

## ORIGINAL ARTICLE

# The role of melatonin in preventing radiation-induced intestinal injury

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## Summary

**Purpose:** Despite the therapeutic effects of radiotherapy on tumor cells, it has potential severe adverse effects on the surrounding normal tissues. Acute or chronic intestinal adverse effects that are likely to occur in patients undergoing radiotherapy for pelvic and abdominal cancers lead to increased morbidity, significant impairment of the quality of life, and economic losses. Various biological, chemical and pharmacological agents are being tested to protect from and to treat radiation enteritis. This experimental study aimed to investigate the protective effects of melatonin against radiation-induced intestinal injury when administered before radiation exposure in rats.

**Methods:** In the present study, villus height and the number of villi in the ileum and jejunum of rats receiving two different doses of intraperitoneal melatonin (5 and 10 mg/kg)

prior to a single fraction of radiation given at a dose of 8 Gy to the abdominal region, was evaluated by histopathological examination 3 and 7 days after radiation exposure.

**Results:** At a dose of 5 mg/kg, melatonin was found to be effective in preventing radiation-induced injury to villus height in the jejunum and the number of villi in the ileum and jejunum, and at a dose of 10 mg/kg it was also effective in preventing radiation-induced injury to villus height in the ileum.

**Conclusions:** Melatonin is effective for the prevention of radiation-induced intestinal injury. This outcome can be considered an evidence to test melatonin in clinical trials.

**Key words:** intestinal injury, melatonin, radiation enteritis, radioprotective, radiotherapy

## Introduction

Radiotherapy is performed as part of curative or palliative care in more than half of cancer patients [1]. Despite the favorable therapeutic effects of radiotherapy on tumor cells, potential side effects on normal tissues are in question. Owing to the recent technological developments and availability of 3D computer planning systems, it has been possible to determine the size and shape of target tissues more definitely and minimize the damage to the surrounding normal tissues during radiotherapy [1]. On the other hand, the increase in the number of cancer cases due to the negative effects of modern lifestyle and the advances in diagnostic

methods enhanced the interest to the efficacy and safety of cancer therapies. Today, unintended side effects of radiotherapy remain as a challenge. Radiation-induced gastrointestinal injury is a frequent side effect of radiotherapy. Despite developments in technology, short-term enteritis is generally seen in patients receiving radiotherapy for pelvic, abdominal and colorectal cancers. Moreover, nearly 15% of the patients develop chronic intestinal problems [2]. These intestinal side effects, which might be either acute or chronic, lead to increased morbidity, significant impairment in quality of life, and economic losses [3].

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The pathogenesis of radiation-induced intestinal injury is complicated. While ionizing radiation directly causes DNA deletions and breaks, it causes DNA injury through free radicals by its indirect effect. In addition to DNA, lipid and proteins as well are exposed to attack of free radicals produced by ionizing radiation [4]. Finally, intestinal cell injury and cell death occurs. Depending on the dose of radiation, severe crypt-villus loss is seen particularly due to intestinal stem cell death [4].

Radiation-induced damage to the gastrointestinal tract can be minimized by one of the two strategies: technical strategies aiming to physically shift the dose of radiation away from the normal intestinal tissues, or biological strategies aiming at modulating the normal tissue response to radiation or increasing tissue resistance [3]. Within this context, various biological, chemical and pharmacological agents are being tested to protect normal tissues against radiation injury [3]. Melatonin is one of these agents. Melatonin is a hormone widely present in the nature, synthesized locally by various cells and tissues, and produced primarily by the pituitary gland in humans. Melatonin is one of the strongest natural antioxidants [5]. The present experimental study aimed to investigate the protective effects of melatonin against radiation-induced intestinal injury when administered prior to radiation exposure in rats.

## Methods

### Subjects

Male/female Sprague-Dawley rats (n=49) aged 16-20 weeks and with a mean weight of  $200 \pm 25$  g were used in the present study. The rats were kept in quarantine at least 3 days before radiation. Over the entire experimental period, they were kept in cages without windows at  $23 \pm 1^\circ\text{C}$  in a 12:12 hour light/dark cycle. Each cage contained a maximum of 10 rats and they were fed with standard pellet and water. The study was approved by the local Ethics Committee of Ataturk University School of Medicine.

### Procedure

The rats were divided into 7 groups each containing 7 rats. The applied processes in the control group (Group I), radiation only groups (Group IIa and Group IIb) and radiation+ melatonin groups (Group IIIa, Group IIIb, Group IVa and Group IVb) are explained below.

Group I: Control group; only received 0.25 mL normal saline (NS) via intraperitoneal route on Day 1.

