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Low-Dose Gamma Radiation Modulates Liver and Testis Tissues Response to Acute Whole Body Irradiation

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Abstract

Aim: This work aims to investigate whether the pre-exposure to low dose/low dose rate (40 mGy, 2.2 mGy/hour) γ-radiation as a priming dose can produce a protective effect against the subsequent high one (4 Gy, .425 Gy/minute).

Methods: Rats were divided into Group I (control), Group II (L); exposed to 40 mGy, Group III (H); exposed to 4 Gy, and Group IV (L+H); exposed to 40 mGy 24 hours before the exposure to 4Gy. The molecular and biochemical changes related to oxidative stress, DNA damage, apoptosis, and mitochondrial activity in the liver and testis were studied 4 hours after irradiation.

Results: Exposure to 40 mGy before 4 Gy induced a significant increase in the levels of Nrf2, Nrf2 mRNA, TAC, and mitochondrial complexes I & II accompanied by a significant decrease in the levels of LPO, 8-OHdG, DNA fragmentation, TNF-α, caspase-3, and caspase-3 mRNA compared with H group.

Conclusion: Exposure to low-dose γ-radiation before a high dose provides protective mechanisms that allow the body to survive better after exposure to a subsequent high one via reducing the oxidative stress, DNA damage, and apoptosis-induced early after irradiation. However, further studies are required to identify the long-term effects of this low dose.

Keywords

radiation hormesis, low dose radiation, nuclear factor erythroid 2-related factor-2, Tumor necrosis factor-α, caspase-3, complex I& complex II

Introduction

Ionizing radiation (IR) has a very important role in our modern world, especially in nuclear medicine and medical care such as X-ray imaging, for example, computed tomography scans; however, a high dose of IR may be dangerous as it can cause cancer and even death.[1] The biological effects of IR, especially those of low doses, are not yet fully understood. Many studies have discussed the effects of low-doses of radiation; most of them suggested beneficial or no effects, while others reported deleterious effects.[2-4]

Low-dose IR was defined by the UNSCEAR as a total dose of radiation less or equal to 100 mGy, and dose rates below .1 mGy/min as low dose rates.[5] However, many experimental studies have considered IR equal to or below .5 Gy as low-doses.

From the beneficial effects observed after low doses (.01–.5 Gy) of gamma radiation are induction of adaptive responses,[6] enhancement of antioxidant defense,[7] increase resistance to high dose of radiation,[8] activation of mitochondrial dynamics, and upregulation of genes controlling respiratory chain.[9,10] Feinendegen 2016 summarized the results of several studies

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investigating the effects of acute low-doses radiation at the molecular and cellular levels, and attempted to quantify adaptive radioprotections. He reported that this protection may reach 100% at doses below 100 mGy while above 200 mGy, the average degree of protection diminishes and may reach about 10%.[11]

Radioadaptive response (RAR) is a significant phenomenon stimulated by the exposure to low dose radiation and noticeable by a decrease of radiobiological response in cells which have been pre-exposed to a low dose of radiation followed by a subsequent higher dose; but the molecular mode of action of RAR is not yet defined.[12] The consequences of low dose-induced adaptive response have raised a lot of debate. Its beneficial or harmful effect is still controversial. Many studies have suggested that low-dose radiation may induce a protective effect that underlies the low-dose adaptive response. This response can induce cellular resistance to the harmful effects induced by exposure to different toxic agents.[13-15] Previously, it has been indicated that adaptation to radiation can be observed by accelerated DNA repair or by a decrease of some harmful genetic effects such as DNA single- and double-strand breaks, gene mutations, chromosome abnormalities, and formation of micronuclei.[16]

However, Abdelrazzak et al. observed that there is no evidence that 10cGy-irradiation (x-rays) protected the rat liver cells against a subsequent higher dose (2 Gy).[17] This controversy may be related to the type, dose, and dose rate of radiation. The resolution of this controversy requires further studies. Therefore, the current study aims to investigate the effect of exposure to low dose/low dose rate 40 mGy, 2.2 mGy/hour (The dose that falls within the window recommended by UNSCEAR 2012[5]) before exposure to a higher dose (4 Gy, .425 Gy/minute) of ionizing radiation on, first, the genetic expression and protein levels of nuclear factor erythroid 2-related factor-2 (Nrf2) as a transcription factor for stress response and also, the levels of total antioxidant capacity (TAC), and lipid peroxidation (LPO) as oxidative stress markers. Second, the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), DNA fragmentation, and the genetic expression and protein levels of caspase-3 and TNF-α (as markers of DNA damage, apoptosis, and inflammation). Third, the levels of mitochondrial complex I; NADH: ubiquinone oxidoreductase and complex II; succinate dehydrogenase, in liver and testes of rats. We chose these parameters because they are related to the adaptive response mechanisms. The liver and testes were chosen as the liver is the site of most metabolic and detoxification processes that can be affected by ionizing radiation while the testis is one of the most radiosensitive organs.

