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MitoQ ameliorates testicular damage induced by gamma irradiation in rats: Modulation of mitochondrial apoptosis and steroidogenesis

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ABSTRACT

Aims: The deleterious effect of gamma radiation on testicular tissue is a challenging problem in nuclear medicine. This study investigated the potential radioprotective effect of mitoquinol (MitoQ), a mitochondria-targeted antioxidant, against testicular damage induced by gamma irradiation in rats.

Main methods: Rats were allocated into four groups. The first group served as the control, the second group received MitoQ (2 mg / kg / day; i.p.) for seven days, the third group was exposed to gamma radiation (5 Gy as a single dose) and the last group received MitoQ prior to irradiation. Rats were sacrificed. Then, sperm analyses and the serum testosterone were determined. Moreover, evaluation of mitochondrial oxidative stress parameters (SOD, GSH and GPx) as well as apoptosis indicators (cytochrome-c, Bax, Bcl-2 and caspase-3) was performed. Additionally, analysis of steroidogensis biomarkers (StAR, 3β-HSD and 17β-HSD) and histopathological investigations were carried out.

Key findings: MitoQ replenished mitochondrial SOD, GPx and GSH indicating its strong antioxidant effect in addition to its energy preservation manifested by the elevated ATP. MitoQ inhibited the intrinsic apoptosis via diminution of Bax, cytochrome-c and caspase-3 and alleviation of Bcl-2. This antioxidant conferred protection to steroidogenesis as verified by the increase in testosterone and the up-regulation of StAR, 3β-HSD and 17β-HSD expression; these effects might be correlated with its antioxidant/anti-apoptotic potential. Histopathological and sperm analyses corroborated the biochemical findings.

Significance: This study identifies MitoQ as a novel agent for the management of testicular toxicity induced by gamma irradiation.

1. Introduction

Gamma radiation exposure is widespread in the medical field for diagnostic and therapeutic purposes; both patients and health professionals can be exposed. Testis is one of the most vulnerable tissues to radiation [3]; spermatogenesis could be arrested by exposure to even a low dose of gamma radiation [31]. Gamma irradiation increases oxidative stress in testicular tissue through the release of reactive oxygen species (ROS), which eventually leads to apoptosis of spermatogonia, spermatocytes and spermatozoa [30]. Moreover, testosterone level is substantially diminished upon gamma radiation exposure [3]. These findings have prompted researchers to search for effective and safe radioprotective agents that can mitigate the testicular damage caused by gamma irradiation.

Testicular mitochondria regulate different aspects of male reproductive functions, such as spermatogenesis, ATP synthesis and Leydig cell steroidogenesis [37]. In addition, sperm mitochondria are involved in many essential processes in fertility, such as sperm motility, hyperactivation, capacitation and acrosome reaction [32].

Although, the predominant postulation of radiation biology primarily attributes the harmful effects of ionizing radiation to DNA damage in the nucleus, growing evidence posits that the extranuclear radiation effect, particularly that on mitochondria, is considered a potential culprit of ionizing radiation damage [8,23]. Ionizing radiation disturbs mitochondrial functions through a burst of different species with high reactivity and energy, such as e_{aq}^-, $H^\bullet$, $HO^\bullet$ and $O_2^{-\bullet}$.

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Ionizing radiation causes alterations in the expression of genes that affect cell survival [26]. Meanwhile, some mitochondrial proteins are involved in the compensatory mechanisms against radiation hazards [23].

Gamma radiation was reported to induce mitochondrial (intrinsic) apoptosis pathway through two cornerstone proteins Bcl-2 and Bax [39]. Gamma radiation inhibits the anti-apoptotic Bcl-2 protein, which hinders cytochrome-c release from the mitochondria. Meanwhile, gamma radiation sequesters the pro-apoptotic Bax protein, thereby promoting mitochondrial release of cytochrome-c into the cytoplasm and activating the apoptotic caspase cascade [39]. Mitochondrial ROS and apoptosis caused by gamma irradiation interrupt steroidogenesis in Leydig cells particularly in the cholesterol transfer phase [37].

Mitoquinol (MitoQ) is a mitochondria-targeted antioxidant consisting of two moieties: triphenyl phosphonium cation (TPP+) and ubiquinol, the endogenous coenzyme Q10 [25]. MitoQ transfers rapidly through the plasma membrane and mitochondrial membrane due to the lipophilicity of the TPP+ cation, resulting in excessive accumulation in the cell mitochondria. MitoQ quenches mitochondrial oxidative stress and has been shown to protect against various diseases, such as cardiovascular [41] and neurodegenerative disorders [28]. Consequently, this study was conducted to investigate the potential radioprotective activity of MitoQ against whole-body gamma irradiation induced testicular injury in experimental rats to provide an insight into the impact of mitochondrial protection on the effects of gamma radiation in male fertility.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats (180–200 g) were used in this research. Animals were supplied from the Medical Research Center, Ain Shams University, Cairo, Egypt. They were housed for seven days in the laboratory room prior to the experiment. The animals were kept under controlled environmental conditions: a room temperature of 24–26 °C, constant humidity (60 ± 10%), with alternating 12 h dark and light cycle. Water and food were allowed ad libitum. Rats were treated gently; pressure, squeezing and rough handling were avoided. All animal procedures were performed in accordance with the Ethics Committee for Animal Experimentations of the Faculty of Pharmacy, Cairo University (Permit Number: PT 2014), which complies with the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011).

2.2. Chemicals

MitoQ (mitoquinol mesylate) was supplied from Antipodean Pharmaceuticals, Auckland, New Zealand. All other reagents and fine chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless specified herein.

2.3. Irradiation process

Animals were subjected to whole-body gamma irradiation at a single dose of 5 Gy (Gy). The dose rate was 0.676 rad/s. This dose was selected because it induces testicular toxicity [1]. Irradiation was carried out using the biological irradiator (cesium ^{137}Cs source) at the National Center of Radiation and Research Technology (NCRRT), Cairo, Egypt.

