenobiotics and aging processes. *European Journal of Cancer Prevention*: the official journal of the European Cancer Prevention Organisation (ECP), **11**, S8-11.

Ávila-Román, J., Soliz-Rueda, J.R., Bravo, F.I., Aragonès, G., Suárez, M., Arola-Arnal, A., et al. (2021) Phenolic compounds and biological rhythms: Who takes the lead?. *Trends in Food Science & Technology*, **113**, 77-85.

Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M., Coleman, R., et al. (2000) Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E–deficient mice. *The American Journal of Clinical Nutrition*, **71**(5), 1062-1076.

Benchagra, L., Berrougui, H., Islam, M.O., Ramchoun, M., Boulbaroud, S., Hajjaji, A., et al. (2021) Antioxidant Effect of Moroccan Pomegranate (*Punica granatum* L. Sefri Variety) Extracts Rich in Punicalagin against the Oxidative Stress Process. *Foods*, **10**(9), 2219.

Bisso, G.M., Briancesco, R., Michalek, H. (1991) Size and charge isomers of acetylcholinesterase in the cerebral cortex of young and aged rats. *Neurochemical Research*, **16**(5), 571-575.

Ceron, J.J., Tecles, F., Tvarijonaviciute, A. (2014) Serum paraoxonase 1 (PON1) measurement: An update. *BMC Veterinary Research*, **10**(1), 1-11.

Clement, J.G., Erhardt, N. (1990) Serum carboxylesterase activity in various strains of rats: sensitivity to inhibition by CBDP (2-/o-cresyl/4H: 1: 3: 2-benzodioxaphosphorin-2-oxide). *Archives of Toxicology*, **64**(5), 414-416.

De Salvi, R. (2005) *Punica granatum* L. whole fruit extract as a protection against the hydrogen peroxide-induced damage. *Revista Cubana de Plantas Medicinales*, **10**, 1-4.

Droge, W. (2002) Free radicals in the physiological control of cell function. *Physiological Reviews*, **82**(1), 47-95.

Eghbali, S., Askari, S.F., Avan, R., Sahebkar, A. (2021) Therapeutic effects of *Punica granatum* (Pomegranate): An updated review of clinical trials. *Journal of Nutrition and Metabolism*, **2021**, https://doi.org/10.1155/2021/5297162.

Ellman, G.L., Courtney, K.D., Andres Jr, V., Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7**(2), 88-95.

El-Missiry, M.A., Fayed, T.A., El-Sawy, M.R., El-Sayed, A.A. (2007) Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. *Ecotoxicology and Environmental Safety*, **66**(2), 278-286.

Festing, M.F., Altman, D.G. (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR Journal*, **43**(4), 244-258.

Fukami, T., Yokoi, T. (2012) The emerging role of human esterases. *Drug Metabolism and Pharmacokinetics*, DMPK-12.

Gagnaire, B., Bonnet, M., Tchamitchian, S., Cavalie, I., Della-Vedova, C., Dubourg, N., et al. (2019) Physiological effects of gamma irradiation in the honeybee, Apis mellifera. *Ecotoxicology and Environmental Safety*, **174**, 153-163.

Guo, S., Deng, Q., Xiao, J., Xie, B., Sun, Z. (2007) Evaluation of antioxidant activity and preventing DNA damage effect of pomegranate extracts by chemiluminescence method. *Journal of agricultural and Food Chemistry*, **55**(8), 3134-3140.

Gupte, P.A., Mahajan, M.P., Kole, M.S.R., Mandlecha, A.H., Tatke, P.A., Naharwar, V.A., Bhalerao, S.S. (2022) Efficacy and acceptability of pomegranate effervescent granules in patients suffering from acid peptic disorders. *Indian Journal of Pharmacology*, **54**(1), 7.

Harangi, M., Seres, I., Varga, Z., Emri, G., Szilvássy, Z., Paragh, G., Remenyik, É. (2004) Atorvastatin effect on high-density lipoprotein-associated paraoxonase activity and oxidative DNA damage. *European Journal of Clinical Pharmacology*, **60**(10), 685-691.

Hatfield, M.J., Umans, R.A., Hyatt, J.L., Edwards, C.C., Wierdl, M., Tsurkan, L., et al. (2016) Carboxylesterases: General detoxifying enzymes. *Chemico-biological Interactions*, **259**, 327-331.

Hussien, N.A., Abd El-Azez, A.M., Hamza, R.Z. (2015) Assessment of the genotoxic and mutagenic effect of al-taif pomegranate (*Punica granatum* L.) peel extract alone and combined with malathion and atrazine pesticides in liver of male albino mice. *Asian Journal of Pharmaceutical and Clinical Research*, **8**(4), 302-307.