Materials & Methods

Ethics Statement

The current study was approved by the ethics committee of the Egyptian Atomic Energy Authority, National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt (8A/20). All animal procedures performed in this study were in accordance with the ethical standards of the care and use of laboratory animals.

Chemicals

Various chemicals used in this study were purchased from Sigma Pvt. Ltd.

Experimental Design

Healthy Wistar male rats weighing 140–170 g were obtained from the animal house that belongs to the NCRRT. Animals were maintained in plastic cages under standard conditions of light, ventilation, temperature and humidity and were fed on standard pellet diet (IBEX Company, Cairo, Egypt) and supplied with water *ad libitum*. Animals were left to acclimatize for one week before starting the experiment.

Irradiation Processing

High dose irradiation. Single whole-body dose (4 Gy) of gamma radiation and dose rate .425 Gy/minute was done by using an indoor shielded ^{137}Cs radiator (gamma cell-40) installed in the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. The rats (6 rats at the one time) were placed in a ventilated plastic cage. The dose rate of radiation was calculated according to the Radiation Protection and Dosimetry Department in the NCRRT, Cairo, Egypt.

Low Dose Irradiation. Animals were exposed to low dose/low dose rate of 40 mGy and dose rate 2.2 mGy/hour (according to UNSCEAR 2012[5]) using ^{137}Cs gamma source belonging to the Radiation Protection Department, Nuclear & Radiological Regulatory Authority, Cairo, Egypt. In two co-centric cylinders cage rats were kept between the two cylinders and the source is located at the center of the cage.

Animal Groups

Animals were divided to 4 different groups, each with ten rats. Group I (control group): animals in this group were not given any radiation treatment (sham-irradiated). Group II (L group): animals in this group were exposed to whole body gamma-radiation with low-dose 40 mGy and dose rate 2.2mGy/hour. The animals in Group III (H group) were exposed to a whole-body single dose of gamma-radiation (4 Gy, .425 Gy/minute). The animals in Group IV (L+H group) were exposed to gamma-radiation with low-dose 40 mGy as group II and after 24 hrs exposed to a single dose of gamma-radiation (4 Gy) as Group III. Four hours after the last irradiation, all the animals were sacrificed and liver and testes removed immediately. Each tissue was divided into 2 parts; the first one was stored at −70°C for the molecular analysis. The second part was placed

Table 1: Primer Sequences for Quantitative Reverse Transcription PCR (RT-qPCR) Analysis.

Gene	Primer sequence
Nuclear factor erythroid 2-related factor-2	F: 5′-CAGCATGATGGACTTGGA-3′ R: 5′-TGAGACACTGGTCACACT-3′
Tumor necrosis factor-α	F: 5′-AGAACTCCAGGCGGTGTCTGT-3′ R: 5′- CCTTGTCCCTTGAAGAGAACC-3′
Caspase 3	F: 5′-AGCTTCTTCAGAGGCGA CTA-3′ R: 5′-GGACACAATACACGGGATCT-3
GAPDH	F: 5′-GGTCGGAGTCAACGGATTTG-3′ R: 5′-ATGAGCCCCAGCCTTCTCCAT-3′

in ice-cold isotonic saline, then tissue homogenates (10% w/v) were prepared in ice-cold 10mM PBS (phosphate-buffered saline, .15M NaCl), pH 7.4 and used for each of the biochemical estimations, described below.

A. Molecular Analysis

1- Extraction of RNA and Quantitative RT-PCR Analysis

Level of Nrf2, TNF-α, and caspase-3 genes expression were measured using a real-time PCR assay with SYBR Green. First, total RNA was extracted from liver and testes of rats by using PureLink RNA Mini Kit (cat#12183026) (Invitrogen, USA) according to the manufacturer's instructions. Reverse transcription (RT) was then performed on a scale of 20 µL using RevertAid First Strand cDNA Synthesis Kit (cat#K1621) (Thermo Scientific, USA) according to the manufacturer's instructions, using oligo-dT-primers and 1 µg RNA per reaction as a template. Nrf2, TNF-α and caspase-3 mRNA expression levels were measured using ABI 7500 quantitative PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) and 2X Maxima® SYBR Green/ROX qPCR Master Mix (#K0221) (Thermo Fisher Scientific, USA). The qPCR cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec and 72°C for 30 sec. The primer sequences are listed in Table 1. The relative expression of Nrf2, TNF-α and caspase-3 were normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and calculated according to the 2-ΔΔCq method described by *Livak and Schmittgen*.[18] A 2-fold increased (≥2) or decreased (≤.5) value was considered mRNA overexpression or downregulation, respectively.