2.4. Experimental design

Rats were randomly allocated into four groups each consisting of six rats: the first group, which served as the control, was injected i.p. with sterile pyrogen-free water (Egypt Otsuka Pharmaceutical Co., Cairo, Egypt); the second group was injected i.p. with MitoQ (2 mg/kg/day) for seven days (MitoQ group); the third group was injected i.p. with sterile pyrogen-free water and then irradiated at a dose of 5 Gy as a single dose (IR group), and the last group was injected i.p. with MitoQ (2 mg/kg/day) for seven days followed by 5 Gy gamma radiation as a single dose (MitoQ + IR group). The MitoQ was dissolved in sterile pyrogen-free water. The MitoQ dose was selected after a pilot study through sperm, histopathological and caspase-3 analyses after different doses of MitoQ (0.5, 1, 2, 5 and 10 mg/kg/day). The data of the pilot study are not shown.

Table 1
The sequences of primers used for real-time PCR analysis.

mRNA species	Primer sequence	Accession no.
StAR	F: GGGCATACTCAACAACCAG R: ACCTCCAGTCGGAACACC	NM-031558.3
3β-HSD	F: TGTGCCAGCCTTCATCTAC R: CTTCTCGGCCATCCTTTT	NM-001007719.3
17β-HSD	F: GACCGCCGATGAGTTTGT R: TTTGGGTGGTGCTGCTGT	NM-054007.1
Bax	F: CCCTGTGCACTAAAGTGCCCG R: TTCTTCCAGATGGTGAGTG	NM-017059.2
Bcl-2	F: CATGCCAAGGGGGAAACACCAGAA R: GTGCTTTGCATTCTTGGATGAAGG	NM-016993.1
β actin	F: GACGGTCAGGTCATCACTAT R: CTTCTGCATCCTGTCAGCAA	NM_031144.3

2.5. Sample collection

The rats were weighed and sacrificed seven days after gamma irradiation by cervical dislocation under light urethane (1.2 mg/kg) [16]. Blood samples were obtained by heart puncture and centrifuged, and then, the serum was frozen at −80 °C to determine the testosterone level. The cauda epididymis was dissected to evaluate sperm features. The testes were isolated and weighed to calculate the relative testes/body weight ratio. Testis tissues were divided into two portions. The first part was stored with buffered formalin (10% *v*/v) for histopathological examination, and the other part was frozen at −80 °C. For determination of the different parameters, the testis tissues were minced and homogenized with 10 mM HEPES–KOH, 1 mM EGTA buffer (pH 7.5), 0.25 mM sucrose, and inhibitors of protease and phosphatase. Then, centrifugation was performed by a cooling centrifuge (Hettich Universal 32A, Kirchlengern, Germany) at 20000 g for 10 min at 4 °C. The animal bodies were frozen untill they were incinerated.

2.6. Sperm motility determination

Cauda epididymis fluid was diluted to 2 ml using Tris buffer. Motility was estimated under a light microscope within 2–4 min (×400 magnification). Data are presented as a percentage of motile sperms toward the total sperm count [9].

2.7. Sperm abnormalities determination

Three hundred sperm were counted per slide and microscopically examined at a magnification of ×400, and sperm abnormalities were evaluated using the criteria provided by Evans and Maxwell [15].

2.8. Mitochondrial isolation

Homogenized testes were centrifuged with 0.25 M sucrose at 2000 g for 10 min at 4 °C. Pellets were discarded, and 0.75 M sucrose in HEPES buffer was added to the supernatant and then centrifuged at 10,000 *g* for 30 min. HEPES buffer was added to mitochondrial pellets after the supernatant was discarded. Further centrifugation was carried out at 10,000 g for 10 min, and the supernatant was discarded. Thereafter,

