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Radioprotective Effects of Melatonin on Gamma Radiation- Induced Tissue Damage in Rats

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Abstract

Background & Objective: Gamma rays are widely used in medicine despite their harmful effects on health. Our study evaluated the protective effects of melatonin on kidney, heart, and liver tissues.
Materials & Methods: Seventy-two adult male Wistar rats were categorized into nine groups. Groups 2 and 3 only received whole-body γ-ray irradiation (WBI) (2.0 Gy), no melatonin, interval time (IT) 8hr and 24hr. Groups 4 and 5 received WBI (8.0 Gy, no melatonin, 8hr and 24hr). Groups 6 and 7 received melatonin at 60 minutes (min) before WBI (2.0 Gy). Groups 8 and 9 received melatonin before WBI (8.0 Gy). All the rats were sacrificed 8 or 24 h after the experiments for laboratory and histopathological analysis. Serum levels of Blood Urea Nitrogen (BUN), Creatinine (Cr), Lactate dehydrogenase (LDH), Potassium (K), Gamma-glutamyl Transferase (GGT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic-Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), c-reactive protein (CRP), Troponine (TPO) and histological features of liver, heart and kidney tissues were assessed. Statistical analysis was performed by One-Way ANOVA.
Results: Our data did not indicate significant differences in BUN, Cr, K, and CRP between groups with or without melatonin treatment ($P>0.05$); but differences were significant for LDH, GGT, SGOT, SGPT, ALP, and TPO ($P<0.05$). The results showed that radiation-induced histopathological effects on the liver, heart, and kidneys were mitigated in the groups six to nine.
Conclusion: The existence of significant differences in serum levels of LDH, GGT, SGOT, SGPT, ALP, and TPO in groups (6 to 9) and amelioration of the histopathological effects of irradiation on the liver, heart, and kidneys in the groups six to nine showed that the melatonin (100 mg/kg) is able to protect the body in gamma-radiation (2.0 Gy and 8.0 Gy).

Keywords: Melatonin, Oxidative stress, Whole-Body Irradiation, Histology

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Introduction

Antioxidants and the enzymes involved in their production are the natural lines of defense against the overproduction of free radicals and cellular oxidative stress (1). Nowadays, Gamma (γ) rays are widely used in medicine, industry, and other technological applications despite their harmful effects on vital human tissues (2). In addition to direct damage to cells, especially germline cells, gamma irradiation indirectly causes irreparable damage to vital tissues (such as the heart and the liver) via inducing free radicals' production (3, 4). The destructive effects of radiation probably remain for several years in the body (5).

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The increased production of free radicals after gamma irradiation (2) causes damage to cellular membranes, nucleotides in deoxyribonucleic acid (DNA), critical sulfhydryl bonds in proteins, etc. (6). Gamma irradiation in doses higher than 2 gray (Gy) is the most lethal factor to mammalians' bone marrow cells (7). Some organs, such as the liver, are involved in the body's antioxidant defense. However, the antioxidant functions of the liver, such as glutathione (GSH) production, may not suffice to fight overproduced free radicals, requiring the administration of external sources of antioxidants such as melatonin to protect vital organs against irradiation.

The peptide hormone melatonin is secreted by the pineal gland and plays an important role in the regulation of the sleep-wake cycle. This hormone regulates the immune system and reduces free radicals' production. As age increases, the production of melatonin decreases (8–11). In vitro studies showed that the antioxidant properties of melatonin are more than that of vitamin C (ascorbic acid), and the hormone seems to be more effective in protecting vital organs against the blood oxidants induced by gamma irradiation (12, 13). It's recognized that cardiac damage in diabetics that is related to high serum levels of antioxidants and low function of the reduced-enzymes, ameliorates when melatonin is administered with vitamins C and E (14–16).

The amount, time, and duration of taking antioxidants are thought to be the determinants of their protective effects on many tissues such as muscles, against oxidative stress. However, excessive amounts of antioxidants (such as vitamin E) may lead to organ dysfunction, such as heart attack. As an explanation, some studies have shown that oxidants play a role in signal transduction in muscles, so antioxidants can disrupt the transmission of cellular messages and subsequently cellular functions (14). In this study, we investigated the protective effects of melatonin against the acute complications (i.e., 8 and 24 hours) of 2.0 Gy and 8.0 Gy-gamma radiation in rats.

Material and Methods

Our study was approved by the Ethics Committee of Zabol University of Medical Sciences, Zabol, Iran. All ethical principles of working with laboratory animals were carried out, including maintenance of the animals in special cages with adequate and dry space (suitable temperature, adequate water, and food). Our project has used the proficiency of co-authors expert in working with laboratory animals and in Clinical Psychology. To minimize the harassment of animals, only one injection was used.

Animals

Seventy-two adult male Wistar rats (mean ± standard deviation (SD) of weight: 190±20 g, mean ±SD of age: 68±2 days) were obtained from the animal house of Zabol University of Medical Sciences. The rats were kept in special cages at a temperature of 20-22°C and a 12-hour dark/light cycle. The diet of the animals contained a standard formulation without any additives. The rats were divided into nine groups of eight animals each (Table 1).

Irradiation and Melatonin Treatment

The selection of 60 minutes (min) interval between melatonin injection and irradiation, as well as melatonin concentrations and the doses of γ-ray were based on previous experiences from the studies performed in our laboratory (17). The rats were exposed to sublethal (2.0 Gy) and lethal (8.0 Gy) WBI emitted from a source with a distance of 100 cm from the skin at the fixed field size of 35 cm^2 at room temperature (20 ± 2°C) using a 6 mega electron volt (MeV) γ-ray linear accelerator machine (Elekta Compact 6 MV, China). Before irradiation, to ensure the output of the accelerator, dosimetry and calibration were performed using an ionizing chamber based on the International Atomic Energy Agency (IAEA) TRS-398 standard (17, 18). The length of time in melatonin treatment (8 and 24 hours) in the present

study, was according to Take et al. (2009).

In the control group, rats did not receive melatonin (100 mg/kg) or irradiation. In the experimental groups of 2 (no melatonin, with 2.0 Gy and 8 hr), 3 (no melatonin, with 2.0 Gy and 24 hr), 4 (no melatonin, with 8.0 Gy and 8 hr) and 5 (no melatonin, with 8.0 Gy and 24 hr), rats were evaluated after 8 or 24 hr (Table 1).

In the experimental groups of 6 (with melatonin, 2.0 Gy and 8 hr), 7 (with melatonin, 2.0 Gy and 24 hr),8 (with melatonin, 8.0 Gy and 8 hr) and 9 (with melatonin, 8.0 Gy and 24 hr), the rats received 100 mg/kg melatonin (dissolved in 500 µL of phosphate buffer saline=PBS) intraperitoneally 60 min before radiation (12, 18). Then, they were evaluated after 8 or 24 hr (Table 1).

Ketamine (87 mg/kg) and xylazine (13 mg/kg) were used for anesthetizing the rats. Due to the low solubility of melatonin in water and its high solubility in fat, it was initially dissolved in 25 µL of absolute ethanol and then diluted in 475 µL of PBS for injection.

Table 1. Experimental Groups, Melatonin Administration, Radiation Doses, and Interval Times Used in This Study hours.