2- DNA Fragmentation Assay

Genomic DNA was extracted from liver and testes of rats by using GeneJET™ Genomic DNA Purification Kit (cat# K0721) (Thermo Scientific, USA) according to the manufacturer's instructions. The isolated DNA was measured at 260/280 nm to assess DNA concentration and purity, then 500 ng of DNA was pipetted onto a 1% agarose gel containing 100 ng/mL ethidium bromide, and electrophoresis was performed.

B. Biochemical Analysis

1- Lipid Peroxidation (LPO) and Total Antioxidant Capacity (TAC)

LPO was measured by following the method of Wills.[19] Tissue homogenates (10% w/v) were mixed with 10% TCA (ice cold) then centrifuged at 800g for 10 min. After that the supernatant was mixed with Thiobarbituric acid (TBA) and to develop the color; boiled for 10 min at 100°C, then cooled at room temperature, finally, optical density was read at 532nm.

TAC was measured by using total antioxidant capacity (colorimetric method) kit (cat# TA 25 13) (Biodiagnostic, Inc., Egypt) according to the protocol of manufacturer.

2- Nuclear Factor Erythroid 2-Related Factor-2 (Nrf2), Tumor Necrosis Factor Alpha (TNF-α) and Caspase-3 Proteins

Nrf2 protein was quantified in liver and testes tissues using a Rat Nuclear Factor Erythroid 2-Related Factor 2 ELISA Kit (cat# MBS012148) (MyBiosource, USA) according to the manufacturer's instructions. TNF-α level was determined by using Quantikine Rat TNF-alpha ELISA kit (cat# RTA00) (R&D Systems, Inc., USA) according to the manufacturer's instructions. Caspase-3 activity was determined by Rat caspase-3 ELISA Kit (cat# MBS743552) (MyBioSource, Inc., USA) according to the manufacturer's instructions.

3- NADH: Ubiquinone Oxidoreductase (Complex I), Succinate Dehydrogenase (Complex II) and 8-Hydroxy-2′-deoxyguanosine (8-OHdG)

The activity of complex I was measured by using Complex I Enzyme Activity Assay Kit (cat# ab109721) (Abcam, Inc., USA); while the activity of complex II was measured by using Succinate Dehydrogenase (SDH) (Rat) ELISA Kit (cat# E4596-100) (BioVision, Inc) according to the manufacturer's instructions.

The level of 8-OHdG was determined by 8-hydroxy 2 deoxyguanosine (8-OHdG) ELISA Kit (cat#ab201734) (Abcam, Inc., USA) according to manufacturer's instructions.

C. Statistical Analysis

Statistical analysis was performed using IBM SPSS software (version 23.0; IBM Corp., Armonk, NY, USA), and data were presented as means ± S.D. One-way ANOVA was used to determine statistically significant differences between group's means and post hoc test (LSD) for pairwise comparisons. Pearson correlation coefficients were calculated to evaluate the association between relevant parameters. The criterion for significance was $p<.05$.

Results

1- Oxidative Stress Markers

Results of oxidative stress markers in both liver and testes tissues were shown in Figure 1; our results revealed that the levels of LPO were significantly increased in H group when compared to control group, while they were significantly decreased in L+H group when compared to H group (Figure 1A). Meanwhile, the levels of TAC and Nrf2 were significantly decreased in H group when compared to control group and significantly decreased in L+H group when compared to H group (Figure 1B and C). These results were confirmed by data obtained from quantitative RT-PCR analysis as the expression levels of Nrf2 mRNA in the liver and testes of rats were also significantly decreased in H group when compared to control group and significantly increased in L+H group when compared to H group; as shown in Figure 1D. The exact values for all biomarkers were shown as supplementary data Tables 1 and 2.

2- DNA Damage, Inflammation, and Apoptosis Markers

Analysis of agarose gel electrophoresis revealed that DNA obtained from liver and testes tissues of L group rats was completely intact, while it was slightly fragmented in liver and testes samples of L+H group, and degree of DNA fragmentation was elevated in liver and testes samples of H group (Figure 2B). By measuring the levels of 8-OHdG (which is a biomarker of oxidative DNA damage) in the liver and testes tissues, our results showed that they were increased in H group when compared to control group; but when compared to H