Group IIa: On Day 1, radiation was given 30 min after intraperitoneal administration of 0.25 mL NS and tissue sample was obtained on Day 3 for histopathological examination.

Group IIb: On Day 1, radiation was given 30 min after

intraperitoneal administration of 0.25 mL NS and tissue sample was obtained on Day 7 for histopathological examination.

Group IIIa: On day 1, radiation was given 30 min after intraperitoneal administration of 5 mg/kg melatonin and tissue sample was obtained on Day 3 for histopathological examination.

Group IIIb: On day 1, radiation was given 30 min after intraperitoneal administration of 5 mg/kg melatonin and tissue sample was obtained on Day 7 for histopathological examination.

Group IVa: On day 1, radiation was given 30 min after intraperitoneal administration of 10 mg/kg melatonin and tissue sample was obtained on Day 3 for histopathological examination.

Group IVb: On day 1, radiation was given 30 min after intraperitoneal administration of 10 mg/kg melatonin and tissue sample was obtained on Day 7 for histopathological examination.

*Administration of Melatonin:* After being dissolved in alcohol, melatonin (Sigma Chemical Co, St. Louis, MO, USA) was diluted with NS to 2 mg/0.1 mL (0.25 mL in total). It was administered intraperitoneally 30 min before radiation at a single dose predetermined for the group.

*Radiation:* Except for those in the control group, the rats were anesthetized using 50 mg/kg ketamin HCL (Pfizer Pharmaceuticals, Istanbul, Turkey) given by intraperitoneal route, and were placed on the radiation apparatus in supine position. A single dose of radiation at 8 Gy-fraction was applied to a radiation field of 5.5x5 cm in the abdominal region at a source-to-surface distance of 80 cm, using the Picker Cobalt-60 teletherapy machine. The percentage depth dose at the depth of maximum dose was calculated to be in the midline from abdominal skin surface. The productivity ratio of the radiation device was 58.4 cGy/min.

*Histopathological examination:* The rats were sacrificed under high-dose general anesthesia on Day 3 or 7 depending on the group. After the abdomen was opened, 10 cm tissue samples were obtained from the proximal jejunum and distal ileum. These tissue samples were fixed with 10% buffered formalin. Sections 2 mm in thickness were obtained from both end and middle parts of the tissue samples. They were dehydrated passing through different grades of alcohol solutions, and were embedded in paraffin. Sections, 4  $\mu$  in thickness, obtained using a microtome, were stained with hematoxylin and eosin and examined under a light microscope. The number of villi in each 1X magnification field was counted, and the mean value was calculated. In addition, villus height was morphometrically measured using Samba Technology and the mean values were obtained.

### Statistics

SPSS statistical package, version 11 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Between-group comparisons were performed using analysis of variance (ANOVA) and in the presence of difference, Duncan test was performed for further analysis. The level of statistical significance was set at  $p < 0.05$ .

**Results**

Height and the number of villi in the ileum and jejunum were evaluated using histological examination. Table 1 shows villus height in the ileum and jejunum of the study groups.

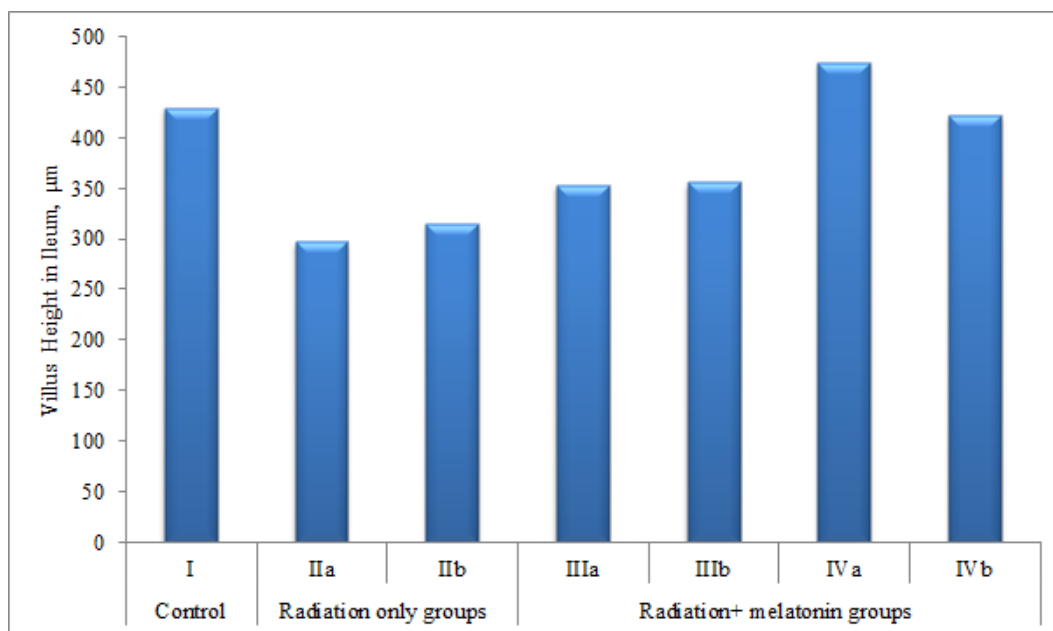
ANOVA analysis revealed a significant difference between the groups in terms of villus