Group No.	Melatonin administration	Gamma Radiation	Time to measuring outcomes
1	No	None	8 hr
2	No	2.0 Gy	8 hr
3	No	2.0 Gy	24 hr
4	No	8.0 Gy	8 hr
5	No	8.0 Gy	24 hr
6	Yes	2.0 Gy	8 hr
7	Yes	2.0 Gy	24 hr
8	Yes	8.0 Gy	8 hr
9	Yes	8.0 Gy	24 hr

* 2.0 Gy = 2 Gray Gamma Radiation, 8.0 Gy= 8 Gray Gamma Radiation, 8 hr= 8 hours, 24 hr = 24 hours

Biochemical Analyses

In this study, serum levels of Blood Urea Nitrogen (BUN), Creatinine (Cr), Gamma-Glutamyl Transferase (GGT), Glutamic Oxaloacetic Transferase (SGOT), Glutamic Pyruvic Transferase (SGPT) and Alkaline Phosphatase (ALP) detected using the Sphera-Autoanalyzer-Set with diagnostic kits of Pars Azmoon Co. Serum level of Lactate Dehydrogenase (LDH) was evaluated by LDH-manual kit of Pars Azmoon Co. on the basis of photometric method with Eppendorf Ecom-E 6125 Set. Mouse C-Reactive Protein (CRP) ELISA Kit (Cat:80634) and Microlyte Reader-Audicom AC-9800 Model was used for Potassium (K) measurement. Troponin I (TPO) serum level was also measured using Chemiluminescence Assay.

Pathological Analyses

The rats' tissue blocks (kidney, heart, and liver) were prepared for histological examination. The tissues were cut into 5-µm-thick sections via a microtome and stained by Hematoxylin and Eosin (H &E). The staining process includes the following steps: Xylene steps (15 min), Ethanol 96% (3 min), Ethanol 80% (3 min), Ethanol 70% (5 min), Distilled Water (DW), Hematoxylin (15 min), 1% alcohol acid (prepare 70% alcohol and pour 1 cc of concentrated hydrochloric acid), 1% saturated lithium carbonate, Eosin (4 min), 70% alcohol (2 min), alcohol 80% (2 min), alcohol 96% (2 min) Xylene (15 min). The slides were assessed by an optical microscope equipped with Olysia software (19).

Statistical Analysis

Statistical analysis of data was performed in SPSS 24 software using one-way analysis of variance (One-Way ANOVA) based on the scheffe test. The P value of <0.05 was considered to designate a statistically significant difference.

Melatonin Effects on Serum Levels of BUN and Cr

There was no significant difference in the serum levels of BUN (Chart 1) and Cr (Chart 2) between the experimental groups and the control group ($P>0.05$).

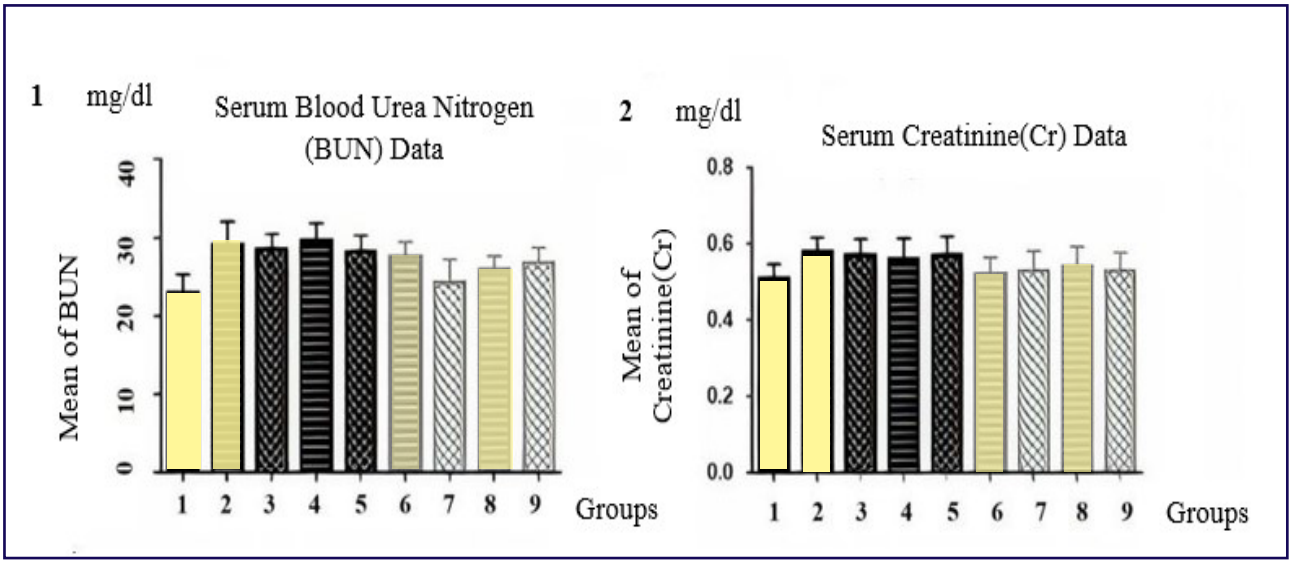


Chart 1. Increases in the mean±SD of the BUN serum levels were insignificant between the experimental groups (2, 3, 4 and 5) compared to the control group. In addition, there was an increase in the mean±SD of the BUN serum levels of groups (6, 7, 8 and 9) compared to the control group, which was insignificant ($P>0.05$). **Chart 2.** There was an insignificant decrease in the mean±SD of the Cr serum levels in groups 6 to 9 compare with groups 2 to 5, but it was an insignificant increase compared to the control group ($P>0.05$). But in experimental groups (2, 3, 4 and 5), there was an increase in the mean±SD of the Cr serum levels compared to the control group, which were not significant ($P>0.05$)

Melatonin Effects on Serum LDH Level

Mean±SD serum level of LDH showed a significant difference comparing the second (2449.8±494.4), fourth (3433.0±681.25), and fifth (3192.0±471.02) groups with the control group (504.28±79.23) (P<0.05). Likewise, there was a significant difference comparing the fifth group with the sixth (966.8±198.8), seventh (977.0±317.2), and eighth groups (1515.1±190.5) (P<0.05). Moreover, there was a significant difference comparing the fourth group with the sixth and seventh groups (P<0.05, Chart 3).