Jam, N., Hajimohammadi, R., Gharbani, P., Mehrizad, A. (2022) Antibacterial activity of *Punica granatum* L. and Areca nut (PA) combined extracts against some food born pathogenic bacteria. *Saudi Journal of Biological Sciences*, **29**(3), 1730-1736.

Kaur, G., Jabbar, Z., Athar, M., Alam, M.S. (2006) *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and Chemical Toxicology*, **44**(7), 984-993.

Khayyal, M.T., Kreuter, M.H., Kemmler, M., Altmann, P., Abdel-Naby, D.H., El-Ghazaly, M.A. (2019) Effect of a chamomile extract in protecting against radiation-induced intestinal mucositis. *Phytotherapy Research*, **33**(3), 728-736.

Khosravi, M., Poursaleh, A., Ghasempour, G., Farhad, S., Najafi, M. (2019) The effects of oxidative stress on the development of atherosclerosis. *Biological Chemistry*, **400**(6), 711-732.

Kimak, E., Hałabiś, M., Baranowicz-Gąszczyk, I., Solski, J., Książek, A. (2011) Association between

moderately oxidized low-density lipoprotein and high-density lipoprotein particle subclass distribution in hemodialyzed and post-renal transplant patients. *Journal of Zhejiang University Science B*, **12**(5), 365-371.

Li, H., Horke, S., Förstermann, U. (2013) Oxidative stress in vascular disease and its pharmacological prevention. *Trends in Pharmacological Sciences*, **34**(6), 313-319.

Licciardello, F., Kharchoufi, S., Muratore, G., Restuccia, C. (2018) Effect of edible coating combined with pomegranate peel extract on the quality maintenance of white shrimps (*Parapenaeus longirostris*) during refrigerated storage. *Food Packaging and Shelf life*, **17**, 114-119.

Matthaiou, C.M., Goutzourelas, N., Stagos, D., Sarafoglou, E., Jamurtas, A., Koulocheri, S.D., et al. (2014) Pomegranate juice consumption increases GSH levels and reduces lipid and protein oxidation in human blood. *Food and Chemical Toxicology*, **73**, 1-6.

Mendonça, A.M.S., Monteiro, C.D.A., Moraes-Neto, R.N., Monteiro, A.S., Mondego-Oliveira, R., Nascimento, C.E.C., et al., (2022) Ethyl acetate fraction of *Punica granatum* and its Galloyl-HHDP-glucose compound, alone or in combination with fluconazole, have antifungal and antivirulence properties against *Candida* spp. *Antibiotics*, **11**(2), 265.

Mustafa, H.A., AbdElrahman, N.H. (2015) Effect of *Punica granatum* juice on lipid profile of hypercholesterolemic albino rats. *EC Ophthalmology*, **2**, 178-184.

Nikolova, N., Ivanova, D., Yaneva, Z. (2022) *In vivo* radioprotective potential of newly synthesized azomethine and styrylquinoline derivatives and a natural polyphenol: A preliminary study. *Life*, **12**(3), 346.

Noda, Y., Kaneyuki, T., Mori, A., Packer, L. (2002) Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin, and pelargonidin. *Journal of Agricultural and Food Chemistry*, **50**(1), 166-171.

Onody, A., Csonka, C., Giricz, Z., Ferdinandy, P. (2003) Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. *Cardiovascular Research*, **58**(3), 663-670.

Rathod, N.R., Biswas, D., Chitme, H.R., Ratna, S., Muchandi, I.S., Chandra, R. (2012) Anti-urolithiatic effects of Punica granatum in male rats. *Journal of Ethnopharmacology*, **140**(2), 234-238.

Rosenblat, M., Draganov, D., Watson, C.E., Bisgaier, C.L., La Du, B.N., Aviram, M. (2003) Mouse macrophage paraoxonase 2 activity is increased whereas cellular paraoxonase 3 activity is decreased under oxidative stress. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **23**(3), 468-474.

Saada, H.N., Ussama, Z.S., Mahdy, A.M. (2003) Effectiveness of Aloe vera on the antioxidant status of different tissues in irradiated rats. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, **58**(12), 929-931.

Sahebkar, A., Simental-Mendia, L.E., Giorgini, P., Ferri, C., Grassi, D. (2016) Lipid profile changes after pomegranate consumption: A systematic review and meta-analysis of randomized controlled trials. *Phytomedicine*, **23**(11), 1103-1112.

Sampson, M.J., Braschi, S., Willis, G., Astley, S.B. (2005) Paraoxonase-1 (PON-1) genotype and activity and in vivo oxidized plasma low-density lipoprotein in Type II diabetes. *Clinical Science*, **109**(2), 189-197.

Sánchez-Lamar, A., Fonseca, G., Fuentes, J.L., Cozzi, R., Cundari, E., Fiore, M., et al. (2008) Assessment of the genotoxic risk of *Punica granatum* L. (Punicaceae) whole fruit extracts. *Journal of Ethnopharmacology*, **115**(3), 416-422.