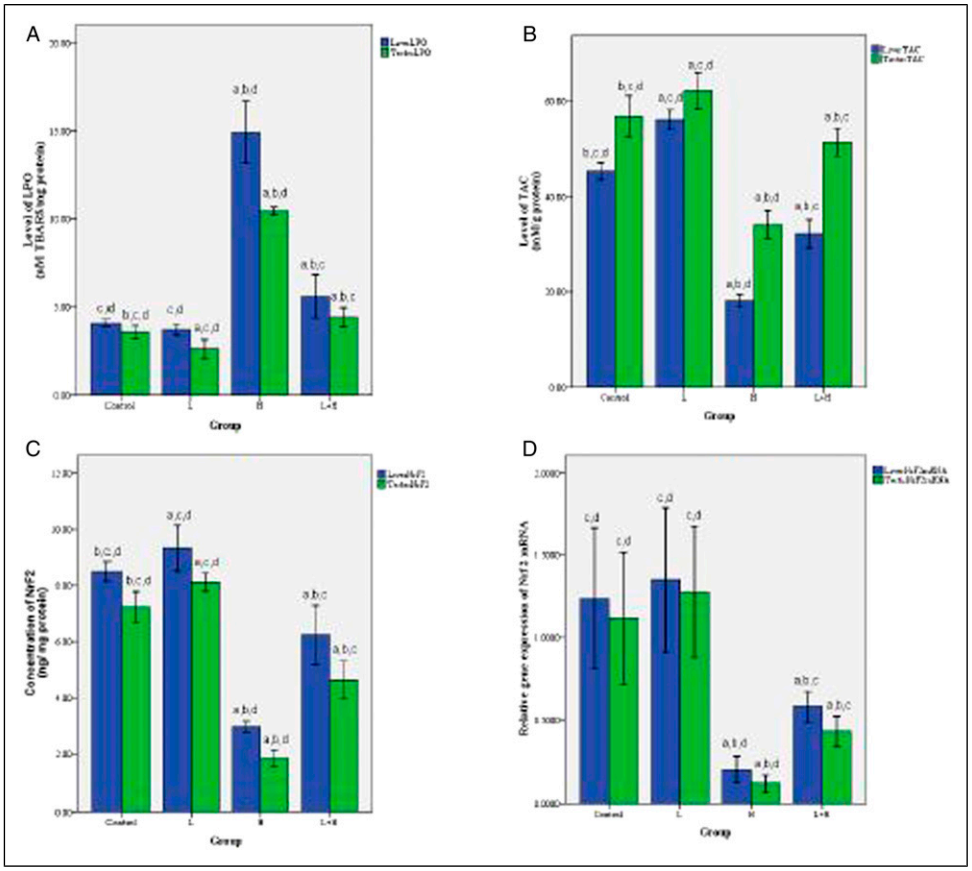

Figure 1. Oxidative stress markers in the liver (blue) and testes (green) of rats. (A): Level of Lipid peroxidation (LPO); (B): Level of Total antioxidant capacity (TAC); (C): Concentration of Nuclear factor erythroid 2-related factor-2 (Nrf2); (D):Fold change of Nrf2 mRNA relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are expressed as mean ± SD (n = 10). a: significant compared to L group, c: significant compared to H group, d: significant compared to L ± H group ($P < .05$).

group, the levels of 8-OHdG in L+H group were significantly decreased (Figure 2A).

Beside the oxidative stress and DNA damage markers, the protective effect of low dose before high dose exposure was proven by determination of TNF-α concentration as an inflammatory marker and the activity of caspase-3 as an apoptosis marker in the liver and testes tissues of rats. Our result indicated that both TNF-α concentration and caspase-3 activity were significantly increased in H group when compared to control group; while they were significantly decreased in L+H group when compared to H group; as shown in Figures 3A and B. The same results were obtained by measuring the expression levels of TNF-α and caspase-3 mRNA in the liver and testes, which were also significantly increased in H group when compared to control group and significantly decreased in L+H group when compared to H group; As shown in Figures 3C and D.

3- Mitochondrial Markers

The activities of NADH: ubiquinone oxidoreductase (complex I) and succinate dehydrogenase (complex II) were measured as mitochondrial biomarkers. Our results revealed that the exposure to low dose before high dose radiation had a protective effect as the activities of both complex I and II were significantly increased in L+H group when compared to H group; as shown in Figures 4A and B.

Finally as a summary, from the results of all studied biomarkers we could conclude that the exposure to low dose before high dose radiation significantly decrease the activity of caspase-3 and the levels of LPO, TNF-α, 8-OHdG, DNA fragmentation, TNF-α mRNA, and caspase-3 mRNA, while it significantly increase the activities of complex I and II and the levels of TAC, Nrf2, and Nrf2 mRNA when compared to H group ; as shown in Table 2.

4- Correlation of Nrf2 and 8-OHdG

Data recorded in Table 3 showed the correlation matrix of Nrf2 and 8-OHdG with the different measured parameters in the liver and testes of rats, results revealed that there is a significant negative correlation between Nrf2 with LPO, TNF-α, caspase-3, and 8-OHdG, while there is a significant positive correlation between Nrf2 with TAC, complex I, and complex II in both liver and testes. From these results, it was found that in the liver and testes of rats there is a significant negative correlation between 8-OHdG with TAC, Nrf2, complex I, and complex II, while there is a significant positive correlation between 8-OHdG with LPO, TNF-α, and caspase-3.