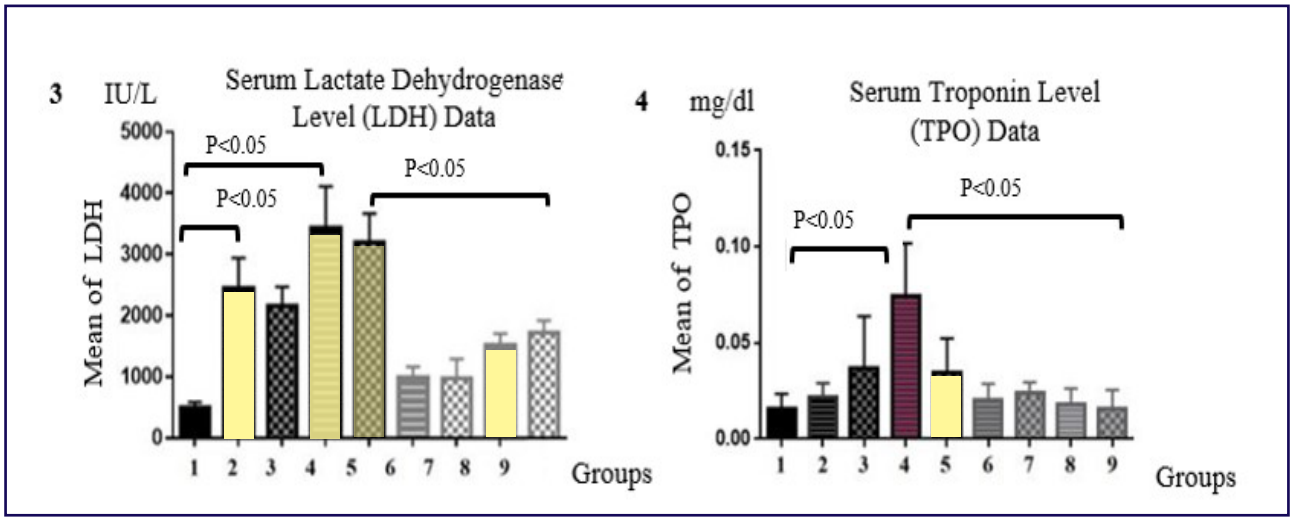


Chart 3. The mean±SD of serum LDH levels in the experimental groups were significantly different from those in the control group (P<0.05). **Chart 4.** The mean±SD of serum TPO levels showed a significant increase in the fourth group compared with the other groups (P<0.05)

Melatonin Effects on Serum TPO Level

There was a significant increase in Mean±SD serum TPO level in the fourth group (0.074±0.027) compared with the other groups, except for the fifth group (0.034±0.018) (P<0.05, Chart 4).

Melatonin Effects on Serum Potassium(K) Level

Data analysis indicated that serum K levels slightly and insignificantly decreased in the sixth (4.82±0.28) and seventh (4.74±0.33) groups compared with the control (4.9±0.73) group (P>0.05, Chart 5).

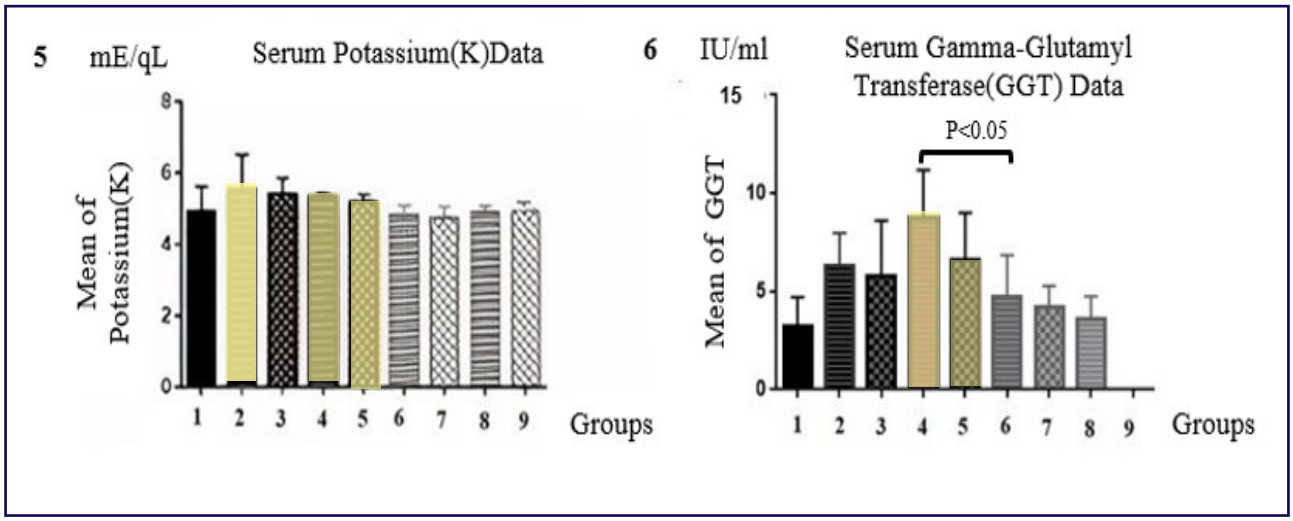


Chart 5. The mean±SD of serum K levels did not differ significantly between the experimental groups and the control group (P>0.05). **Chart 6.** The mean±SD of serum GGT values did not differ significantly between the experimental groups and the control group, but there was a significant difference between the fourth and sixth groups (P<0.05)

Melatonin Effects on Serum GGT Level

According to data analysis, Mean±SD of GGT serum level showed a significant difference between the fourth group (8.80±2.38) and the sixth group (4.71±2.13) (P<0.05, Chart 6).

Melatonin Effects on Serum SGOT Level

There was a significant increase in Mean±SD serum SGOT level in the fourth (262.6±12.92) and fifth (236.8±37.08) groups compared with the control group (125.0±13.73) (P<0.05). In addition, there was a significant difference between the third (148.28±13.28), sixth (143.75±15.15), seventh (134.0±14.02), and ninth (131.0±11.04) groups compared with the fourth group (P<0.05). Moreover, there was a significant difference between the ninth and the fifth groups (P<0.05, Chart 7).

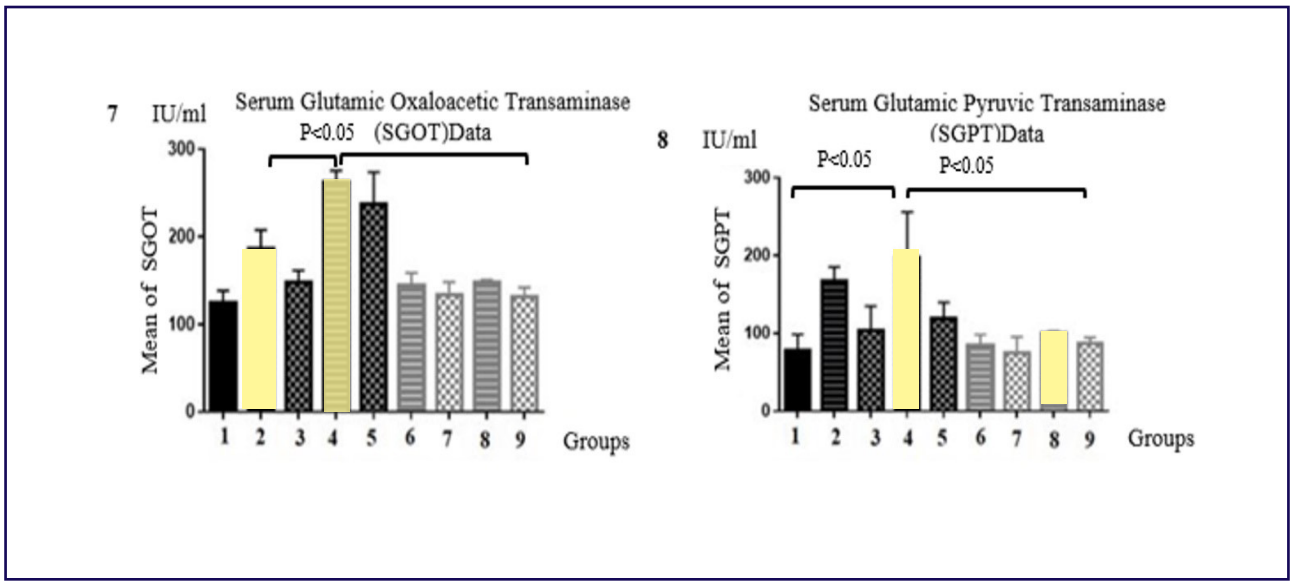


Chart 7. The mean±SD of serum SGOT concentration was significantly different between the third, sixth, seventh, and ninth groups with the fourth group (P < 0.05). **Chart 8.**The mean±SD of serum SGPT concentration showed a significant difference between the study groups (P<0.05)

Melatonin Effects on Serum SGPT Level

There was a significant difference in the mean serum levels of SGPT in the fourth group (197.80±57.72) compared with the control group (77.57±21.01). There was also a significant difference between the second (167.60±17.40) and third groups (103.28±31.57) (P < 0.05). Moreover, there were significant differences comparing the fourth group with the third and the fifth (118.80±21.09) with ninth (86.83±7.73) groups (P<0.05). There were also significant differences comparing the second group with the sixth (84.60±13.81), seventh (75.14±20.30), and ninth groups (P<0.05, Chart 8).