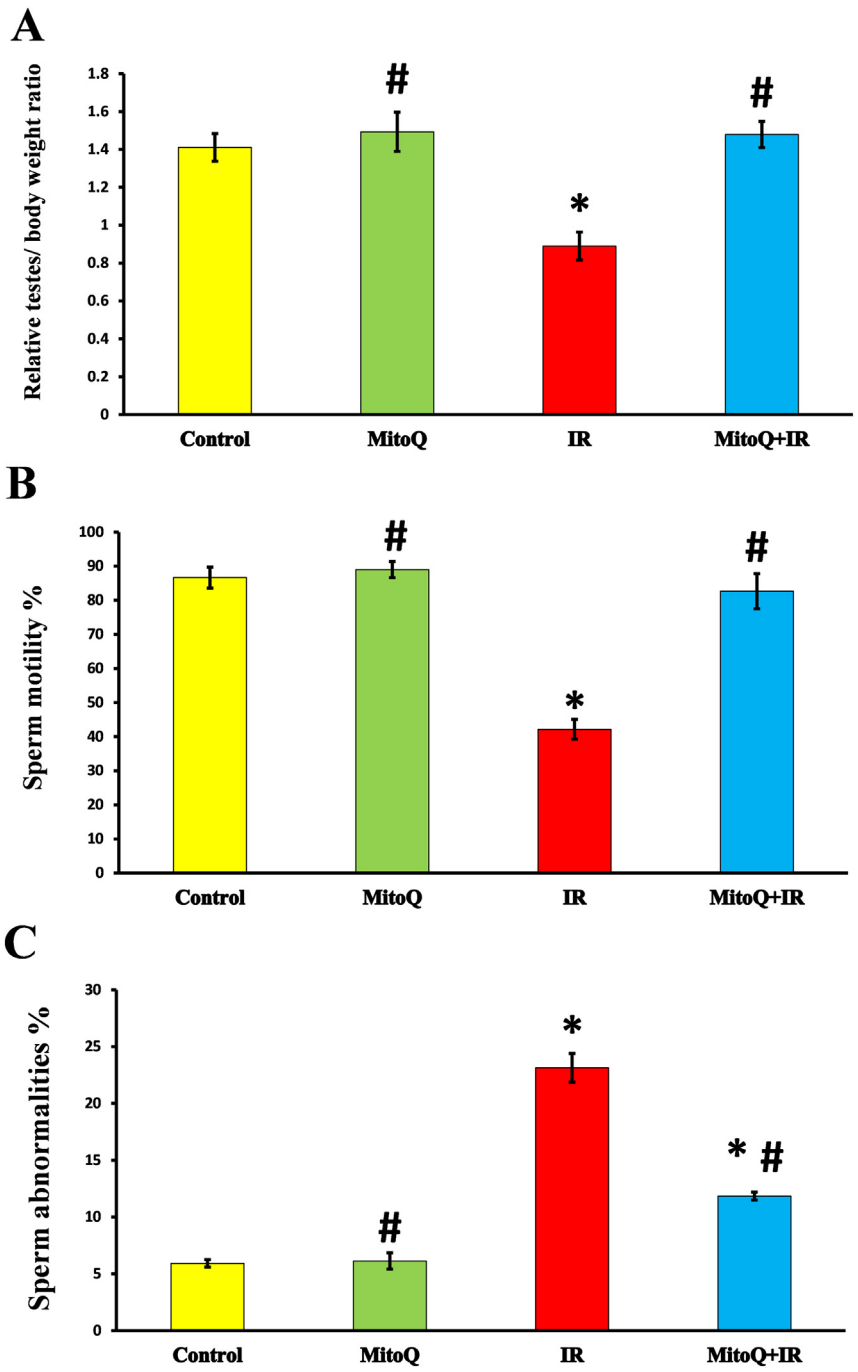

Fig. 1. Effect of MitoQ on testicular damage induced alterations in the relative testes/body weight ratio (A), sperm motility (B) and sperm abnormalities (C). Each bar represents the mean of six rats per group, and error bars represent the SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$; *vs the control group, # vs the irradiated group.

PBS was added to the final mitochondrial pellets [38]. The samples were stored at −80 °C till the determination of the following parameters.

2.8.1. Mitochondrial superoxide dismutase enzyme (SOD) determination

SOD enzyme assay is dependent on its capability to inhibit the nitroblue tetrazolium dye reduction produced by phenazine methosulphate. The absorbance change was determined at 560 nm. Data are presented as U/mg protein [34].

2.8.2. Mitochondrial reduced glutathione (GSH) determination

Mitochondrial GSH content was evaluated by measuring the yellow compound produced from reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) by the SH group in the structure of GSH. The yellow colour was measured colourimetrically at 405 nm [10].

2.8.3. Mitochondrial glutathione peroxidase (GPx) determination

Mitochondrial GPx was measured with the method developed by Paglia and Valentine [36]. This method relies on GSH oxidation by hydrogen peroxide (H_2O_2) in the presence of GPx. The absorbance decrease was determined at 340 nm as NADPH was converted to NADP +, which reflects the oxidized glutathione content resulting from GPx activity. The GPx activity is expressed as U/mg protein where one unit indicates the GPx content that reacts with 1 mmol NADPH per minute

