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Radio-protective effects of melatonin therapy against testicular oxidative stress: a systematic review and meta-analysis of rodent models

Niloofar Dehdari Ebrahimi, MD, Alireza Sadeghi, MD, MPH*, Kimia Falamarzi, MD, Mohammad Amin Shahlaee, MD, Negar Azarpira, MD*

Background: Radiation exposure is a concern in today's world, given the widespread use of electronic devices and medical procedures involving ionizing and non-ionizing radiation. Radiations may cause male infertility by inducing oxidative stress in testicular tissue. Melatonin has antioxidant properties.

Methods: The authors systematically reviewed the literature for the studies that have investigated the effects of melatonin therapy on radiation-induced oxidative stress in rodents' testicular tissue. PubMed, Scopus, and Web of Science were searched for relevant animal trials. Standardized mean difference and 95% CIs were used to pool the data. Subgroup and sensitivity analyses were done. The risk of bias was assessed using SYRCLE tool.

Results: Outcomes: histopathology and sperm analyses (testicular apoptotic cells, Johnsen's testicular biopsy score, seminiferous epithelial height, tubular diameter, sperm motility, viability, count, and morphology, concentration of spermatid, spermatocyte, and spermatogonia), body and testes weights (absolute and relative body and testicular weights), reproductive hormones (serum prolactin, FSH, and testosterone), and oxidative stress tissue markers (TBARS, CAT, GSH, GSH-Px, MDA, SOD, and XO, and total antioxidant capacity). Rats and mice were exposed to electromagnetic radiations (gamma, roentgen, microwave, radiofrequency, and high-power line energy) and particle waves (radioiodine and carbon-ion). Melatonin therapy was significantly associated with improved male reproduction.

Conclusion: Radiation exposure harms male fertility, but melatonin, as an antioxidant, is potentially associated with improved male reproductive function in rodents. Inconsistencies in research require further investigations.

Keywords: male infertility, melatonin, oxidative stress, radiation, sperm, testicular tissue

Background

We are surrounded by various types of radiations, which can be divided into ionizing and non-ionizing radiations (IR and non-IR)[1]. IR, including α, β, γ, UV, and X radiations, are extensively utilized in medical practice for diagnostic and therapeutic applications like medical imaging and cancer-related radiotherapy, affecting both patients and healthcare providers. IR may cause severe tissue damage directly by breaking the DNA strands or indirectly by inducing oxidative stress, eventually resulting in genomic instability, cell dysfunction, and death[2,3]. Non-IR are characterized into extra-low frequency (ELF) and radiofrequency[4–6]. Mobile phones, laptops, microwave ovens, and Wi-Fi, which have become a substantial part of everyday life, are the sources of non-ionizing radiation[7]. Although non-IR are not as harmful as IR, long-term exposure to non-IR might lead to detrimental effects on different organs[5]. Non-IR may cause changes in the structure of temperature-sensitive proteins due to their thermal effects, produce reactive oxygen species (ROS), and induce oxidative stress due to their non-thermal effects on various tissues, including testis[5,7,8].

Testis is one of the most radiosensitive organs because of its high proliferation activity[1]. Various in-vitro and in-vivo studies

HIGHLIGHTS

- Radiation exposure causes male infertility via oxidative stress in testicular tissue.
- Melatonin has antioxidant properties and mitigates radiation-induced male infertility.
- Melatonin improved rat reproduction after radiation.
- The studies had significant methodological and statistical heterogeneity and quality.
- More research is needed on melatonin's effect on testicular oxidative stress.

Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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**Corresponding author. Address: Shiraz University of Medical Sciences, Shiraz 7193635899, Iran Islamic Republic of Iran. Tel.:+98 917 624 9615. E-mail: alireza.sadeghi.md@gmail.com (A. Sadeghi); Research Tower, Shiraz University of Medical Sciences, Shiraz 7193635899, Iran Tel.: +98 917 317 6294 E-mail: negarazarpira@yahoo.com (N. Azarpira).*

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have demonstrated the hazardous effects of radiation on spermatogenesis, sperm parameters (such as count, motility, and morphology), male reproductive hormones, testis histological structure, and antioxidant system of testicular tissue and ultimately result in impaired male fertility which its increasing rate is a major issue worldwide[1,5,7,9,10]. Radiations induce testicular damage by decreasing antioxidant capacity, stimulating oxida tive stress, inflammation, and apoptosis; hence discovering radio protector agents is important to ameliorate radiation-induced testicular injuries[11,12].

Melatonin, a neurohormone mainly rhythmically secreted from the pineal gland, regulates circadian rhythm, immune responses, and reproductive functions[13,14]. Also, melatonin is a potent antioxidant, reduces oxidative stress by scavenging free radicals, inducing anti-oxidative enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and inactivating pro-oxidant enzymes[3,11,15–17]. Due to its amphipathic nature, melatonin can cross biological membranes and barriers like the blood-testis barrier and exerts its effects via activating melatonin receptors MT1 and MT2 (which are mem bers of G-protein coupled receptors superfamily)[11,18]. Melatonin receptors are present in the male reproductive tract, including the testes, epididymis, and seminal vesicles, and also detected in all types of cells in the testes, such as germ cells, Leydig and Sertoli cells[19,20]. Accordingly, melatonin is a potential protector of testicular tissue from radiation-induced injuries via its anti oxidant activities, repairing DNA damage, inhibiting germ cell apoptosis, preserving sperm qualities, and regulating testosterone synthesis[14,21,22].