Melatonin Effects on Serum ALP Level

According to our results, Mean±SD of ALP serum level showed a significant difference between the fourth group (59.0±10.02) and the other groups (P<0.05). In addition, there was a significant difference comparing the fifth group (48.0±7.40) with the seventh (15.40±1.63), eighth (13.85±1.50), and ninth (14.0±3.21) groups (P<0.05, Chart 9).

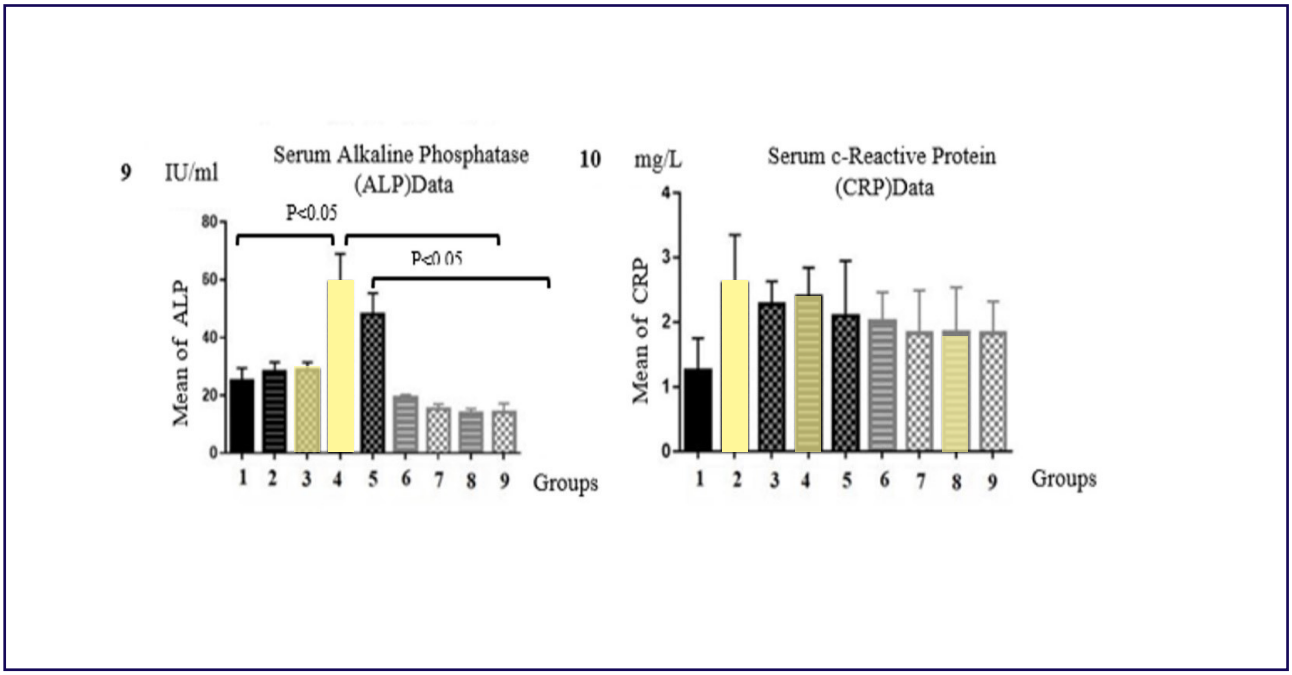


Chart9. The mean±SD of serum concentration ALP showed significant differences between some of the study groups (P<0.05).
Chart 10. The mean±SD of serum CRP concentration showed no significant difference between the experimental groups and the control group (P<0.05)

Melatonin Effects on Serum CRP Level

There was an insignificant increase in Mean±SD serum CRP level in all the experimental groups compared with the control group (P>0.05, Chart 10).

Melatonin Effects on Pathological Changes in the Kidney, Heart, and Liver

Based on our observations and histological analyses, the most significant histological lesions were observed in the fifth group (i.e., gamma radiation exposure at the dose of 8.0 Gy within 24 hr intervals without melatonin treatment). In contrast, histopathological analysis of the tissues obtained from groups 6 to 9 (i.e., gamma irradiation+treatment with melatonin) showed mild lesions compared to the groups without melatonin treatment.

Kidney histological analysis in the fifth group showed proliferative lesions in glomeruli, as well as infiltration by mononuclear cells. An increase was also observed in the partial wall thickness of the Bowman capsule. In contrast, mild cellular atrophy and inflammatory cell infiltration was observed in group 9 (Figure 1A and B).

The histological analysis of the heart in the fifth group showed hypertrophy, nuclei enlargement, and a moderate level of fibrosis in muscle fibers (Figure 2A). In contrast, a decrease in the thickness of cardiac fibers was also observed in group 9 (Figure 2B).

Liver histopathological examination in the fifth group revealed hepatocyte swelling (ballooning), hepatocyte ablation, and pyknotic transformation of nuclei (Figure 3A). In contrast, occasional pyknotic nuclei and mild hepatocyte ballooning was observed in those treated with melatonin (group 9)(Figure 3B).