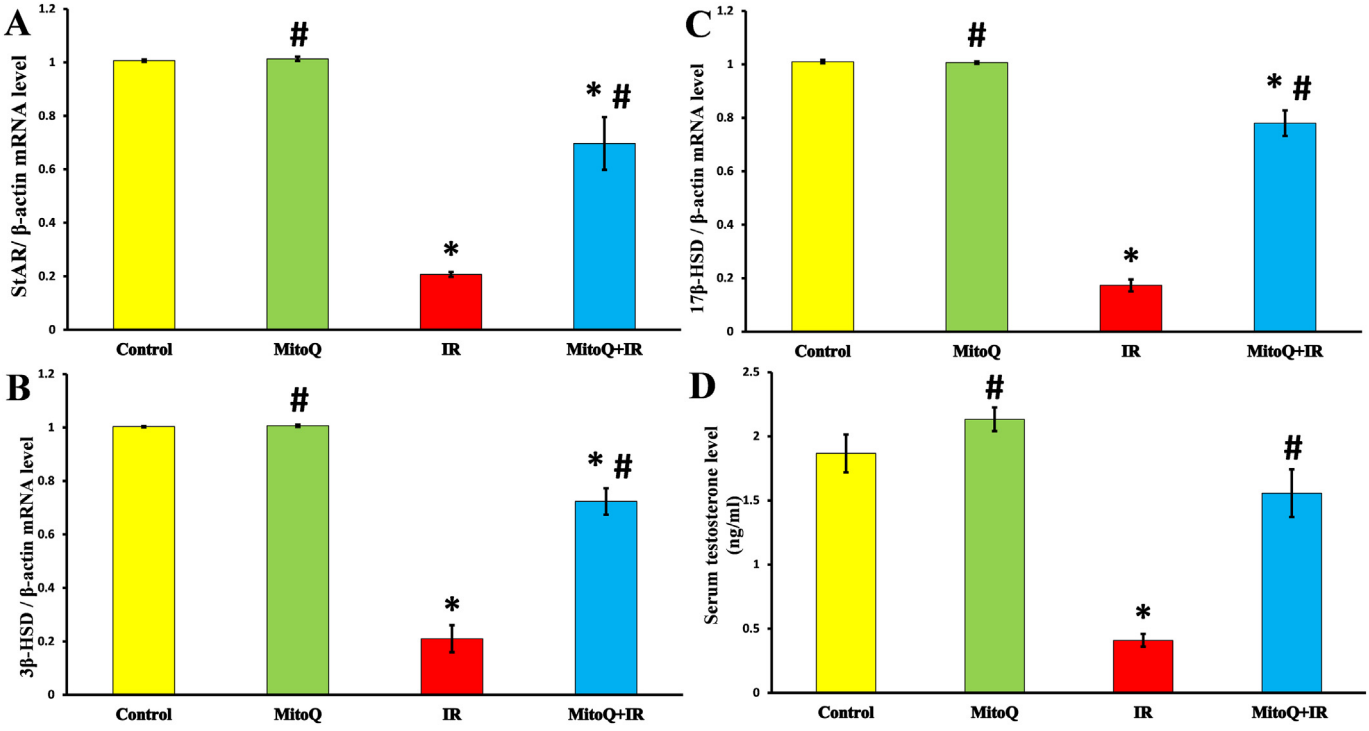

Fig. 2. Effect of MitoQ on testicular damage-induced alterations in the gene expression of StAR (A), 3β-HSD (B) and 17β-HSD (C) as well as the serum testosterone level (D). Each bar represents the mean of six rats per group, and the error bars represent the SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$; *vs the control group, # vs the irradiated group.

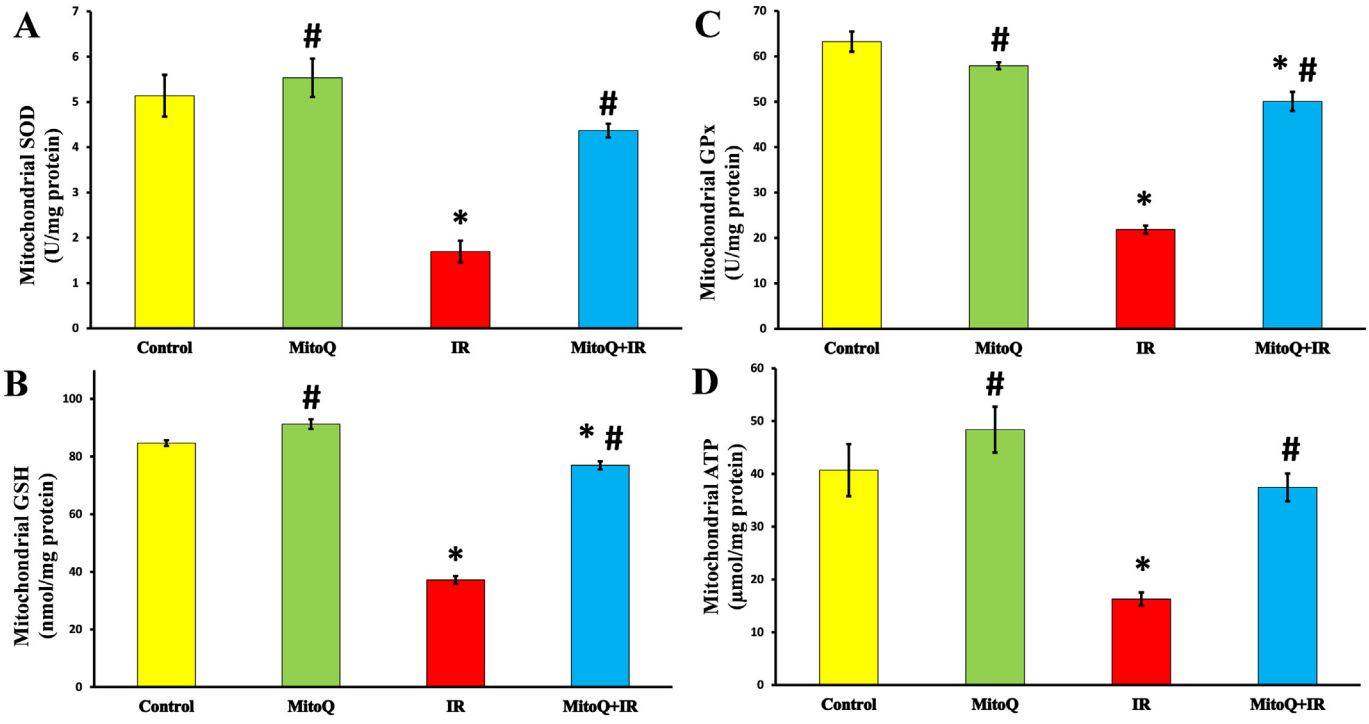

Fig. 3. Effect of MitoQ on testicular damage-induced alterations in mitochondrial SOD (A), GSH (B), GPx (C) and ATP (D) in testicular tissue. Each bar represents the mean of six rats per group, and error bars represent the SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$; *vs the control group, # vs the irradiated group.

[36].

2.8.4. Mitochondrial ATP determination

Mitochondrial ATP content was measured using Abnova's ATP Assay Kit (Abnova, Taipei, Taiwan) which relies on ATP phosphorylation of glycerol and the resulting compound was easily measured colourimetrically at 570 nm. The data are presented as μmol/mg protein.