height in the ileum ( $p < 0.001$ ), and Duncan test was performed. There was no difference between the groups IIa, IIb, IIIa and IIIb and between the groups I, IVa and IVb in terms of villus height in the ileum. However, mean villus height in the groups I, IVa and IVb was significantly higher than that in group IIa, IIb, IIIa and IIIb (Figure 1).

**Table 1.** Villus height in the ileum and jejunum of the study groups

Groups	Villus Height in Ileum, $\mu\text{m}$ Mean $\pm$ SD	Villus Height in Jejunum, $\mu\text{m}$ Mean $\pm$ SD
I	429.8 $\pm$ 66.3	757.3 $\pm$ 117.9
IIa	296.9 $\pm$ 84.3	361.9 $\pm$ 29.6
IIb	315.2 $\pm$ 41.7	412.4 $\pm$ 93.7
IIIa	353.4 $\pm$ 24.7	430.7 $\pm$ 64.1
IIIb	355.8 $\pm$ 32.2	546.8 $\pm$ 123.1
IVa	475.0 $\pm$ 86.3	595.0 $\pm$ 28.5
IVb	422.2 $\pm$ 20.2	644.4 $\pm$ 140.9
P value (ANOVA)*	<0.001	<0.001

\*Analysis of variance



Group	Villus Height in Ileum, $\mu\text{m}$	
Control	I	429.8
Radiation only groups	IIa	296.9
	IIb	315.2
Radiation+ melatonin groups	IIIa	353.4
	IIIb	355.8
	IVa	475.0
	IVb	422.2

**Figure 1.** Villus height in the ileum according to the study groups.

ANOVA analysis revealed a significant difference between the groups in terms of villus height in the jejunum ( $p < 0.001$ ), and Duncan test was performed. While there was no difference between the groups IIa, IIb and IIIa, and the groups IIIb, IVa and IVb, mean villus height in the groups IIIb, IVa and IVb was significantly higher than that in group

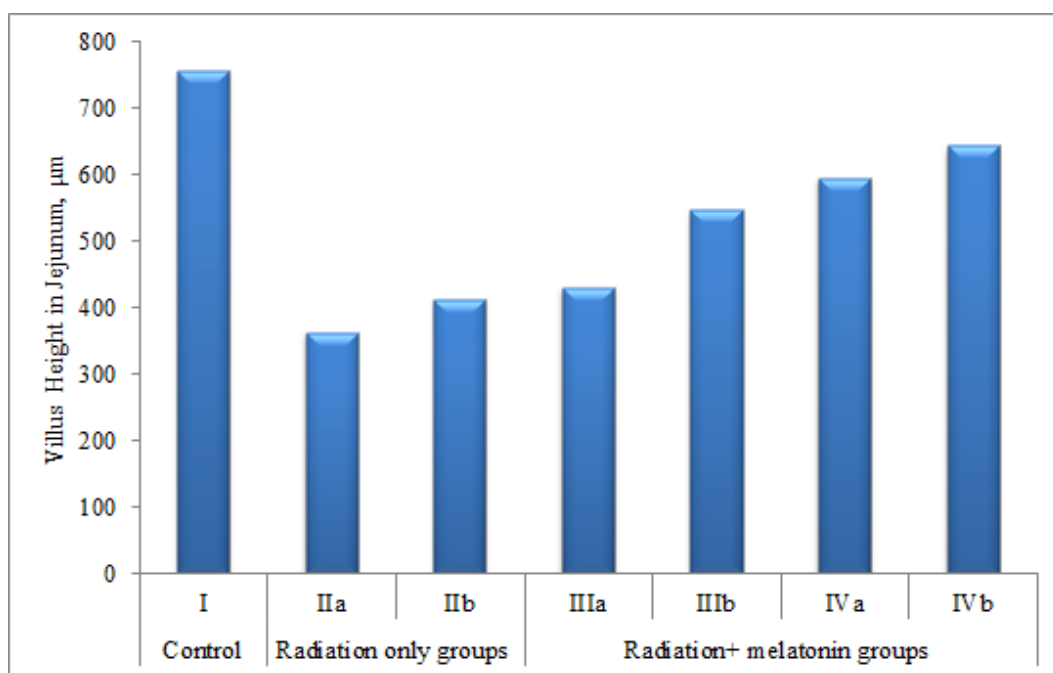
IIa, IIb and IIIa. In jejunum, the maximum villus height was observed in group I (Figure 2). Table 2 illustrates the number of villi in the ileum and jejunum in the study groups.