Discussion

Exposure to low doses of ionizing radiation has been shown to provide benefits for biological systems. Previous studies indicated that low doses of ionizing radiation would stimulate different mechanisms that have effects on different cell types of physiological systems. Therefore, it has been accepted that the radiation hormesis phenomenon exists, which means that exposure to low dose ionizing radiation stimulates beneficial biological effects[20] however, exposure to a high dose induces critical cellular responses in different organs in the body. The testis is one of the most radiosensitive organs, and the liver is the main organ that controls systemic metabolism and maintains homeostasis in response to external stimuli, so they are easily affected by ionizing radiation.

The present study was conducted to understand the effects of exposure to low dose/low dose rate of γ-radiation (40 mGy, 2.2 m Gy/hour) before exposure to a higher dose (4 Gy, .425 Gy/minute), at the first time to the best of our knowledge, on oxidative stress, DNA damage, mitochondrial activity, and apoptotic markers in the liver and testes of rats.

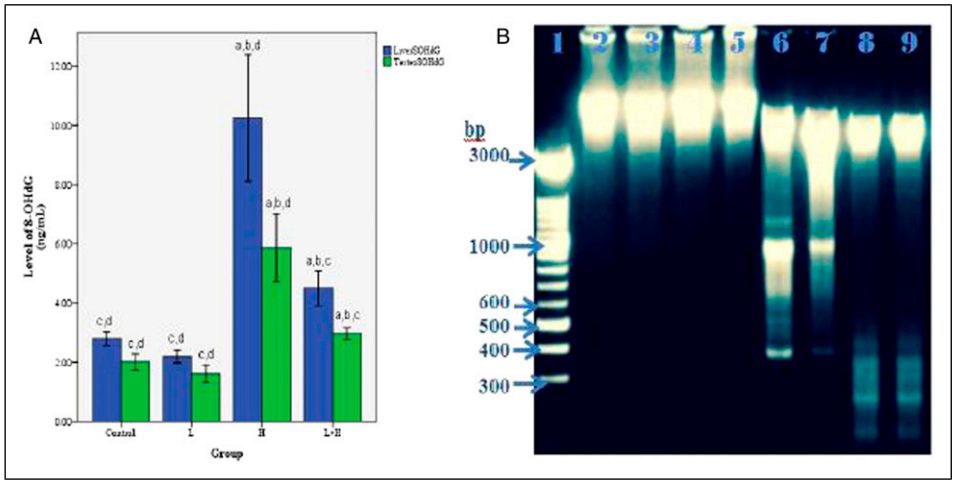

Figure 2. Damage of DNA markers in the liver (blue) and testes (green) of rats. (A): level of 8-hydroxy-2'-dexyguanosine (8-OHdg). Data are expressed as mean ± SD (n = 10). a: significant compared to L group, c: significant compared to H group, d: significant compared to L ± H group ($P < .05$). (B); DNA fragmentation agarose gel electrophoresis. Lane 1: DNA ladder; Lane 2, 4, 6 & 8: DNA from liver of rats of control, L, H and L+H groups; respectively. Lane 3, 5, 7 & 9; DNA from testes of rats of control. L, H and L ±H groups, respectively.