2.8.5. Serum testosterone determination

The serum testosterone level was estimated via an ELISA kit

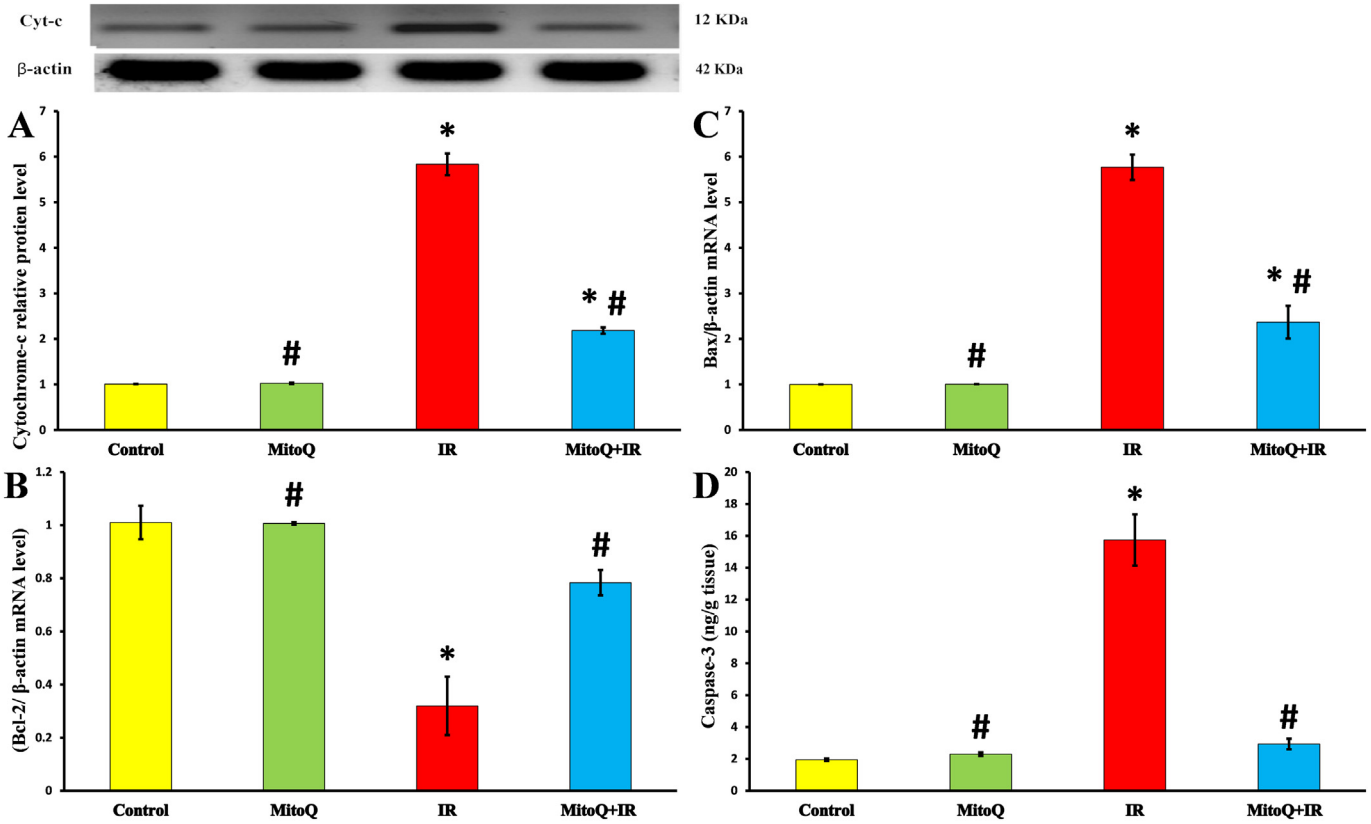

Fig. 4. Effect of MitoQ on testicular damage induced alterations in the protein expression of cytochrome-c (A), the gene expression of Bcl-2 (B) and Bax (C) and the caspase-3 content (D) in testicular tissue. Each bar represents the mean of six rats per group, and error bars represent the SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$; *vs the control group, # vs the irradiated group.

(MyBioSource, San Diego, CA, USA). The colour intensity produced was evaluated at 450 nm by means of a microplate reader. Testosterone concentration was determined from a standard curve.

2.9. Testicular StAR, 3β-HSD, 17β-HSD, Bax and Bcl-2 determination using RT-qPCR

The total RNA was extracted from testis homogenate by the RNeasy purification reagent (Qiagen, Valencia, CA, USA). The total RNA purity was determined with a spectrophotometer. First-strand cDNA was generated using a reverse transcription reaction (Superscript II, Gibco Life Technologies, NY, USA). RT-qPCR was performed on an Applied Biosystem with programme version 3.1 (StepOne™, CA, USA) using the SYBR Premix EX Taq II kit (Applied Biosystems, CA, USA). Data were evaluated by the comparative cycle threshold (CT) method. The primer specificity was confirmed by the melting curve analysis. The relative expression of each target gene was quantified with β-actin as the housekeeping gene. All these steps comply with the MIQE guidelines [13]. The primer sequences of different target genes are shown in Table 1.

2.10. Testicular cytochrome-c determination using western blotting

Total proteins were extracted from testis homogenate by RIPA lysis buffer PL005 (Bio BASIC Inc., Ontario, Canada). The concentration of total proteins in the mixture was quantified by a Bradford Protein Assay Kit (Bio BASIC Inc., Ontario, Canada). The protein samples (20–30 μg total protein) were loaded onto a 12% SDS–polyacrylamide gel (10% acrylamide gel) via a Bio-Rad Mini-Protean II system (USA). Then, the proteins were transferred to polyvinylideine difluoride (PVDF) membranes (Thermo Fisher Scientific, Rockford, IL, USA). The membranes were blocked after washing with 5% (*w/v*) skim milk powder in PBS for 1 h at 25 °C and subsequently incubated with primary anti-cytochrome-c antibody (Biosynthesis Biotechnology, Beijing, China, 1:500). Anti-β-actin (Thermo Fisher Scientific, Rockford, Illinois, USA, 1:1000) served as an internal control. Analysis of bands was performed via a ChemiDoc™ imaging system with Image Lab™ programme version 5.1 (Bio-Rad Laboratories, CA, USA). The relative protein level of cytochrome-c was determined after normalization by the protein level of β-actin.

2.11. Testicular caspase-3 determination

Caspase-3 content in testis homogenate was determined using a rat caspase-3 ELISA kit (MyBioSource, San Diego, CA, USA). The yellow colour produced was measured at 450 nm using a microplate reader.

2.12. Histopathological examination

Rat testes were fixed in buffered formalin and sectioned. Then, the testis sections were stained using haematoxylin and eosin staining. Histopathological investigation was performed with an electric light microscope as described by Bancroft and his colleages [7]. Spermatogenesis was evaluated by Johnsen's mean testicular biopsy score (MTBS) criteria. The microscopic scoring was done through evaluating tubules in ten sequential x400 fields and categorized mild (+), moderate (++) and severe (+++) ([21,40]. In addition, spermatogonia, spermatocytes, spermatids and Leydig cells were counted according to [4].