ANOVA analysis revealed a significant difference between the groups in terms of the number of villi in the ileum ( $p < 0.001$ ), and Duncan test

**Table 2.** The number of villi in the ileum and jejunum of the study groups

Groups	Number of Villi in Ileum	Number of Villi in Jejunum
	Mean±SD	Mean±SD
I	8.3 ± 0.7	8.9 ± 0.3
IIa	7.1 ± 0.3	6.9 ± 0.3
IIb	5.4 ± 0.9	5.3 ± 0.7
IIIa	8.4 ± 0.9	9.1 ± 0.8
IIIb	8.9 ± 0.3	9.1 ± 1.8
IVa	9.1 ± 0.8	8.7 ± 0.7
IVb	9.1 ± 0.3	8.1 ± 0.6
P value (ANOVA)*	<0.001	<0.001

\*Analysis of variance



	Group	Villus Height in Jejunum, µm
Control	I	757.3
Radiation only groups	IIa	361.9
	IIb	412.4
Radiation+ melatonin groups	IIIa	430.7
	IIIb	546.8
	IVa	595.0
	IVb	644.4

**Figure 2.** Villus height in the jejunum according to the study groups.

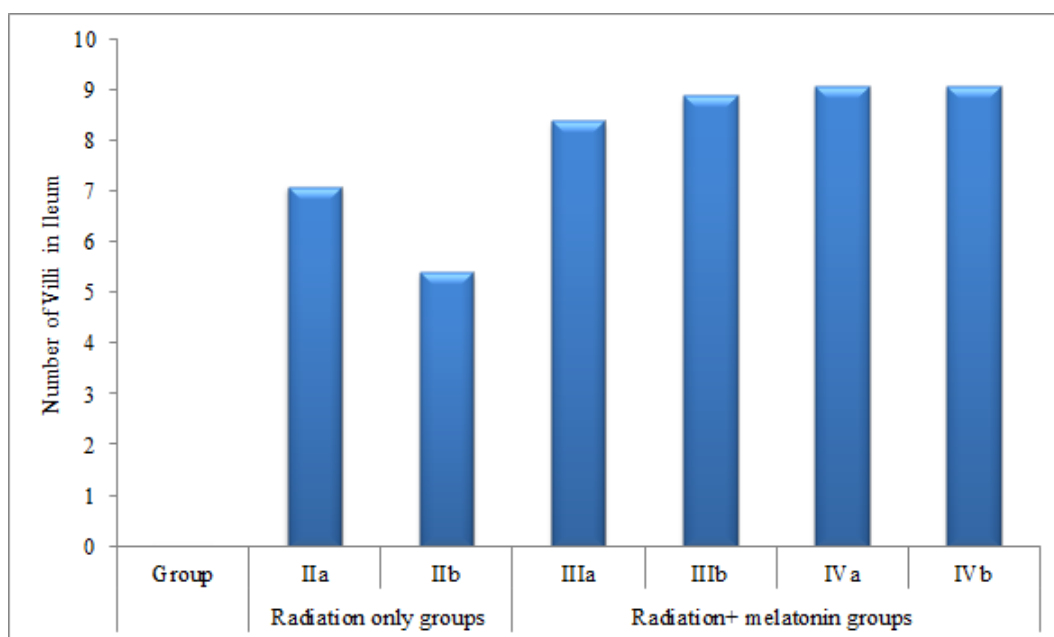
was performed. There was a significant difference between group IIa and group IIb in terms of the number of villi in the ileum. Although there was no difference between groups I, IIIa, IIIb, IVa and IVb in terms of the number of villi, the number of villi in the groups I, IIIa, IIIb, IVa and IVb was significantly higher than that in group IIa and Group IIb (Figure 3).

ANOVA analysis revealed a significant difference between the groups in terms of the number of villi in the jejunum ( $p < 0.001$ ), and Duncan test was performed. Although there was no difference between groups I, IIIa, IIIb, IVa and IVb in terms of the number of villi in the jejunum, the number of villi in groups I, IIIa, IIIb, IVa and IVb was higher than that in group IIa and group IIb (Figure 4).