We previously demonstrated melatonin's beneficial effects on rodent testis damaged by physical injuries, environmental pollutants, metabolic disorders, and anti-cancer agents[4,23–25]. Radio-protective roles of melatonin have been investigated in different organs (including the liver, brain, skin, and lung)[26–30]. Nevertheless, evidence of melatonin's radio-protective effects in testicular tissue is scattered. Therefore, in the current study, the protective roles of melatonin on rodents' irradiated testes were reviewed systematically.

Material and methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, Supplemental Digital Content 10, http://links.lww.com/MS9/A624) statement[31] to conduct this systematic review and meta-analysis, which is part of a series of systematic reviews that examine how melatonin protects rodent testes from oxidative stress[4,23–25]. The protocol was registered in the International prospective register of sys tematic reviews (PROSPERO) with registration code (CRD42023411200).

Data sources and search

We searched three databases (PubMed, Scopus, and Web of Science) for relevant studies. Two reviewers (N.D.E. and A.S.) looked for relative records. We also checked the references of the included studies for additional citations. We did not limit our search by language, and our search strategy is in Supplementary material 1, Supplemental Digital Content 1, http://links.lww.com/MS9/A615.

Study selection and eligibility criteria

Following the removal of duplicate records, two independent reviewers (N.D.E. and A.S.) screened the remaining records by titles and abstracts using the Rayyan online tool for managing systematic reviews[32]. Full-text screening of the relevant records was conducted to meet the eligibility criteria, and any discrepancies were resolved through discussions. Studies that satisfied the following requirements were included: (1) animal trials, (2) administrated melatonin-based regimens to at least one intervention group, (3) rodents exposed to IR and non-IR (such as electromagnetics and beta-rays) resulting in oxidative stress to testicular tissue, (4) reported hallmarks of testicular tissue (such as sperm, histopathologic, and biochemical analyses), and (5) at least one control group with comparable stress.

Any trials conducted on non-rodent animals or humans were ruled out. The same applied to studies that involved exposure to stressors other than radiation, like physical trauma, chemotherapy, toxins, heavy metals, and metabolic disorders. In-vitro and ex-vivo studies were also not considered. Moreover, the review only looked at studies where melatonin was used alone as a treatment; trials involving a combination of melatonin with other drugs were deemed unsuitable. Studies that failed to report relevant outcomes were also excluded, as were trials that used healthy controls without radiation-induced stress. Finally, treatments that involved derivatives of melatonin were not included in the review.

Data extraction and risk-of-bias assessment

The data included (1) study characteristics (first author and publication year), (2) rodent characteristics (age, species, radiation type, and sample size), (3) melatonin (dose, treatment duration, timing, and administration route), and (4) tissue, plasma, and histopathological indices.

We used the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool for animal intervention studies[33] to assess the risk of bias in the studies. Two reviewers (N.D.E. and M.A.S.) independently rated each article as high, low, or unclear for each bias domain. They discussed disagreements and consulted a third reviewer (A.S.) if needed.

Data synthesis and statistical analysis

We employed Stata MP Version 16 (StataCorp) to conduct meta-analyses using the random DerSimonian–Laird model. The effect measure used was standardized mean difference (SMD, Cohen's d), and statistical significance was set at a P value of less than 0.05.

To assess between-study heterogeneity, I^2 and P values were examined. An I^2 value of less than 25% indicated low heterogeneity, while values between 25 and 50% suggested moderate heterogeneity. Heterogeneity greater than 50% was considered substantial[34]. In cases where data were missing, we reached out to the authors via e-mail and waited for at least one month for responses. If the missing data were essential, we excluded the study from the analysis. To prevent overcalculations, we combined intervention arms for studies with common control arms using Cochrane's formula[35].

We conducted subgroup analyses when at least two separate study arms were available for each subgroup to explore potential sources of heterogeneity. Additionally, sensitivity analyses were performed using the leave-one-out method to evaluate the

robustness of our results. Due to the limited number of studies, it was not feasible to assess for publication bias as per Cochrane's guidelines[35].

Results

General characteristics of the included studies

Figure 1 displays the PRISMA flow diagram that outlines the literature search process. A comprehensive overview of each study's specific attributes is provided in Table 1, while supplementary information detailing the strategy employed for inducing stress and administering melatonin can be found in an accompanying Supplementary material 2, Supplemental Digital Content 2, http://links.lww.com/MS9/A616.

Outcomes

A total of 25 outcomes were extracted and analyzed. These outcomes were categorized into four groups: histopathology and sperm analyzes [testicular apoptotic cells, Johnsen's testicular biopsy score (JTBS), seminiferous epithelial height and tubular diameter, sperm viability, count, motility, and abnormal morphology, and concentration of spermatid, spermatocyte, and spermatogonia], body and testes weights (absolute and relative body and testicular weights), reproductive hormones [serum prolactin, follicle-stimulating hormone (FSH), and testosterone levels], and tissue markers of oxidative stress [thiobarbituric acid reactive substances (TBARS), CAT, Glutathione (GSH), GSH-Px, Malondialdehyde (MDA), SOD, and Xanthine oxidase (XO) activity, and total antioxidant capacity]. The pooled estimates of effect sizes for each outcome are gathered into Figure 2. The detailed forest plot for each outcome is presented in Supplementary materials 3-6, Supplemental Digital Content 3, http://links.lww.com/MS9/A617, Supplemental Digital Content 4, http://links.lww.com/MS9/A618, Supplemental Digital Content 5, http://links.lww.com/MS9/A619, Supplemental Digital Content 6, http://links.lww.com/MS9/A620.