2.13. Statistical analysis

Data are expressed as the mean ± SEM. Comparisons between means were performed using one-way ANOVA followed by Tukey-

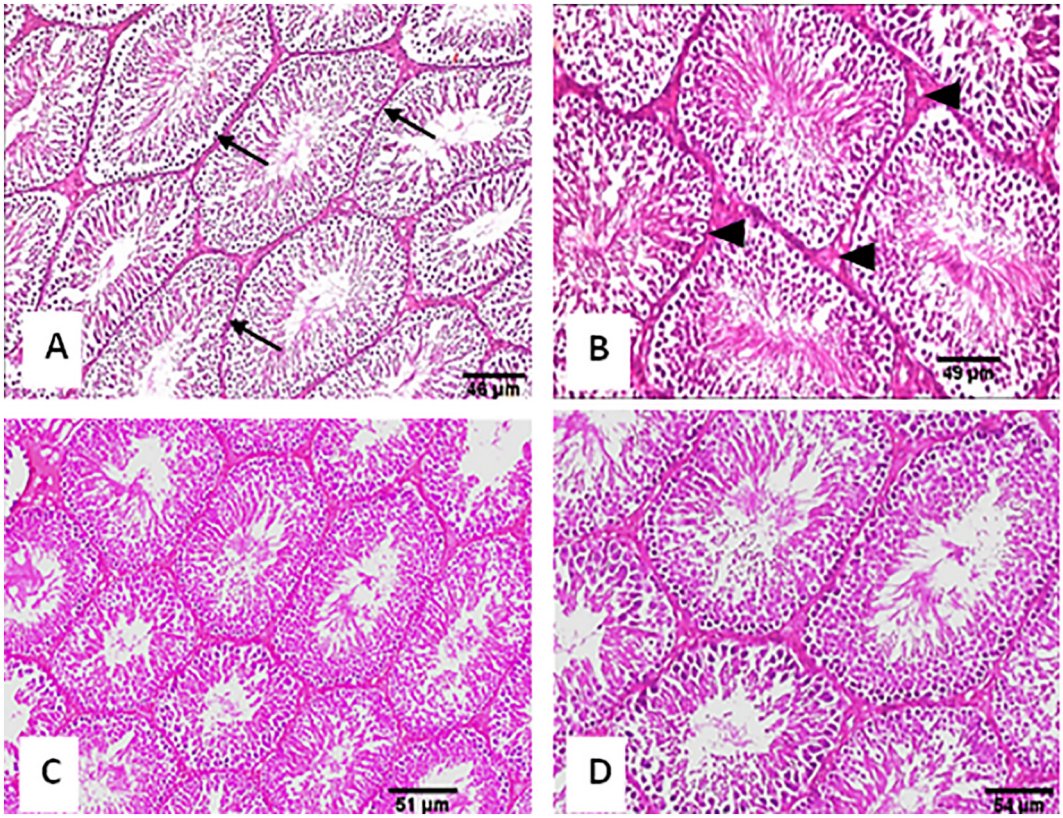

Fig. 5. Histopathological sections of control and MitoQ-treated rat testes (A-D) stained with H&E. Testicular sections of control rats show active spermatogenesis in normal-size seminiferous tubules with thin basement membranes (↑, ×200) (A) and interstitial cells in between the tubules (◀, ×400) (B). Testicular sections of the MitoQ group show regularly arranged spermatogenic cells within the seminiferous tubules (×200) (C) and a normal tubular lumen containing spermatids (×400) (D).

Kramer multiple comparisons test. Statistical analysis was performed via SPSS (version 14). A probability level < 0.05 was accepted as statistically significant.

3. Results

No significant differences were detected in any of the tested markers between control animals and those given MitoQ without being irradiated, indicating MitoQ administration was safe.

3.1. Effect of MitoQ on sperm features and relative testes/body weight ratio

The irradiated group displayed a significant 37% decline in the relative testes/body weight ratio compared to that of the control value. MitoQ pretreatment nearly normalized this ratio. Likewise, irradiated rats showed a significant decline in sperm motility by 51% compared to the control value; MitoQ reverted sperm motility to its normal percentage. In addition, gamma radiation-exposed rats exhibited a 4-fold increase in sperm abnormalities, which was reduced by nearly 50% following the MitoQ pretreatment (Fig. 1 A, B, C).

3.2. Effect of MitoQ on steroidogenesis

The irradiated animals presented a notable decrease in the serum testosterone level (80%) and down-regulation of StAR, 3β-HSD and 17β-HSD at the mRNA level compared to their normal littermates. MitoQ up-regulated the expression of these key proteins in testosterone biosynthesis by 229%, 243% and 359%, respectively. Therefore, a 4-fold increase in the serum testosterone was observed (Fig. 2A, B, C, D).

3.3. Effect of MitoQ on mitochondrial oxidative stress biomarkers and ATP content

Gamma radiation induced mitochondrial oxidative stress and dysfunction as shown by a significant decrease in SOD (67%), GSH (56%), GPx (65.5%) and ATP (60%). A 2-fold increase in these parameters was observed after MitoQ administration, indicating improvement in mitochondrial oxidant status and function (Fig. 3A, B, C, D).

3.4. Effect of MitoQ on mitochondrial apoptosis indicators

The present results revealed gamma radiation-induced apoptosis, as evidenced by up-regulation of the pro-apoptotic cytochrome-c protein and Bax gene expression by approximately 6-fold together with down-regulation of Bcl-2 gene expression by 68%. In addition, caspase-3 was elevated (8-fold). Treatment with MitoQ before irradiation quenched the intrinsic apoptotic pathway, as shown by the decline in cytochrome-c protein level (62.5%), down-regulation of Bax gene expression (60%) and up-regulation of Bcl-2 gene expression (145%), accompanied by mitigation of caspase-3 by 81% relative to that in irradiated rats (Fig. 4A, B, C, D).