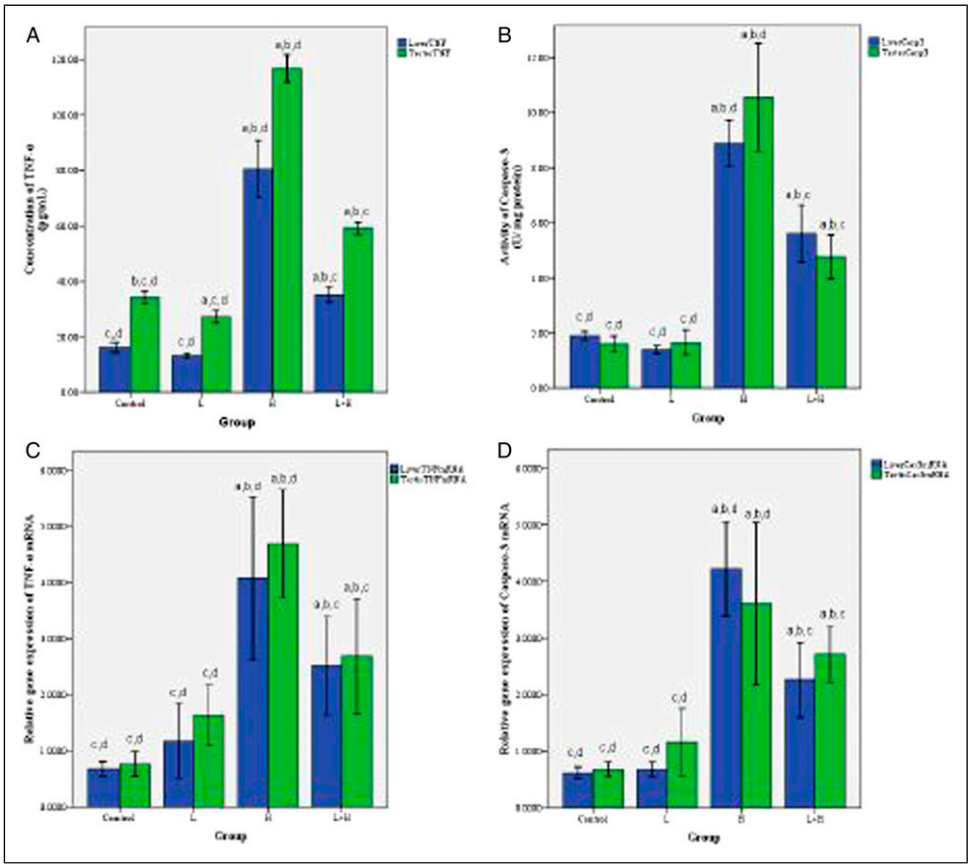

Figure 3. Inflammation and apoptosis markers in the liver (blue) and testes (green) of rats. (A): Concentration of tumor necrosis factor alpha (TNF-α); (B): Activity of Capase-3; (C): Fold change of TNF-α mRNA relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH); **(D):** Fold change of Caspase-3 mRNA relative toGlyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are expressed as mean ± SD (n = 10). a: significant compared to L group, c: significant compared to H group, d: significant compared to L ± H group ($P < .05$).

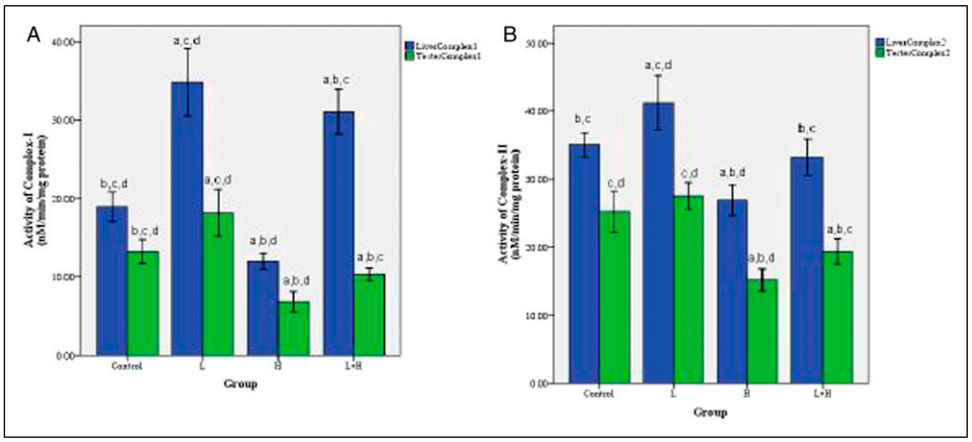

Figure 4. Mitochondrial markers in the liver (blue) and testes (green) of rats. (A): Activity of NADH: ubiquinone oxidoreductase (complex-I); (B): Activity of Succinate dehydrogenase (Complex–II). Data are expressed as mean ± SD (n = 10). a: significant compared to L group, c: significant compared to H group, d: significant compared to L ± H group ($P < .05$).

The results of the present study showed that whole-body gamma irradiation (4 Gy) induced a significant increase in LPO associated with significant decreases in TAC, the concentration of Nrf2, and the expression level of Nrf2 mRNA in the liver and testes of rats, compared to the control group (Figure 1 and Table 2), indicating an imbalance between prooxidants and antioxidants. These results come in accordance with previous studies which indicated that exposure to ionizing radiation

Table 2: An Overview of all Studied Biomarkers in L+H Group When Compared to H Group Indicating the Protective Role of the Exposure to Low Dose Before High Dose Radiation.

(A) Oxidative stress markers				
Parameter	LPO	TAC	Nrf2	Nrf2 mRNA
Response	↓	↑	↑	↑
(B) DNA damage markers				
Parameter	DNA fragmentation		8-OHdG	
Response	↓		↓	
(C) Inflammation and apoptosis markers				
Parameter	TNF-α	TNF-α mRNA	Caspase-3	Caspase-3 mRNA
Response	↓	↓	↓	↓
(D) Mitochondrial markers				
Parameter	Complex I		Complex II	
Response	↑		↑	

Abbreviations: Nrf2, Nuclear factor erythroid 2-related factor-2; TAC, Total antioxidant capacity; LPO, Lipid peroxidation; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; TNF-α, Tumor necrosis factor-α.