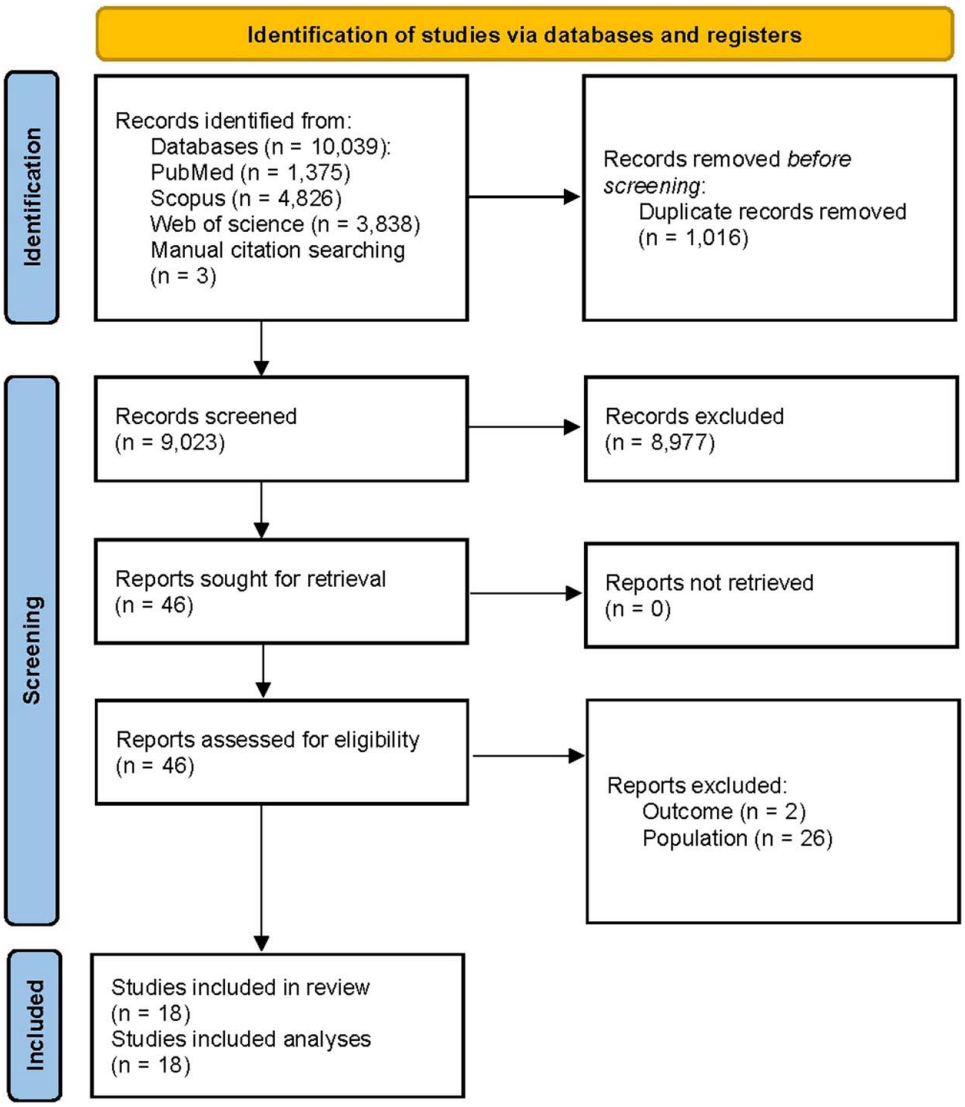

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram illustrating the process of selection of the studies.

Table 1

General characteristics of the included studies.

First author [year]	Rodent	Age	Melatonin/control size	Radiation	Type of radiation
Cebi sen [2018]	Rat	NA	12/12	Beta (radioiodine)	Ionizing radiation
Shokri *et al.* [2020]	Mouse	NA	8/8	Electromagnetic (not specified)	Non-ionizing radiation
Sokolovic [2015]	Rat	6–8 weeks	21/21	Electromagnetic (microwave)	Non-ionizing radiation
Tajabadi [2020]	Mouse	6–8 weeks	5/5	Electromagnetic (gamma)	Ionizing radiation
Yalcinkaya [2009]	Rat	10–12 weeks	10/10	Electromagnetic (gamma)	Ionizing radiation
Hussein [2006]	Rat	NA	20/20	Electromagnetic (roentgen)	Ionizing radiation
Khan *et al.* [2015]	Mouse	8–9 weeks	15/15	Electromagnetic (gamma)	Ionizing radiation
Kushwaha [2021]	Mouse	8–10 weeks	6/6	Electromagnetic (gamma)	Ionizing radiation
Liu [2009]	Mouse	8–10 weeks	24/8	Beta (carbon-ion)	Ionizing radiation
Oksay [2012]	Rat	16 weks	8/8	Electromagnetic (not specified)	Non-ionizing radiation
Meena [2013]	Rat	10 weeks	6/6	Electromagnetic (microwave)	Non-ionizing radiation
Ozmen [2021]	Rat	16–24 weeks	16/16	Electromagnetic (by high-power lines)	Non-ionizing radiation
Panday and Giri [2018]	Mouse	10–12 weeks	5/5	Electromagnetic (radiofrequency)	Non-ionizing radiation
Kamal El-Dein and Anees [2020]	Rat	NA	6/6	Electromagnetic (gamma)	Ionizing radiation
Tawfik [2006]	Mouse	7–9 weeks	18/6	Electromagnetic (gamma)	Ionizing radiation
Hussein [2006]	Rat	3 months	12/12	Electromagnetic (roentgen)	Ionizing radiation
Amer *et al.* [2022]	Rat	8 weeks	10/10	Electromagnetic (gamma)	Ionizing radiation
Seyman [2020]	Rat	NA	6/6	Electromagnetic (radiofrequency)	Non-ionizing radiation

A list of included studies' citations is available in the Supplementary material 2, Supplemental Digital Content 2, http://links.lww.com/MS9/A616.
NA, not available.