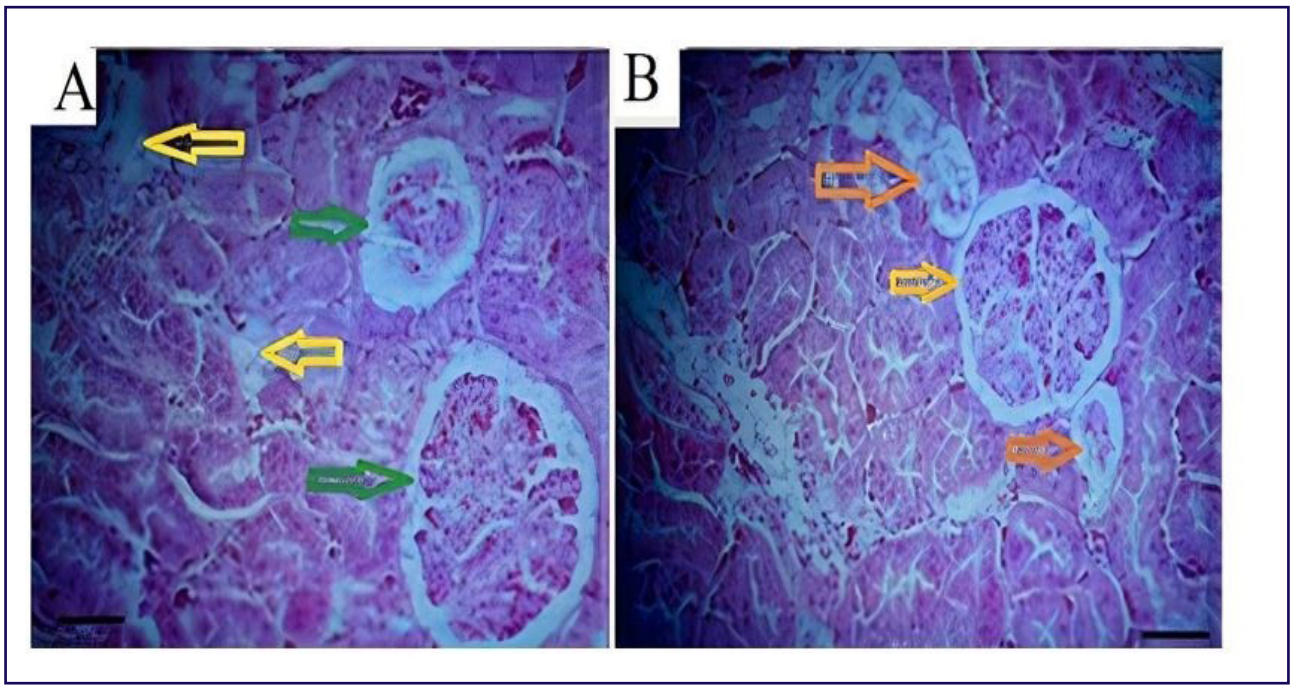


Figure 1. Histopathological analysis of the rat's kidneys after gamma-irradiation exposure(8.0 Gy), **A**). Cell atrophy and infiltration (yellow arrows), enlarged urine spaces (green arrows) in the untreated melatonin group compared to **B**) treated with melatonin (magnification 400X)

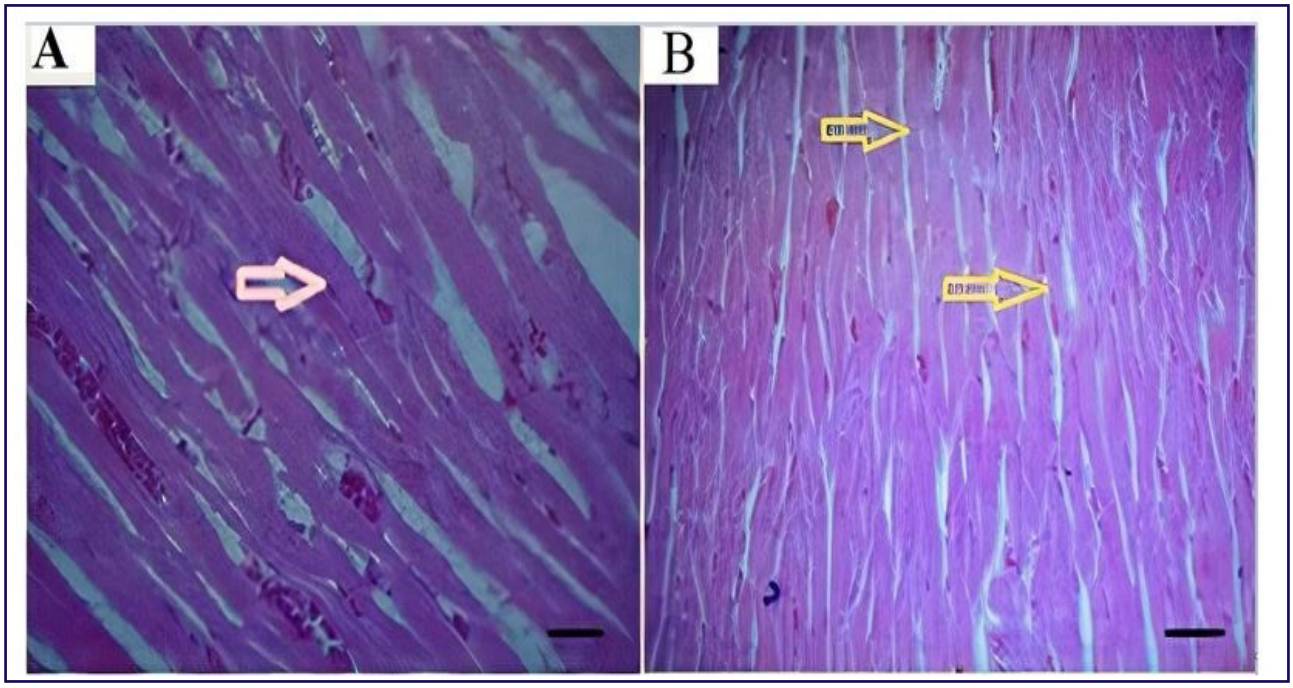


Figure 2. Histopathological analysis of the rat's hearts after gamma-irradiation exposure (8.0 Gy), **A**) Thickening of heart fibers without treatment with melatonin (white arrow) compared with **B**) a decrease in the thickness of heart fibers treated with melatonin (yellow arrows)(magnification 400X)