Schmid, W. (1975) The micronucleus test. *Mutation Research*, **31**, 9-15.

Seeram, N.P., Adams, L.S., Henning, S.M., Niu, Y., Zhang, Y., Nair, M.G., Heber, D. (2005) *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of Nutritional Biochemistry*, **16**(6), 360-367.

Seeram, N.P., Lee, R., Heber, D. (2004) Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice. *Clinica Chimica Acta*, **348**(1-2), 63-68.

Seifabadi, S., Vaseghi, G., Ghannadian, M., Javanmard, S.H. (2019) Standardized *Punica granatum* pericarp extract, suppresses tumor proliferation and angiogenesis in a mouse model of melanoma: Possible involvement of PPARα and PPARγ pathways. *Iranian Journal of Pharmaceutical Research: IJPR*, **18**(1), 348.

Shah, T.A., Parikh, M., Patel, K.V., Patel, K.G., Joshi, C.G., Gandhi, T.R. (2016) Evaluation of the effect of *Punica granatum* juice and punicalagin on NFκB modulation in inflammatory bowel disease. *Molecular and Cellular Biochemistry*, **419**(1), 65-74.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, **175**(1), 184-191.

Singh, A.P., Singh, A.J., Singh, N. (2011) Pharmacological investigations of *Punica granatum* in glycerol-induced acute renal failure in rats. *Indian Journal of Pharmacology*, **43**(5), 551.

Sisakht, M., Darabian, M., Mahmoodzadeh, A., Bazi, A., Shafiee, S.M., Mokarram, P., Khoshdel, Z. (2020) The role of radiation induced oxidative stress as a regulator of radio-adaptive responses. *International Journal of Radiation Biology*, **96**(5), 561-576.

Song, R.X., Ruan, H.J., Jing, X.Y., Liu, Z.T. (2007) Genotoxicity of Sodium Pentachlorophenol. *Carcinogenesis, Teratogenesis & Mutagenesis*, 5.

Sudheesh, S., Vijayalakshmi, N. R. (2005) Flavonoids from *Punica granatum*—potential antiperoxidative agents. *Fitoterapia*, **76**(2), 181-186.

Tan, F.C., Yay, A.H., Yildiz, O.G., Kaan, D. (2022) Protective effect of pycnogenol against gamma radiation-induced lung injury in rat: DNA damage, lipid peroxidation, antioxidant levels, and histopathological changes. *Journal of Cancer Research and Therapeutics*, [Epub ahead of print]. Available from: https://www.cancerjournal.net/preprintarticle.asp?id=322704

Targhi, R.G., Homayoun, M., Mansouri, S., Soukhtanloo, M., Soleymanifard, S., Seghatoleslam, M. (2017) Radio protective effect of black mulberry extract on radiation-induced damage in bone marrow cells and liver in the rat. *Radiation Physics and Chemistry*, **130**, 297-302.

Thresiamma, K.C., Kuttan, R. (1996) Inhibition of liver fibrosis by ellagic acid. *Indian Journal of Physiology and Pharmacology*, **40**(4), 363-366.

Titenko-Holland, N., Windham, G., Kolachana, P., Reinisch, F., Parvatham, S., Osorio, A.M., Smith, M.T. (1997) Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay *in vitro* and *in vivo*: A study of malathion-exposed workers. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **388**(1), 85-95.

Wu, S., Tian, L. (2017) Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (*Punica granatum*). *Molecules*, **22**(10), 1606.

Xiang, A.C., Xie, J., Zhang, X.J. (2008) Acetylcholinesterase in intestinal cell differentiation involves G2/M cell cycle arrest. *Cellular and Molecular Life Sciences*, **65**(11), 1768-1779.

Yang, R., Wang, J., Liu, Z., Pei, X., Han, X., Li, Y. (2011) Antioxidant effect of a marine oligopeptide preparation from chum salmon (*Oncorhynchus keta*) by enzymatic hydrolysis in radiation injured mice. *Marine drugs*, **9**(11), 2304-2315.

Yi, J., Zhu, J., Zhao, C., Kang, Q., Zhang, X., Suo, K., et al. (2021) Potential of natural products as radioprotectors and radiosensitizers: Opportunities and challenges. *Food & Function*, **12**(12), 5204-5218.

Zhao, L., Bao, C., Shang, Y., He, X., Ma, C., Lei, X., et al. (2020) The determinant of DNA repair pathway choices in ionising radiation-induced DNA double-strand breaks. *BioMed Research International*, **2020**, 4834965.

Zhao, W., Jiang, X., Deng, W., Lai, Y., Wu, M., Zhang, Z. (2012) Antioxidant activities of *Ganoderma lucidum* polysaccharides and their role on DNA damage in mice induced by cobalt-60 gamma-irradiation. *Food and Chemical Toxicology*, **50**(2), 303-309.