Table 3. Pearson Correlation Coefficients of Nuclear factor erythroid 2-related factor-2 and 8-Hydroxy-2′-Deoxyguanosine with Other Parameters in the Liver and Testes of Rats.

	Correlation coefficient (r) of Nrf2		Correlation coefficient (r) of 8-OHdG	
Parameter	In Liver	In Testes	In Liver	In Testes
LPO	−.889**	−.915**	.884**	.934 **
TAC	.946**	.925**	−.889**	−.877 **
Nrf2	1		−.908**	−.902 **
TNF-α	−.943**	−.953**	.892**	.962**
Caspase-3	−.956**	−.926**	.935**	.974**
8-OHdG	−.908**	−.902**	1	
Complex I	.629**	.886**	−.683**	−.739**
Complex II	.823**	.914**	−.761**	−.869**

Abbreviations: Nrf2, Nuclear factor erythroid 2-related factor-2; TAC, Total antioxidant capacity; LPO, Lipid peroxidation; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; TNF-α, Tumor necrosis factor-α.
**: Correlation is significant at the .01 level.
*: Correlation is significant at the .05 level.

induced LPO, decreased antioxidant enzymes activities, and Nrf2 expression in liver tissues.[21,22] The findings of Wang et al. indicated that the defects in the Nrf2 signaling pathway may have contributed to the increased sensitivity to oxidative stress damage induced by gamma radiation.[23] Previously, Khan et al. observed that whole-body irradiation (5 Gy) induced a marked decrease in TAC and an increase in thiobarbituric acid reactive substances (TBARS) in testicular tissue 2, 4, and 8 hours post-irradiation, and demonstrated significant differences between 2 to 4 hours and non-significant differences between 4 and 8 hours. The authors suggested that TBARS increased significantly during a period of time till it reached its maximum level at 4 hours.[24]

Remarkably, oxidative stress and ROS production can also destroy proteins, and nucleic acids, resulting in DNA single or double-strand breaks. Under extreme conditions, the abnormally repaired DNA may affect the cell signal transduction pathway leading to uncontrolled cell proliferation, apoptosis, and even carcinogenesis.[25] The correlation matrix of 8-OHdG with the different measured parameters in the liver and testes of rats revealed that there is a significant positive correlation between 8-OHdG and LPO while, there is a significant negative correlation between 8-OHdG and TAC (Table 3).

Our results indicated that exposure to 4 Gy induced cellular and subcellular injuries leading to DNA damage, mitochondrial disturbances, and apoptosis. IR elevated the level of 8-OHdG, DNA fragmentation, the concentration and expression levels of caspase- 3, and TNFα associated with a significant decrease in mitochondrial complex I and complex II in the liver and testes, compared to the control group (Figures 2-4 and Table 2). Comparable results have been recorded in previous studies of Khan et al., Marzban et al. and Moustafa et al. using different doses (2–6 Gy).[24,26,27] The disturbances of these parameters may be associated with mitochondrial injury. Mitochondria are known to be a target of radiation toxicity that disrupts the metabolic pathway of oxidative phosphorylation responsible for energy production, increases the generation of mitochondrial superoxide, and activates apoptosis. caspase-3 is the main executioner of apoptosis activated by other caspases (−8, −9, or −10). Once activated, it stimulates the breakdown of many proteins related to DNA fragmentation, nuclear collapse and condensation, and margination of chromatin.[28,29] It has been found that the inhibition of caspase-3 is related to the characteristics of necrosis, which revealed the importance of caspase-3 activity in apoptosis occurrence following ionizing radiation exposure.[30,31] In addition, TNFα is a pleiotropic cytokine widely involved in apoptosis and immune and inflammatory reactions. It plays many roles as a signaling molecule within the cell and as an effector molecule in cell proliferation or cell death.[32,33]

Previously, it was demonstrated that low doses of chemical and physical agents induce beneficial effects while the opposite occurs at higher doses, a phenomenon known as hormesis.[34] The results of the current study showed that exposure to low dose/low dose rate gamma-radiation (40 mGy, 2.2 m Gy/hour) induced a significant increase in Nrf2 and TAC levels associated with a decrease in LPO in the liver and testes compared to the control group (Figure 1), indicating an enhancement of endogenous antioxidant defense machinery. These results are in agreement with the findings of Sharma et al. who showed that exposure to low-dose X-rays (.2 Gy) provides cellular protection 6 and 24 hours after irradiation by enhancing the cellular antioxidant mechanisms, particularly the glutathione system.[20] In addition, it has been concluded that 50 mGy of gamma-radiation was incapable of inducing significant in vivo genomic instability in the bone marrow cells of mice 1 and 4 hours postirradiation.[35] Consequently, it is suggested that low doses

of radiation generate a low level of reactive oxygen species in the exposed tissue, which are neutralized by the action of the enhanced endogenous antioxidant system. Moreover, it has been demonstrated that exposure to low doses of ionizing radiation provided resistance to oxidative injuries in different tissues triggered by high-dose radiation, doxorubicin, and snake venom.[7,36,37] Also, low doses intensified the antioxidant, antiapoptotic, and anti-inflammatory effects of exogenous antioxidants administered to rats against liver and testis injuries induced by different toxicants.[13,38,39] As well, the clinical trial recorded by Yu et al. showed that low dose radiation-mediated radioadaptive response can alleviate high dose radiation toxicities, improve the short-term efficacy of radiotherapy, and improve the survival of the lung cancer patients.[40]