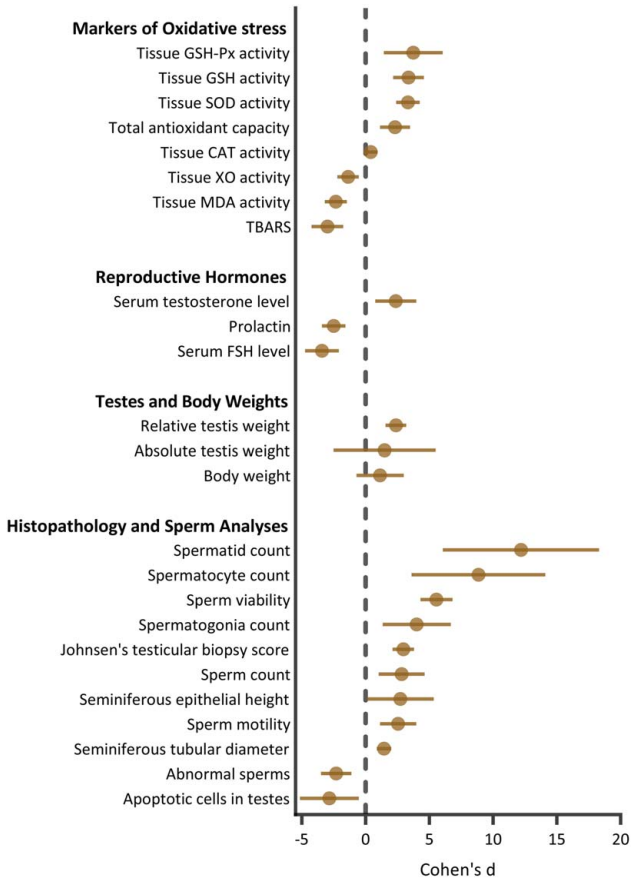

Figure 2. Summary of the effect sizes for each outcome. Effect sizes are provided in tissue markers of oxidative stress, reproductive hormones, body and testis weights, and histopathology and sperm parameters. CAT, catalase; FSH, follicle-stimulating hormone; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; XO, xanthine oxidase.

According to our analysis, melatonin therapy was significantly associated with improved histopathology and sperm parameters. The pooled SMDs are as follows: testicular apoptotic cells (SMD = 2.83, 95% CI: 1.03–4.63, $P < 0.01$), JTBS (SMD = 2.96, 95% CI: 2.12–3.80, $P < 0.01$), seminiferous epithelial height (SMD = 2.73, 95% CI: 0.12–5.34, P 0.04) and tubular diameter (SMD = 1.44, 95% CI: 0.87–2.01, $P < 0.01$), sperm count (SMD = 2.83, 95% CI: 1.03–4.63, $P < 0.01$), motility (SMD = 2.55, 95% CI: 1.13–3.97, $P < 0.01$), viability (SMD = 5.56, 95% CI: 4.3–6.82, $P < 0.01$), and abnormal morphology (SMD = –2.30, 95% CI: –3.49 to –1.11, $P < 0.01$), and concentration of spermatid (SMD = 12.19, 95% CI: 6.06–18.32, $P < 0.01$), spermatocyte (SMD = 8.85, 95% CI: 3.60–14.10, $P < 0.01$), and spermatogonia (SMD = 4.01, 95% CI: 1.33–6.69, $P < 0.01$).

Reproductive hormones

Three outcomes were extracted and classified as reproductive hormones, all of which were significantly associated with melatonin therapy. The pooled SMDs for each outcome are as follows: serum prolactin (SMD = –2.51, 95% CI: –3.43 to –1.58, $P < 0.01$), FSH (SMD = –3.42, 95% CI: –4.74 to –2.10, $P < 0.01$), and testosterone levels (SMD = 2.37, 95% CI: 0.76–3.97, $P < 0.01$).

Markers of oxidative stress

Except for tissue CAT activity, the association of melatonin therapy with these outcomes was statistically significant: TBARS (SMD = –3, 95% CI: –4.24 to –1.75, $P < 0.01$), CAT (SMD = 0.4, 95% CI: –0.14 to 0.93, P 0.15), GSH (SMD = 3.37, 95% CI: 2.16–4.57, $P < 0.01$), GSH-Px (SMD = 3.74, 95% CI: 1.44–6.04, $P < 0.01$), MDA (SMD = –2.33, 95% CI: –3.20 to –1.47, $P < 0.01$), SOD (SMD = 3.32, 95% CI: 2.39–4.24, $P < 0.01$), and XO activity (SMD = –1.38, 95% CI: –2.22 to –0.53, $P < 0.01$), and total antioxidant capacity (SMD = 2.31, 95% CI: 1.13–3.49, $P < 0.01$).