3.5. Histopathological findings

The parenchyma of the testis of control rats revealed seminiferous tubules of variable sizes; all of them were surrounded by an outer thin layer of connective tissue and lined with spermatogenic and Sertoli cells. The spermatogenic cells are comprised of several layers (spermatogonia, primary and secondary spermatocytes as well as spermatids). The interstitial cells (Leydig cells) originated mostly in groups between the seminiferous tubules; they appeared large and ovoid with

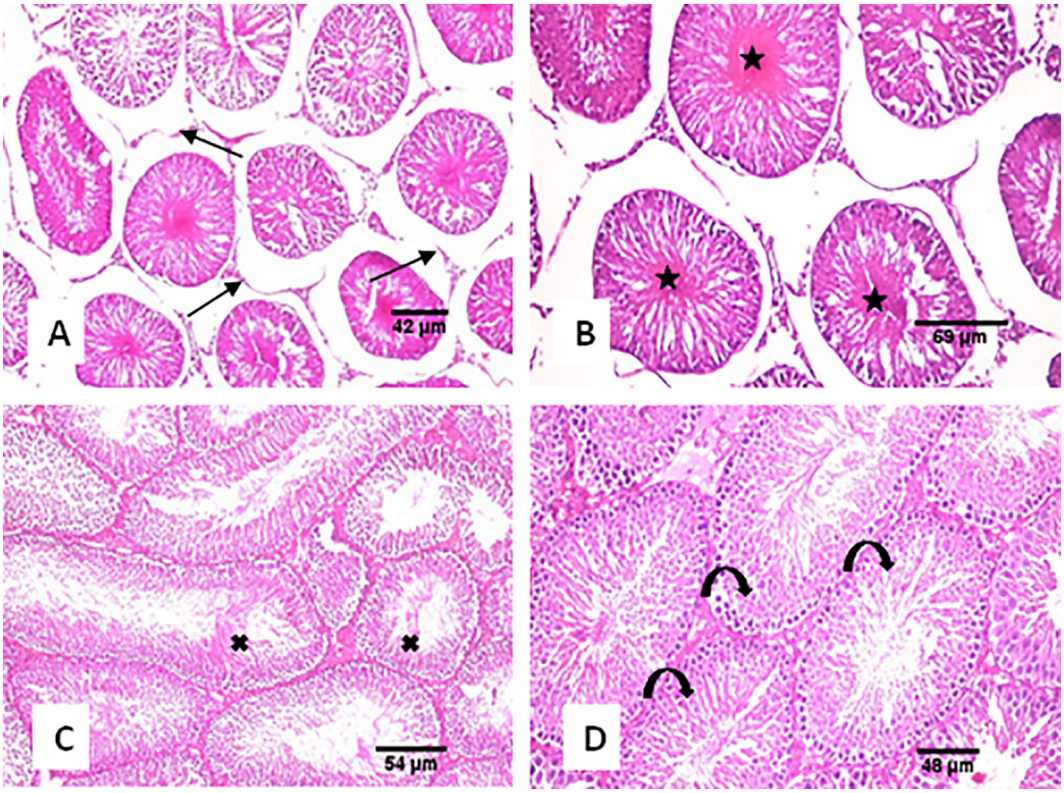

Fig. 6. Effect of MitoQ on gamma radiation-induced testicular histopathological alterations (A-D) stained with H&E. Testicular sections of irradiated rats show atrophy of the seminiferous tubules as well as widening of the interstitial space (arrow, ×200) (A) and reductions in the number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids (star, ×400) (B). Testicular sections of the MitoQ + IR group show regularly arranged spermatogenic cells within the seminiferous tubules (×, ×200) (C) and a normal tubular lumen containing spermatids (curved arrow, ×400) (D).

Table 2
Degrees of some histopathological lesions of testicular sections from rats [mild (+), moderate (++), severe (+++)], $n = 6$.

Parameters	Control	MitoQ	IR	MitoQ + IR
Desquamation in germinal cells	ND	ND	+++	+
Disorganization in germinal cells	ND	ND	+++	+
Interstitial oedema	ND	ND	+	+
Degeneration in germinal cells	ND	ND	+++	+
Reduction in germinal cell counts	ND	ND	++	+

ND: Not detected.

rounded nuclei (Fig. 5 A, B). In contrast, the histopathological sections of irradiated rats showed that seminiferous tubules appeared atrophied with a widening of the interstitial spaces and a decrease in the interstitial cell count. Disorganization of spermatogenic cells within the seminiferous tubules was observed. Moreover, the spermatogenic cells exhibited a significant decline in the number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Some spermatogenic cells appeared necrotic, and hyperactivity of Sertoli cells was observed (Fig. 6A, B) (Table 3).

Notably, rats that received MitoQ without being irradiated showed a normal histological appearance (Fig. 5C, D). In MitoQ-pretreated rats, most of the histopathological lesions observed in the testes were reverted, and many seminiferous tubules exhibited a nearly normal appearance. The spermatogenic cells appeared regularly arranged within the seminiferous tubules. Similarly, a normal tubular lumen containing spermatids was demonstrated (Fig. 6C, D).

Johnsen's scoring system was used to compare the degrees of some histological lesions between groups, and revealed that administration of MitoQ before exposure to gamma radiation significantly ameliorated the histological perturbations compared to those of irradiated rats (Table 2).

4. Discussion

To our knowledge, this is the first study demonstrating the protective potential of MitoQ against testicular toxicity induced by gamma radiation. MitoQ decreased oxidative stress in testis mitochondria, which probably led to inhibition of apoptosis and restoration of

Table 3
Effect of MitoQ on testicular damage induced decline in number of spermatogonia, spermatocytes, spermatids and leydig cells.