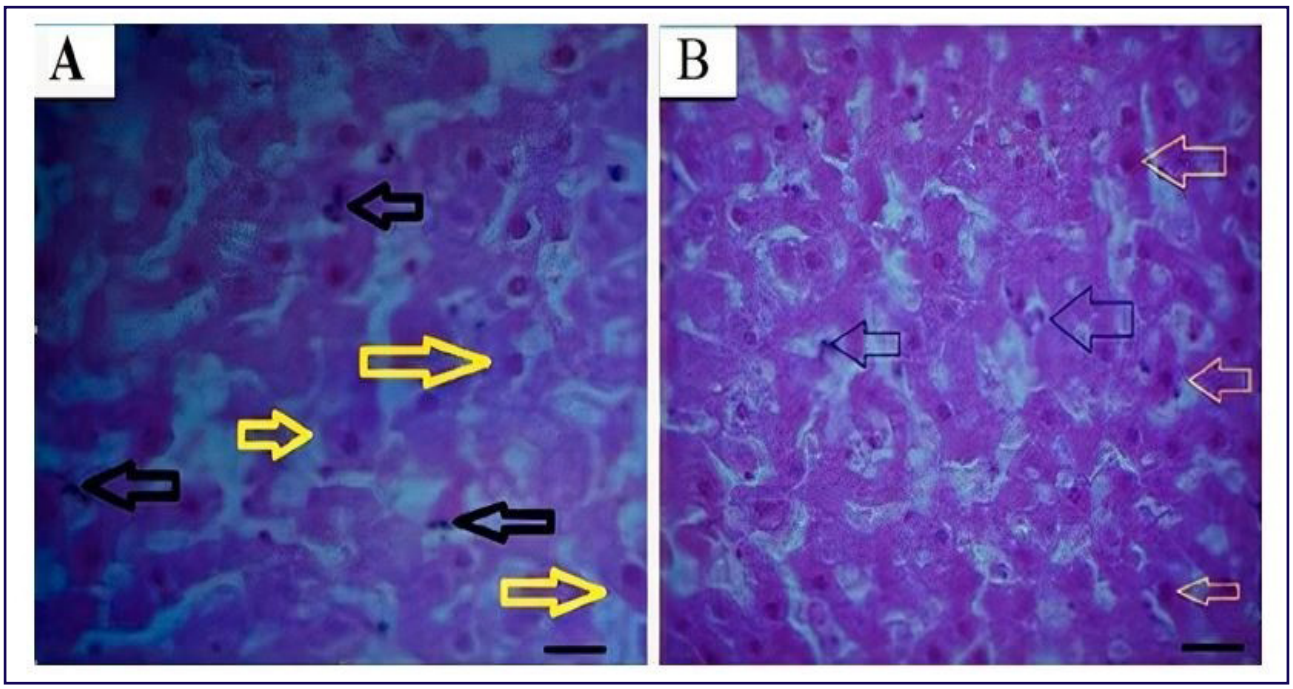


Figure 3. Histopathological analysis of the rat's livers after gamma-irradiation exposure (8.0 Gy), **A**) pyknotic nuclei and cellular ballooning in hepatocytes (bold black and yellow arrows) not treated with melatonin compared to **B**) occasional pyknotic nuclei and mild ballooning of hepatocytes treated with melatonin (black and yellow arrows)(magnification 400X)

Discussion

In our study, we investigated the protective effects of melatonin against whole body γ-irradiation (2.0 Gy and 8.0 Gy) in rats. Melatonin was injected intraperitoneally, and the rats were evaluated at 8 and 24 hr after irradiation. In a similar study, Koc et al. in 2003 examined the effects of WBI (6.0 Gy) on rats' liver function. The result of Gamma-irradiation was an increase in the serum level of MDA, and a decrease in GPx function (20).

Regarding serum BUN and Cr levels, our findings showed that the majority of the study groups without melatonin treatment had an insignificant increase compared with the control group (P>0.05). Also, Soliman et al. in 2019 examined the nephrotoxicity effects of 8.0 Gy γ-irradiation on rats and reported significant increases in Cr and BUN (21). The increasing serum levels of BUN and Cr indicate renal dysfunction (22). Ali et al. in 2020 reported that WBI (6.5 Gy) in rats decreased kidney size and induced renal dysfunction evidenced by serum Cr elevation (23).

We observed an insignificant decrease in the serum levels of BUN and Cr in the sixth to ninth groups (i.e., with melatonin treatment) compared with groups two to five (P>0.05). El-Missiry et al. in 2007 showed that the intraperitoneal injection of melatonin (10 mg/kg) four days prior to WBI (2.0 Gy and 4.0 Gy) decreased serum levels of BUN and Cr (24). In addition to the irradiation dose (2.0 Gy or 8.0 Gy) in this study, the time of exposure is also important (8 or 24 hr). We noticed a decrease in the serum levels of BUN and Cr in the groups receiving no melatonin after 24 hr compared with 8 hr in the groups treated with 2.0 Gy γ-radiation (P>0.05). The same occurrence is also seen in group 5 (8.0 Gy, 24 hr) as compared to group 4 (8.0 Gy, 8hr) (P>0.05).

The choice of 24-hour and 8-hour measurements is based on the study by Take et al., 2009, who reported apoptosis-reduction after intraperitoneal injection of melatonin (10 mg/kg) with 24 hr interval after γ-irradiation (8.0 Gy) (25). Erol et al. (2004) and Canyilmaz et al. (2016) also reported that intraperitoneal injection of melatonin (100 mg/kg) with intervals

(five days and 30 min) prior to irradiation respectively, had the protective effects on mouse kidneys and brain. Tubular atrophy and diffuse intertubular fibrosis appeared in pathological assessments (13, 26). Pathological findings in the fifth group were glomerular proliferative lesions, infiltration of mononuclear cells, and increase in the Bowman's capsule thickness. Gu et al. (2020) also observed the same pathological changes in the mice intestinal tissue after total body gamma (6.0, 7.0 and 8.0 Gy) irradiation (TBI) that caused weight loss, reduction in food intake and severe damage to intestinal villi (27). Pathological assessment of the ninth group (with melatonin, 8.0 Gy, 24 hr) showed mild cellular atrophy, low cellular infiltration, and urinary space expansion that was in line with Canyilmaz et al.'s findings.

In addition, an assessment on the serum level of K ion showed that although there were no significant differences between the groups, there was a mild increment in the majority of the groups receiving no melatonin compared with the control group. Moreover, our findings showed an insignificant decrease in serum K in the sixth, seventh, and eighth groups (with melatonin) compared with the control group ($P>0.05$). Hyperkalemia is considered a risk factor for heart arrest (28). Kaločayová et al. in 2019 showed that 25 Gy γ-irradiation in rats caused dysfunction of the Na^+/K^+ adenosine triphosphatase (ATPase) channel and imbalanced serum levels of these ions (29). Lanz et al. (2009) reported that the rats exposed to 3.0 Gy γ-irradiation had decreased levels of urine K^+ (30), which may be due to the dysfunction of the Na^+/K^+ ATPase channel after γ-irradiation (29). Some previous studies have also revealed that whole body irradiation promotes K^+ leakage from erythrocytes, boosting serum levels of this ion (28).

Regarding the serum level of LDH, there was a significant increase in the second to fifth groups compared with the control group, supporting the idea that irradiation has a destructive effect on tissues such as the heart, liver, as well as on erythrocytes and other cells ($P<0.05$). Moreover, our pathological findings showed hypertrophy, nuclei enlargement, and a moderate level of fibrosis in cardiac muscle fibers in the fifth group. Similarly, Freitas et al. (2013) showed that whole body irradiation in mice (a single dose of 6.0 Gy) caused heart damage and increased LDH serum level (31). In the same direction, Unthank et al. in 2015 evaluated the effects of TBI-gamma-irradiation (at a dose close to 8.0 Gy) in mouse models and observed an increase in fibrosis and collagen deposition (32).

In our study, a continuous decrease in serum LDH levels was seen in the sixth to the ninth groups (with melatonin treatment) compared to the control group ($P<0.05$). Our pathological findings in the groups six to nine exhibited a decrease in cardiac fiber thickeness.

This is important as LDH along with troponin can be the indicators of cardiac muscle damage (33). According to many studies, the main origin of LDH is the reticuloendothelial system (34); therefore, the increased concentration of serum LDH in the groups 1 to 5 compared with the control group probably indicates the irradiation-induced damage of the reticuloendothelial tissue. In a similar study, Freitas et al. showed that whole body irradiation by a single dose of 6.0 Gy in rats for 24 hr increased the serum level of LDH to 600 U/L (31).

Our findings also showed that irradiation elevated TPO serum level in the majority of the experimental groups compared to the control group ($P<0.05$). The fourth group (without melatonin, 8.0 Gy, 24 hr) demonstrated the highest serum level of TPO ($P<0.05$). Similar to our study, Soliman et al. (2018) showed that the mean serum level of TPO was 0.22 ng/mL in irradiated mice (6.0 Gy) (35). Also, Ibrahim et al. (2017) showed that WBI (0.5 Gy) in rats increased TPO serum level (36).

In the sixth to ninth groups (with melatonin treatment), a gradual decrease in TPO serum levels was observed compared to the control group ($P<0.05$), suggesting that melatonin prevented troponin elevation in the groups with melatonin administration

Garau et al. in 2011 noted that TBI had detrimental effects on vital organs such as the liver and heart and increased the serum level of troponin (37).