Testes and body weights

Relative testis to body weight was significantly higher in melatonin-therapy arms (SMD = 2.38, 95% CI: 1.57–3.19, $P < 0.01$). However, absolute values of testes (SMD = 1.49, 95% CI: −2.51 to 5.49, P 0.47) and body weight (SMD = 1.14, 95% CI: −0.71 to 2.99, P 0.23) were not significantly associated with melatonin regimen.

Subgroup analyses

Subgroup analyses were done to explore the sources of between-study variability and explore the relationship between the effect sizes and moderators. By grouping based on the study characteristics, subgroup analyses revealed some between-group differences which may help explain some of the observed between-study heterogeneity. The significant between-group differences include difference between IRs and non-IRs in sperm motility and count ($P < 0.001$ and 0.047, respectively), timing of intervention (relative to injury) in sperm count and serum FSH and testosterone levels (P 0.003, 0.015, and < 0.001, respectively), showing higher effects of melatonin when administered after the induction of injury. Also, the results were significantly different in rats and mice regarding abnormal sperm morphology (P 0.009). The detailed results of subgroup analyses are presented in Table 2.

Sensitivity analyses

To examine the robustness of our results and the impact of different levels of heterogeneity on the them, sensitivity analyses were done utilizing leave-one-out method. Regarding P values, all of the outcomes with sufficient number of contributing studies rejected the null hypothesis, showing robustness of the results. The results of sensitivity analyses are presented in Supplementary material 7, Supplemental Digital Content 7, http://links.lww.com/MS9/A621.

Risk-of-bias assessment

Overall results of risk-of-bias assessment are presented in Supplementary material 8, Supplemental Digital Content 8, http://links.lww.com/MS9/A622, showing the low quality of studies, which is a result of a blurred process of design, performance, and reporting. The detailed results of the risk-of-bias assessment are presented in Supplementary material 9, Supplemental Digital Content 9, http://links.lww.com/MS9/A623.

Discussion

The results from this systematic review and meta-analysis highlight the significant roles of melatonin in preserving male rodents' reproductive systems following radiation exposure. According to our results, melatonin induced anti-oxidative enzymes, improved spermatogenesis, sperm morphology, and function, maintained testicular tissue structure, and increased testosterone levels. To the best of our knowledge, this is the first systematic review and meta-analysis performed on the protective effects of melatonin on rodents' irradiated testes, and the outcomes are discussed thoroughly.

Effects of melatonin on oxidative stress

Although ROS production is essential for mammalian sperms and regulation of capacitation, binding to oocytes, acrosomal reaction, and epididymal maturation, excessive ROS generation cause reproductive tissue damage and impaired male fertility[36]. Melatonin is a natural antioxidant and a potent free radical scavenger, and its metabolites also have free radical scavenging properties[37]. Due to its special structure, melatonin is considered to be a sub stantially efficient antioxidant, removing four reactive species by only one molecule[38]. It has been reported that melatonin alle viates oxidative stress damage via activating the SIRT1/Nrf2 sig naling pathway that leads to Nrf2 upregulation and consequently increases antioxidant enzymes' expression[39]. Inflammation, mostly caused by ionizing radiation, and oxidative stress are clo sely related and one may simply induce the other[11,40]. Melatonin is also capable of decreasing inflammation by regulating the expression of nuclear factor kappa B (NF-κB) and mitogen-acti vated protein kinases, production of pro- and anti-inflammatory cytokines, modulating COX2 expression in macrophages, and interacting with the NLRP3 inflammasome; therefore, leading to an increase in antioxidant enzymes (like CAT and SOD) and reduction in lipid peroxidation in testicular tissues[11,40].

Results from our study demonstrated that melatonin administration increased testicular total antioxidant capacity and antioxidant enzymes activities, including GSH, GSH-PX, and SOD, while the decreased activity of XO (a pro-oxidative enzyme), TBARS, and MDA (indicators of lipid peroxidation) in rodents irradiated testicular tissue. These results were consistent with our previous meta-analyses, which reported the protective roles of melatonin against oxidative stress[4,23–25].

Our results showed an increase in CAT activity after melatonin administration, although it was not statistically significant. This result is consistent with the two prior meta-analyses conducted by Morvaridzadeh and colleagues and Sumsuzzman and colleagues, which reported no changes in CAT activity after melatonin intake; however, previous meta-analyses revealed increased CAT activity in rodents' testicular tissue following melatonin administration[24,25,41,42]. These inconsistencies might be due to the limited number of studies included, different types of species, variations in dose and duration of melatonin administration, and types of toxic agents that induce oxidative stress[37]. Hence, fur ther investigations are required to examine the precise effects of melatonin on oxidative stress and anti-oxidative enzymes and also to determine the optimal dose of melatonin in humans.

Effects of melatonin on testis and sperm parameters

Radiation exposure is associated with a reduction in spermatogenic cells, sperm concentration, motility, and viability, as well as an increase in apoptotic cells and abnormal sperms[1,11]. Also, alterations in testicular tissue, such as the decrease in seminiferous tubular diameter and germinal epithelial thickness, were detected due to irradiation[1,22]. Results of the current study reveal the beneficial roles of melatonin on sperm parameters and histo pathology of irradiated testes, including increased spermatogen esis, sperm count, motility, viability, seminiferous diameter, and epithelial height as well as a decrease in abnormal sperms and apoptosis.