Number of	Control	MitoQ	IR	MitoQ + IR
Spermatogonia	154.3 ± 3.9	155.8 ± 5.0$^{\#}$	102.1 ± 4.4*	136.1 ± 3.1$^{*\ \#}$
Spermatocytes	572.8 ± 9.4	587.3 ± 3.7$^{\#}$	341.3 ± 16.4*	461.6 ± 8.7$^{*\ \#}$
Spermatids	885.1 ± 11.6	894.1 ± 3.8$^{\#}$	563.8 ± 15.9*	778.5 ± 11.4$^{*\ \#}$
Leydig cells	70.6 ± 3.4	72.8 ± 1.3$^{\#}$	52.8 ± 1.1*	63.5 ± 1.4$^{\#}$

Values are expressed as the mean ± SEM of total cell count in six sections per group. The total cell count enumerated from ten different fields (×400 magnification). Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$; *vs the control group, # vs the irradiated group.

steroidogenesis, effects that were emphasized by the histopathological examination.

In this study, treatment with MitoQ significantly diminished mitochondrial ROS generation in testicular tissue, as evidenced by the elevation of mitochondrial SOD, GPx and GSH. Inevitably, ROS have been regarded as a crucial culprit of testicular malfunction [37]. MitoQ accumulates more than a hundred-fold within cell mitochondria relative to the cytoplasm [35]. Therefore, MitoQ exerts its rechargeable antioxidant action in testis mitochondria, the major cellular source of ROS. The rechargeable antioxidant effect of MitoQ is due to its reduction to ubiquinol (Coenzyme Q10) by complex II in the inner mitochondrial membrane. Thereafter, this molecule is oxidized to ubiquinone, which is reduced by complex II in turn to regenerate ubiquinol. The active antioxidant ubiquinol protects mitochondria by scavenging free radicals through accepting electrons [33]. Notably, mitochondria-targeted antioxidants, such as MitoQ, are more efficient in diminishing oxidative stress than a large cohort of cytosolic antioxidants, including vitamin E [35].

MitoQ replenished mitochondrial SOD, which is important for dampening superoxide anions and subsequent lipid peroxidation [14]. Furthermore, MitoQ increased mitochondrial glutathiones, endogenous antioxidant that scavenges free radicals and moderates cellular redox status [18]. GSH level is essential for spermatogenesis because Sertoli cells convert glutathione to amino acids that are required in spermatogenesis [24]. Likewise, GPx has multiple functions and represents the central linkage between selenium and sperm [18]. A previous study indicated that MitoQ prevented the inhibition of SOD and glutathiones resulting from free radicals in cardiac tissue [41].

Notably, MitoQ not only prevented mitochondrial oxidative stress but also conserved its main role in energy production, as MitoQ-pretreated rats showed elevated ATP levels. The increase of ATP is incontrovertibly vital for mitochondrial function. This finding is consistant with the results of Vergeade and his colleagues, who reported that MitoQ alleviated ATP reduction induced by cocaine in the left ventricle [41].

Consistent with its antioxidant effects, MitoQ exerted strong anti-apoptotic properties as revealed by down-regulation of pro-apoptotic Bax along with up-regulation of the anti-apoptotic Bcl-2 gene. Meanwhile, this molecule ameliorated the cytochrome-c and caspase-3 levels, providing another justification for its radioprotective effect demonstrated herein. Bax is a cytosolic protein that can modify its conformation, incorporates into the outer mitochondrial membrane and oligomerizes. These oligomers can increase mitochondrial membrane permeability [6] causing cytochrome-c release, which prompts apoptotic caspase activation [20]. Conversely, the anti-apoptotic Bcl-2 can reverse the effects of Bax by inhibiting its activation [12]. Caspase-3 is one of the main downstream executioner caspases that has a pivotal role in the proteolytic cleavage of several proteins produced in cell death. MitoQ was shown to prevent caspase-dependent neuronal death [29].

In the current study, MitoQ elevated the testosterone level and upregulated the expression of StAR, 3β-HSD and 17β-HSD, which are proteins necessary for steroidogenesis. StAR enhances cholesterol transfer from the outer to the inner membrane of the mitochondrion [22], 3β-HSD produces Δ4-androstenedione from dehydroepiandrosterone, and 17β-HSD produces testosterone from Δ4-androstenedione [17]. Gamma radiation disrupts Leydig cell function by generating ROS that lead to a decrease in testosterone production, which ultimately causes disruption of spermatogenesis, depletion of spermatozoa, and testicular atrophy [2,19]. The results denoted that MitoQ conferred remarkable protection against the decrease in serum testosterone concentration and up-regulated the expression of StAR, 3β-HSD and 17β-HSD, most likely due to the anti-apoptotic and antioxidant effects reported herein. Therefore, MitoQ efficiently ameliorates steroidogenesis via the protection of mitochondria.

MitoQ's impact on steroidogenesis incorporated with the improvement of sperm motility and the reduction of sperm abnormalities. Sperm motility is an evident sign of fertilizing capacity. A solid linear relationship exists between decreased sperm motility and excessive ROS [27]. In addition, gamma radiation-induced ROS increases sperm abnormalities because of the high susceptibility of sperm DNA to denaturation [5]. MitoQ substantially attenuated sperm motility and abnormalities possibly due to its ability to diminish mitochondrial oxidative stress and apoptosis. The increase in testicular weight produced by MitoQ administration could be a reasonable consequence of its effects on steroidogenesis and sperm features. The testis weight unquestionably relies on the masses of differentiated spermatogonia, spermatocytes and spermatozoa that are regulated by testosterone hormone [11].

5. Conclusion

This study identifies a radioprotective effect of MitoQ on gamma radiation-induced testicular injury which could be associated with antioxidant/anti-apoptotic effects that at least in part reduce mitochondrial dysfunction, oxidative stress and steroidogenesis arrest. In addition, our study sheds light on the pivotal role of mitochondria in radiation protection against male infertility.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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