Other enzymes, including GGT, AST, ALT, and ALP, also changed after WBI. In this regard, GGT (an important enzyme in the development of oxidative stress) (38) increased in the second to fifth groups compared with the control group ($P<0.05$). Interestingly, a decrease in GGT serum level was noticed in the sixth to ninth groups compared to the control ($P<0.05$). In the same direction, El-Missiry et al. in their study showed that the rats receiving two doses (2.0 Gy and 4.0 Gy) of γ-irradiation had elevated serum levels of GGT, and the administration of melatonin before irradiation ameliorated GGT serum level and activity (24). In parallel, our study highlighted that the groups receiving melatonin, except for the seventh group, had reduced serum levels of GGT compared with the control group ($P<0.05$). In addition, serum GGT level in the eighth and ninth groups markedly decreased compared with the control group after 8 and 24 hr of irradiation, respectively ($P<0.05$). The findings of El-missary et al. also indicated that melatonin administration for four days before radiation remarkably decreased GGT serum level compared with the control group (24). The SGOT (or aspartate aminotransferase (AST) and SGPT (or alanine aminotransferase (ALT) enzymes participate in the metabolism of glutamate in the liver tissue. It seems that γ-irradiation can promote its detrimental effects on the liver by intensifying the oxidative status, leading to a significant increase in the concentrations of these enzymes, which is generally accompanied by a decline in the concentration of antioxidants such as glutathione (GSH) (24). Our findings showed an increase in serum AST level in all the experimental groups compared with the control group ($P<0.05$). However, serum AST level in the sixth to ninth groups (i.e., with melatonin treatment) was remarkably lower compared with the second to fifth groups (i.e., without melatonin treatment) ($P<0.05$). In addition, ALT serum levels showed a gradual increase in the groups without melatonin treatment. Regarding the sixth to ninth groups (treated with melatonin), the reduction in ALT was more pronounced in the seventh group (2.0 Gy, 24 hr exposure, melatonin-treated) compared with the control group ($P<0.05$). Our findings showed that the administration of melatonin 8 and 24 hours before irradiation markedly prevented liver damage. El-Missiry et al. also stated that the activity of AST and ALT decreased in γ-irradiated (2.0 Gy and 4.0 Gy) rats without melatonin administration. However, melatonin treatment markedly increased AST activity compared with the control group (24).

Our findings also clarified a gradual boost in serum ALP level in the second to fifth groups, which received no melatonin. This observation was consistent with the finding of El-Missiry et al. (2007) who employed 2.0 Gy and 4.0 Gy γ-irradiation (24). We also encountered a reduction in serum ALP level in the sixth to ninth groups (with melatonin) ($P<0.05$), suggesting a protective role for melatonin in preserving the production and function of the ALP enzyme in the rats exposed to 2.0 Gy and 8.0 Gy γ-irradiation for 8 and 24 hr. In accordance with our findings, El-Missiry et al. also reported that melatonin treatment decreased ALP levels in the rats exposed to 2.0 Gy and 4.0 Gy γ-irradiation (24). In parallel with biochemical examinations, liver pathology analyses showed hepatocyte anisocytosis, blastic transformation of hepatocytes, metamorphosis, and nuclear deterioration with pycnotic transformation in the fifth group. In the melatonin-treated groups, especially in the ninth group, nuclear anisocytosis was minimal. In accordance with our research, Sayed et al. (2019) assessed the protective effects of a plant extract against the deteriorating effects of 6.0 Gy gamma TBI in mice and found mild hepatocyte swelling and narrowing of hepatic sinusoids in the mice treated with the plant extract (39).

Srinivasan et al. (2014) examined the effects of 10.0 Gy X-ray TBI on rat liver and reported hepatocyte anisocytosis, blastocyst deformation, nucleus destruction, and pyknotic nuclei (40).

Regarding serum CRP levels, we witnessed an insignificant increase in groups 1 to 5 (no melatonin) ($P>0.05$). On the other hand, an insignificant decrease was noticed in serum CRP level in the sixth to ninth groups (with melatonin) compared with the groups receiving no melatonin ($P>0.05$). In accordance with our study, Desouky et al. (2017) found that WBI significantly elevated CRP serum level in rats compared with the control group (41).

Conclusion

Our goal was to evaluate the protective effects of melatonin against γ-ray-induced tissue damage in the rats exposed to 2.0 Gy and 8.0 Gy radiation for either 8 or 24 hr. The existence of insignificant difference in serum levels of BUN, Cr, K, and CRP between groups either melatonin or without it and significant differences of LDH, GGT, SGOT, SGPT, ALP, and TPO in melatonin groups (6 to 9) showed that the melatonin is able to protect the body in gamma-radiation (2.0 Gy and 8.0 Gy). The radiation-induced histopathological effects on the liver, heart, and kidneys were also mitigated in groups six to nine. Therefore, radioprotection with dosages of 2.0 Gy and 8.0 Gy gamma radiation by melatonin (100 mg/kg) administration is possible.

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Conflict of interest

The authors declare no conflict of interest.

References

1.Farid A, El-Dewak M, Safwat G DA. Anti-apoptotic and antioxidant effects of melatonin protect spleen of whole body γ-irradiated male Sprague-dawley rats. Int J Radiat Res. 2021;19(4):861-72.
2.Liu XR, Zhu N, Hao YT, Yu XC, Li Z, Mao RX, et al. Radioprotective effect of whey hydrolysate peptides against γ-radiation-induced oxidative stress in balb/c mice. Nutrients. 2021; 13(3):1–20.
3.Hosseinimehr SJ, Zakaryaee V, Froughizadeh M. Oral oxymetholone reduces mortality induced by gamma irradiation in mice through stimulation of hematopoietic cells. Mol Cell Biochem. 2006;287(1–2):193–9.
4.Zhou Y, Mi MT. Genistein stimulates hematopoiesis and increases survival in irradiated mice. J Radiat Res. 2005;46(4):425–33.
5.Reiter RJ, Tan DX, Herman TS, Thomas CR. Jr. Melatonin as a radioprotective agent: A review. Int J Radiat Oncol Biol Phys. 2004;59(3):639–53.
6.Dennis JM WP. Protective role for antioxidants in acute kidney disease. Nutrients. 2017;9(7):718–43.
7.Katsanos KH, Briasoulis E, Tsekeris P, Batistatou A, Bai M, Tolis C, et al. Randomized phase II exploratory study of prophylactic amifostine in cancer patients who receive radical radiotherapy to the pelvis. J Exp Clin Cancer Res. 2010;29(1):1–9.
8.Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan DX, et al. Melatonin and pregnancy in the human. Reprod Toxicol. 2008;25(3):291–303.
9.Voiculescu SE, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo fetal development. J Med Life. 2014;7(4):488–92.
10.El-Missiry MA, Shabana S, Ghazala SJ, Othman AI AM. Melatonin exerts a neuroprotective effect against γ-radiation-induced brain injury in the rat through the modulation of neurotransmitters, inflammatory cytokines, oxidative stress, and apoptosis. Environ Sci Pollut Res. 2021;28:31108-21.
11.Moradpour R, Shokri M, Abedian S AF. The protective effect of melatonin on liver damage induced by mobile phone radiation in mice model. Int J Radiat Res. 2020;18(1):133-41.
12.Purushothaman A, Sheeja AA, Janardanan D. Hydroxyl radical scavenging activity of melatonin and its related indolamines. Free Radic Res. 2020;54(5):373–83.
13.Erol FS, Topsakal C, Ozveren MF, Kaplan M, Ilhan N, Ozercan IH, et al. Protective effects of melatonin and vitamin E in brain damage due to gamma radiation: An experimental study. Neurosurg Rev. 2004;27(1):65–9.
14.McGinley C, Shafat A, Donnelly AE. Does Antioxidant Vitamin Supplementation Protect against Muscle Damage? J Pineal Res. 2009;47(2):184–91.
15.Farhood B, Aliasgharzadeh A, Amini P, Saffar H, Motevaseli E, Rezapoor S, et al. Radiation-induced dual oxidase upregulation in rat heart tissues: protective effect of melatonin. Medicina (B Aires). 2019;55(7):317–27.