In addition to its antioxidant and anti-inflammatory properties, melatonin has anti-apoptotic features through inhibiting pro-apoptotic markers including p53, p21, cytochrome C, Bax, caspase-3 and

Table 2

Subgroup analyses.

Outcome	Subgroup		No. effect sizes	SMD [95% CI]	I^2 (%)	*P**
Tissue XO activity	Overall		4	−1.38 [−2.22, −0.53]	47.65	
	Melatonin cumulative dose	< 85 mg/kg	2	−0.81 [−1.71, 0.09]	24.13	0.066
		> 85 mg/kg	2	−2.04 [−2.99, −1.08]	—	
Tissue MDA activity	Overall		7	−2.33 [−3.20, −1.47]	70.17	
	Type of radiation	Ionizing	3	−2.74 [−4.40, −1.07]	82.63	0.51
		Non-ionizing	4	−2.07 [−3.15, −0.99]	62.78	
	Melatonin cumulative dose	≤ 80 mg/kg	4	−2.00 [−3.01, −0.99]	63.78	0.423
		> 80 mg/kg	3	−2.82 [−4.54, −1.09]	79.83	
	Rodent	Mouse	3	−1.81 [−2.41, −1.22]	—	0.242
		Rat	4	−2.95 [−4.76, −1.14]	83.58	
Tissue GSH activity	Overall		8	3.37 [2.16, 4.57]	77.21	
	Type of radiation	Ionizing	5	2.52 [1.36, 3.68]	69.10	0.079
		Non-ionizing	3	4.98 [2.50, 7.47]	72.71	
	Melatonin cumulative dose	< 127.5 mg/kg	4	2.65 [1.35, 3.95]	69.65	0.155
		> 127.5 mg/kg	4	4.79 [2.14, 7.44]	84.62	
	Route of melatonin administration	IP	4	2.66 [1.62, 3.69]	62.40	0.176
		Oral	4	5.06 [1.74, 8.38]	86.46	
	Timing of melatonin administration	After injury	2	5.12 [2.36, 7.87]	52.24	0.144
		Before injury	6	2.87 [1.65, 4.10]	76.77	
	Rodent	Mouse	4	3.38 [1.57, 5.19]	82.41	0.916
		Rat	4	3.52 [1.58, 5.47]	77.06	
TBARS	Overall		7	−3.00 [−4.24, −1.75]	66.00	
	Type of radiation	Ionizing	4	−2.72 [−4.66, −0.77]	68.21	0.584
		Non-ionizing	3	−3.38 [−4.74, −2.01]	45.03	
	Melatonin cumulative dose	≤ 100 mg/kg	5	−2.54 [−3.94, −1.15]	60.27	0.244
		> 100 mg/kg	2	−3.92 [−5.76, −2.08]	47.67	
	Route of melatonin administration	IP	5	−3.53 [−4.74, −2.32]	34.63	0.168
		Oral	2	−1.81 [−3.94, 0.32]	74.46	
	Rodent	Mouse	4	−3.23 [−4.38, −2.07]	—	0.646
		Rat	3	−2.64 [−4.85, −0.43]	84.09	
Sperm viability	Overall		6	5.56 [4.30, 6.82]	15.98	
	Radiation cumulative dose	< 5 Gy	2	7.59 [5.08, 10.10]	—	0.111
		≥ 5 Gy	3	4.35 [2.62, 6.08]	—	
	Melatonin cumulative dose	< 108 mg/kg	4	5.20 [3.23, 7.17]	33.66	0.505
		≥ 108 mg/kg	2	6.07 [4.47, 7.66]	—	
	Route of melatonin administration	IP	4	5.02 [3.76, 6.27]	—	0.072
		Oral	2	7.59 [5.08, 10.10]	—	
	Timing of melatonin administration	After injury	2	7.59 [5.08, 10.10]	—	0.072
		Before injury	4	5.02 [3.76, 6.27]	—	
	Rodent	Mouse	3	4.35 [2.62, 6.08]	—	0.082
		Rat	3	6.37 [4.90, 7.84]	—	
Sperm motility	Overall		10	2.55 [1.13, 3.97]	87.66	
	Type of radiation	Ionizing	8	3.41 [1.95, 4.87]	78.77	**< 0.001**
		Non-ionizing	2	−0.49 [−1.20, 0.22]	—	
	Radiation cumulative dose	< 5 Gy	3	5.62 [0.74, 10.49]	90.27	0.275
		≥ 5 Gy	4	2.22 [0.95, 3.49]	38.99	
	Melatonin cumulative dose	≤ 104 mg/kg	5	2.90 [1.37, 4.43]	61.66	0.594
		> 104 mg/kg	5	2.18 [0.02, 4.34]	92.56	
	Route of melatonin administration	IP	7	1.79 [0.28, 3.30]	87.29	0.139
		Oral	3	5.64 [0.77, 10.50]	89.56	
	Timing of melatonin administration	After injury	2	8.33 [1.69, 14.97]	79.33	0.055
		Before injury	8	1.71 [0.42, 3.00]	85.31	
	Rodent	Mouse	4	2.18 [0.92, 3.44]	43.60	0.640
		Rat	6	2.78 [0.58, 4.98]	92.23	
Sperm count	Overall		9	2.83 [1.03, 4.63]	92.71	
	Type of radiation	Ionizing	6	3.88 [1.85, 5.91]	89.39	**0.047**
		Non-ionizing	3	0.68 [−1.74, 3.11]	90.01	
	Radiation cumulative dose	≤ 4 Gy	4	3.81 [1.75, 5.87]	78.49	0.143
		> 4 Gy	2	3.77 [−1.93, 9.46]	96.96	
	Melatonin cumulative dose	< 175 mg/kg	5	3.57 [1.38, 5.75]	90.61	0.332
		≥ 175 mg/kg	4	1.85 [−0.83, 4.54]	91.88	