Concerning the radio adaptive effect of low-dose radiation, the obtained results revealed that pre-conditioning low-dose gamma radiation before exposure to high dose have significantly increased Nrf2 and TAC levels and reduced the level of LPO compared to the H group (Figure 1). These results indicated that low-dose can enhance the resistance of cells to a subsequent high dose and stimulate Nrf2-mediated antioxidant response pathway, thus reduce the DNA injury and fragmentation (Figures 1 and 2 and Table 2). Moreover, it reduces the mitochondrial injury, apoptosis, and inflammation as indicated by the increased levels of mitochondrial complexes I and II and the decreased levels and mRNA expression of TNF-α and caspase-3 compared to the H group (Figure 3 and 4 and Table 2). It has been explained that low dose radiation might enhance free radical formation concomitant with the enhancement of antioxidant mechanisms that overwhelmed the formed free radicals and finally predominated. Therefore low dose preconditioned the body to resist the subsequent oxidative damage by different signaling pathways including antioxidant response and DNA repair.[20,41] It is noteworthy that Nrf2 is a central transcription factor responsible for the basal and inducible expression of proteins involved in these pathways. At basal conditions, Nrf2 binds to Keap1 and stays in the cytosol. However, under stress conditions, when reactive oxygen species levels exceed the ability of cells to scavenge them, Nrf2 dissociates from Keap1 and translocates to the nucleus, attaches to the antioxidant response element, and increases the expression of detoxification, antioxidant, and antiapoptotic proteins[42] that ultimately exert anti-inflammatory functions. It has been reported that Nrf2 activation negatively affects the NF-κB signaling pathway and decreases the expression of inflammatory proteins (COX-2 and iNOS) and pro-inflammatory cytokines including TNF, IL-1β, and IL-6. In the same context, the correlation matrix of Nrf2 with the different measured parameters in the liver and testes of rats revealed that there is a significant negative correlation between Nrf2 with LPO, TNF-α, caspase-3, and 8-OHdG, while there is a significant positive correlation between Nrf2 with TAC, complex I and complex II (Table 3).

On the contrary, other studies have indicated that low doses of radiation commonly induced oxidative stress and DNA damages, which led to systemic aberrations.[4,43] It was observed that exposure to .1 Gy of X rays (dose rate 50 mGy/s) induced elevation of DNA damage and apoptosis in murine spleen tissue after 6 hours.[44] Also, it was observed that exposure to .1 and .5 Gy (dose rate 200 mGy/min), induced liver-specific gene alteration leading to inhibition of glucose, and lipid metabolism 24 hours post-irradiation, in neonatal mice.[45] Moreover, Abdelrazzak et al. concluded that the significant effect of .1 Gy-irradiation (dose rate 4 Gy/min) emphasizes the low dose radiation-associated risk and there is no evidence that this dose protected the liver cells against a subsequent higher dose (2 Gy)-induced oxidative stress and DNA damage.[17]

From the above considerations, the effect of low doses of radiation remains controversial. This controversy may be related to the type, dose, rate of radiation dose, and the time after irradiation. Further studies are required using different doses and time intervals.

Conclusion

According to the results obtained in this study, low dose/low dose rate γ-radiation -as a priming dose—may induce a protective effect against the early damaging effect of γ-radiation, challenge dose, in rats. This effect might be attributed to the reduction of DNA damage induced early after irradiation and activation of anti-oxidative and anti-apoptotic mechanisms via modulating the Nrf2-mediated antioxidant response pathway, and mitochondria-mediated caspase activation & apoptosis. However, further studies are required to identify the long-term effects of this low dose.

Appendix

Abbreviations

Nrf2	Nuclear factor erythroid 2-related factor-2
TAC	Total antioxidant capacity
LPO	Lipid peroxidation; 8-OHdG, 8-hydroxy-2′-deoxyguanosine
TNF-α	Tumor necrosis factor-α.

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Ethics Approval

The current study was approved by the ethics committees of the Egyptian Atomic Energy Authority, National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt (8A/20).

Availability of Data of Material

All data generated or analyzed during this study are included in this published article.

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Supplemental Material

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