16.Allagui MS, Feriani A, Bouoni Z, Alimi H, Murat JC, El Feki A. Protective effects of vitamins (C and E) and melatonin co-administration on hematological and hepatic functions and oxidative stress in alloxan-induced diabetic rats. J Physiol Biochem. 2014;70(3):713–23.
17.Valizadeh M, Shirazi A, Izadi P, Bazzaz JT, Rezaeejam H, GTabesh A. Effects of melatonin on repair of DNA double strand breaks caused by ionizing radiation in rat peripheral blood. Biosci Biotechnol Res Commun. 2016;9(4):821–7.
18.Rezaeejam H, Shirazi A, Izadi P, Bazzaz JT, Ghazi-Khansari M, Valizadeh M, et al. Radioprotective effect of melatonin on expression of Cdkn1a and Rad50 genes in rat peripheral blood. J cancer Res Ther. 2018;14(12):1070–5.
19.Jelodar G, Khaksar Z PM. Endocrine profile and testicular histomorphometry at puberty in rat offspring from diabetic mothers. Comp Clin Path. 2010;19(2):135–9.
20.Koc M, Taysi S, Buyukokuroglu ME, Bakan N. Melatonin protects rat liver against irradiation-induced oxidative injury. J Radiat Res. 2003;44(3):211–5.
21.Soliman AF, Saif-Elnasr M, Fattah SM. Platelet-rich plasma ameliorates gamma radiation-induced nephrotoxicity via modulating oxidative stress and apoptosis. Life Sci. 2019;219:238–47.
22.Dawson LA, Kavanagh BD, Paulino AC, Das SK, Miften M, Li XA, et al. Radiation-Associated Kidney Injury. Int J Radiat Oncol Biol Phys. 2010;76(3):S108-15.
23.Ali OS, Amin NE, Abdel Fattah SM, El-Rahman A. Ameliorative effect of kefir against γ-irradiation induced liver injury in male rats: Impact on oxidative stress and inflammation. Environ Sci Pollut Res. 2020;27(28):35161–73.
24.El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol Environ safety. 2007;66(2):278–86.
25.Take G, Erdogan D, Helvacioglu F, Göktas G, Ozbey G, Uluoglu C, et al. Effect of melatonin and time of administration on irradiation-induced damage to rat testes. Brazilian J Med Biol Res. 2009;42(7):621–8.
26.Canyilmaz E, Uslu GH, Bahat Z, Kandaz M, Mungan S, Haciislamoglu E, et al. Comparison of the effects of melatonin and genistein on radiation-induced nephrotoxicity: Results of an experimental study. Biomed reports. 2016;4(1):45–50.
27.Gu J, Chen YZ, Zhang ZX, Yang ZX, Duan GX, Qin LQ, et al. At What Dose Can Total Body and Whole Abdominal Irradiation Cause Lethal Intestinal Injury Among C57BL/6J Mice? Dose-Response. 2020;18(3):1–7.
28.Hirayama J, Abe H, Azuma H, Ikeda H. Leakage of potassium from red blood cells following gamma ray irradiation in the presence of dipyridamole, trolox, human plasma or mannitol. Biol Pharm Bull. 2005;28(7):1318–20.
29.Kaločayová B, Kovačičová I, Radošinská J, Tóthová Ľ, Jagmaševič-Mézešová L, Fülöp M, et al. Alteration of renal Na,K-ATPase in rats following the mediastinal γ-irradiation. Physiol Rep. 2019;7(3):1–11.
30.Lanz C, Patterson AD, Slavík J, Krausz KW, Ledermann M, Gonzalez FJ, et al. Radiation Metabolomics. 3. Biomarker Discovery in the Urine of Gamma- Irradiated Rats Using a Simplified Metabolomics Protocol of Gas Chromatography-Mass Spectrometry Combined with Random Forests Machine Learning Algorithm. Radiat Res. 2009;172(2):198–212.
31.De Freitas RB, Boligon AA, Rovani BT, Piana M, De Brum TF, da Silva Jesus R, et al. Effect of black grape juice against heart damage from acute gamma TBI in rats. Molecules. 2013;18(10):12154–67.
32.Unthan kJL, Miller SJ, Quickery AK, Ferguson EL, Wang M, Sampson CH, et al. Delayed effects of acute radiation exposure in a murine model of the H-ARS: multiple-organ injury consequent to< 10 Gy total body irradiation. Health Phys. 2015;109(5):511–21.
33.Thygesen K, Alpert JS, White HD. Universal Definition of Myocardial Infarction. ESC/ ACCF/AHA/WHF Expert Consensus Document. J Am Coll Cardiol. 2007;50(22):2173–95.
34.Gopcevic K, Rovcanin B, Kekic D, Radenkovic S. Matrix metalloproteinases and membrane damage markers in sera of patients with acute myocardial infarction. Mol Cell Biochem. 2011;350(1–2):163–8.
35.Soliman AF, Anees LM, Ibrahim DM. Cardioprotective effect of zingerone against oxidative stress, inflammation, and apoptosis induced by cisplatin or gamma radiation in rats. Naunyn-Schmiedeberg's Arch Pharmacol. 2018;39(8):819–32.
36.Ibrahim DM, Radwan RR, Abdel Fattah SM. Antioxidant and antiapoptotic effects of sea cucumber and valsartan against doxorubicin-induced cardiotoxicity in rats: The role of low dose gamma irradiation. J Photochem Photobiol B Biol. 2017;170:70–8.
37.i Garau MM, Calduch AL, López EC. Radiobiology of the acute radiation syndrome. Reports Pract Oncol Radiother. 2011;16(4):123–30.
38.Abdelhalim MA, Moussa SA. The biochemical changes in rats' blood serum levels exposed to different gamma radiation doses. African J Pharm Pharmacol. 2013;7(15):785–92.
39.Sayed DF, Nada AS, Mohamed MA, Ibrahim MT. Modulatory effects of Chrysophyllum cainito L.

extract on gamma radiation induced oxidative stress in rats. Biomed Pharmacother. 2019;111:613–23.
40. Srinivasan M, Kalpana KB, Devipriya N, Menon VP. Protective effect of lycopene on whole body irradiation induced liver damage of Swiss albino mice: pathological evaluation. Biomed Prev Nutr. 2014;4(2):87–94.
41. El-Desouky W, Hanafi A, Abbas MM. Radioprotective effect of green tea and grape seed extracts mixture on gamma irradiation induced immune suppression in male albino rats. Int J Radiat Biol. 2017;93(4):433–9.