Table 2

(*Continued*)

Outcome	Subgroup		No. effect sizes	SMD [95% CI]	I^2 (%)	*P**
	Route of melatonin administration	IP	5	1.81 [−0.60, 4.21]	95.01	0.199
		Oral	4	4.39 [1.26, 7.53]	86.78	
	Timing of melatonin administration	After injury	2	6.36 [4.19, 8.54]	—	**0.003**
		Before injury	7	2.01 [0.15, 3.86]	93.22	
	Rodent	Mouse	3	2.61 [1.31, 3.91]	52.59	0.842
		Rat	6	2.91 [0.25, 5.57]	94.91	
Serum testosterone level	Overall		7	2.37 [0.76, 3.97]	89.29	
	Type of radiation	Ionizing	5	1.98 [0.16, 3.80]	0.033	0.271
		Non-ionizing	2	3.18 [2.06, 4.29]	—	
	Melatonin cumulative dose	≤90 mg/kg	4	3.05 [1.33, 4.78]	0.001	0.193
		>90 mg/kg	3	1.23 [−0.92, 3.37]	0.262	
	Route of melatonin administration	IP	4	1.33 [−0.46, 3.12]	0.146	0.180
		Oral	3	4.34 [0.32, 8.37]	0.034	
	Timing of melatonin administration	After injury	2	6.26 [4.13, 8.39]	—	**<0.001**
		Before injury	5	1.25 [−0.16, 2.66]	0.082	
	Rodent	Mouse	2	1.59 [−1.68, 4.86]	0.341	0.532
		Rat	5	2.86 [0.57, 5.15]	0.014	
Serum FSH level	Overall		5	−3.42 [−4.74, −2.10]	68.73	
	Melatonin cumulative dose	≤108 mg/kg	3	−3.19 [−5.00, −1.38]	73.83	0.605
		>108 mg/kg	2	−4.27 [−7.94, −0.60]	80.16	
	Route of melatonin administration	IP	2	−3.15 [−4.19, −2.11]	27.00	0.585
		Oral	3	−4.04 [−7.07, −1.01]	82.16	
	Timing of melatonin administration	After injury	2	−5.42 [−7.33, −3.51]	—	**0.015**
		Before injury	3	−2.65 [−3.82, −1.49]	60.32	
Seminiferous tubular diameter	Overall		4	1.44 [0.87, 2.01]	—	
	Route of melatonin administration	IP	2	1.40 [0.71, 2.09]	—	0.833
		Oral	2	1.53 [0.53, 2.53]	—	
	Timing of melatonin administration	After injury	2	1.53 [0.53, 2.53]	—	0.833
		Before injury	2	1.40 [0.71, 2.09]	—	
Abnormal sperms	Overall		12	−2.30 [−3.49, −1.11]	84.34	
	Type of radiation	Ionizing	9	−2.86 [−3.87, −1.86]	61.83	0.058
		Non-ionizing	3	−0.54 [−2.73, 1.65]	89.04	
	Radiation cumulative dose	<5 Gy	4	−2.95 [−4.76, −1.13]	80.72	0.188
		≥5 Gy	5	−2.67 [−3.78, −1.56]	25.52	
	Melatonin cumulative dose	≤100 mg/kg	7	−2.26 [−3.09, −1.44]	28.29	0.847
		>100 mg/kg	5	−2.02 [−4.37, 0.33]	92.73	
	Route of melatonin administration	IP	8	−2.40 [−4.17, −0.62]	88.80	0.817
		Oral	4	−2.16 [−3.05, −1.28]	22.26	
	Timing of melatonin administration	After injury	2	−1.89 [−3.53, −0.24]	55.83	0.635
		Before injury	10	−2.42 [−3.85, −0.98]	86.70	
	Rodent	Mouse	7	−3.39 [−4.58, −2.20]	55.90	**0.009**
		Rat	5	−0.91 [−2.35, 0.53]	84.17	

FSH, follicle-stimulating hormone; GSH, glutathione; Gy, Gray; IP, intraperitoneal; MDA, malondialdehyde; SMD, standardized mean difference; TBARS, thiobarbituric acid reactive substances; XO, xanthine oxidase.

*P value for between-group differences.

Statistical significance ($P < 0.05$) values are in bold.

caspase-9 as well as inducing anti-apoptotic Bcl-x proteins[38,43]. As mentioned earlier, melatonin increases SIRT1 expression, which leads to the inhibition of p53 de-acetylation and suppressing oxidative stress-induced apoptosis[44]. Furthermore, previous investigations revealed that melatonin exerts its anti-apoptotic effects on male germ cells via inhibiting JNK and p38 MAPK signaling pathways as well as regulating the histone H3K9me3 level, which eventually contributes to spermatogenic regeneration and spermatogonial stem cells protection[45,46].

While our results showed no significant changes in spermatocytes count, previous investigations reported an increase in spermatocyte count[37,47]. This inconsistency might be due to the limited number of studies included. Melatonin also improves sperm motility by regulating the mitochondrial electron transport chain, stabilizing its inner membrane, and modulating ATP production[21,47]. By protecting sperms against apoptotic pathways, activating mitochondrial respiration, and enhancing ATP synthesis, melatonin increases sperm motility and concentration[48,49]. Moreover, melatonin's interactions with calmodulin, an intracel lular regulator of Ca2+, and enhancing Ca2+ influx into sperm cells are associated with increased sperm motility and velocity[50].

Our analysis demonstrated that melatonin does not affect body and absolute testis weight, but it increases relative testes weight. Notably, there are inconsistencies regarding melatonin effects on body and testes weight among previous studies as well since some experiments, including our previous meta-analysis. Therefore, more investigations are needed to elucidate the mentioned controversial results.

Effects of melatonin on reproductive hormone profile

Testosterone, a vital hormone for spermatogenesis, structure, and function of seminiferous tubules, decreases after radiation exposure and consequently leads to deleterious effects on male fertility[1,11]. Current study results reveal that melatonin partially recovers testosterone secretion in rodents after irradiation, which is congruent with other studies[11,51–53]. Melatonin maintained androgen receptors (AR) expression, which was down-regulated in irradiated rat testes[11]. ARs is substantial for Leydig and Sertoli cells functions and testosterone also exerts its effects through ARs[11]. Melatonin improved leydig cell functions by increasing mitochondria numbers and secretory granules resulting in raised testosterone levels[44,54]. Additionally, melatonin upregulates GATA binding factor 4 (GATA-4) expression and induce testos terone production by Leydig cells[44,54]. Although there are some reports indicating that exogenous melatonin prohibited testos terone secretion, these discrepancies might arise from the dose and duration of melatonin administration, which disrupted endogenous melatonin's normal circadian rhythm[14]. Moreover, our results showed a decrease in FSH levels after melatonin intake. Since high serum FSH levels appear to be associated with low sperm counts and germinal epithelium damage, we can come to the conclusion that melatonin's beneficial effects on sperma togenesis and germinal epithelium preservation may result in low FSH levels[55–57].

Increased prolactin level is one of the reversible causes of male infertility since males with azoospermia and oligospermia demonstrated considerably increased levels of prolactin[58,59]. Hyperprolactinemia might lead to decreased FSH, LH, and testosterone levels, defects in spermatogenesis, and impaired male fertility, probably due to prolactin's inhibitory effects on GnRH pulsatile secretion[60,61]. Our results showed that melatonin decreased prolactin concentrations and consequently resulted in enhanced male reproductive function.

As presented in Table 2, subgroup analyses fail to show the effect of cumulative melatonin dosage and timing of melatonin administration in most of the outcomes. However, it should be noted that sperm count and serum testosterone and FSH levels had greater improvements when melatonin was administered after the induction of stress. Although not conclusive, these findings may indicate that melatonin therapy cannot be used as a preventive measure. Also, failure to detect significant differences between different cumulative melatonin doses in subgroup analyses can indicate that the studies usually recruited more than optimal dosages. However, these findings are inconclusive and further studies, especially designed to measure the dose-response relationship, are recommended.

Strengths and limitations

This study represents the first systematic review and meta-analysis examining the protective effects of melatonin against testicular tissue injury caused by exposure to IR and non-IR in rodents. We present strong evidence that may guide future research. Nonetheless, we emphasize caution when drawing conclusions, as the results may not be generalizable to larger animals or humans due to the study's focus on rodents. Despite our attempts to explore sources of heterogeneity, some instances of high residual heterogeneity remained, which can impact the conclusions, given that the studies included in the analysis exhibited significant methodological differences. We recommend future research to recruit unified methodologies, such as dose-response measurements, timing of administration, and radiation stressors. Additionally, it is crucial to pay attention to the robustness of each outcome and the fact that most studies had a high risk of bias when interpreting the results. Finally, it is important to note that none of the studies included in this analysis investigated adverse events related to melatonin therapy, which should be considered when evaluating its effectiveness. Since melatonin is already used extensively for insomnia treatment, there is adequate information regarding its safety and lack of life-threatening events[40,62,63]. However, further investigations and trials are required to investigate its safety and potential side effects while utilized for male irradiated testes and reproductive systems.

Conclusion

IR and non-IR exposure causes harmful effects on male fertility by affecting testicular tissue, sperm parameters, and reproductive hormones. The present study indicates that melatonin, as a potent antioxidant and free radical scavenger, has favorable effects on spermatogenesis, sperm quality, testicular histopathological structure, and hormone profiles. Further investigations are necessary to determine the exact roles of melatonin on irradiated testes, assess its benefits on humans and discover its potential adverse effects. In this regard, unified methodologies are recommended to decrease the heterogeneity between studies. Also, considering the safety of melatonin in human physiology and the fact that it is being used routinely, the authors recommend future observational and experimental studies with human subjects to be conducted.

Ethical approval

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Consent

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Author contribution

N.A. and N.D.E. conceptualized the study. A.S. and N.D.E. designed the study and screened the records. N.D.E. and M.A.S. the data. A.S. performed the analyses. Visualizations by A.S. A.S. and K.F. provided the primary manuscript. A.S. and N.D.E. contributed equally to this work and share the first authorship. All authors contributed to the article and approved the final version.

Conflicts of interest disclosure

All of the authors declare that they have no competing interest.

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Data availability statement

Our study was based on the findings of research included in our literature review. The data used in this study are available in supplementary material.

Provenance and peer review

Not invited.

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