## Discussion

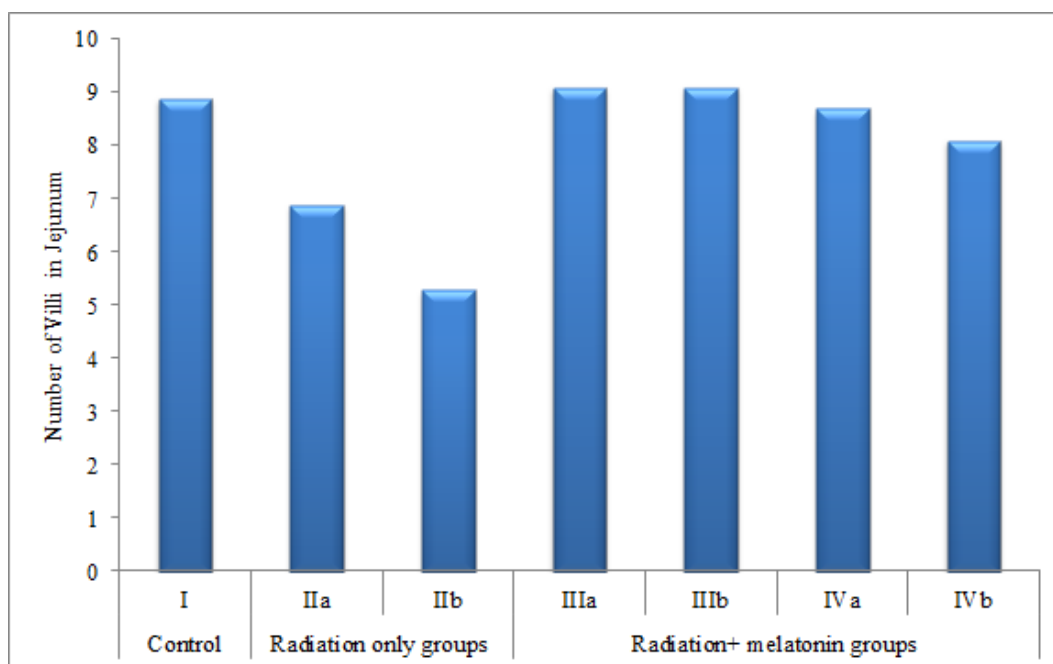
Although radiation-induced enteritis is a frequent side effect encountered in the patients receiving radiotherapy, there is no standard method of treatment [6]. For this reason, it is essential to

protect the small intestine against the effects of radiation. Studies are ongoing to enhance the therapeutic efficacy of ionizing radiation by minimizing its side effects, and radioprotective effects of various agents are being investigated. Melatonin, one of these agents, is a hormone discovered more than 50 years ago, and its physiological and pharmacological effects are being investigated since then [7]. Melatonin has various properties such as regulation of circadian rhythm, sleep-promoting effect and cancer inhibition effect, besides its antioxidant efficacy, which is also relevant to the present study [8]. Protective effects of melatonin against damages associated with various kinds of exposures (ischemia-reperfusion, drug toxicity, radiation, etc.) are being investigated in numerous experimental trials. Protective and curative effects of melatonin in oxidative injury occurring in the organs (liver, lungs and small intestine) due to severe burns [9], in oxidative injury induced by adriamycin, an anti-tumor agent [10], in intestinal ischemia-reperfusion injury [11], and in intestinal dysfunction induced by methotrexate, an anti-tumor agent, were demonstrated in previous studies [12]. In experi-



Group	Number of Villi in Ileum
Radiation only groups	IIa 7.1
	IIb 5.4
Radiation+ melatonin groups	IIIa 8.4
	IIIb 8.9
	IVa 9.1
	IVb 9.1

**Figure 3.** The number of villi in the ileum according to the study groups.



	Group	Number of Villi in Jejunum
Control	I	8.9
Radiation only groups	IIa	6.9
	IIb	5.3
Radiation+ melatonin groups	IIIa	9.1
	IIIb	9.1
	IVa	8.7
	IVb	8.1

**Figure 4.** The number of villi in the jejunum according to the study groups.

mental trials, healing effect of melatonin against radiation-induced injury has been demonstrated in the heart [13], lungs [14], liver [15,16], brain [17], kidneys [18], testicles [19], thyroid [20], epiphysis [21], and skeletal muscle [22] tissues. In the present experimental trial, we as well, investigated the radioprotective effect of melatonin in the small intestine.

The technique and dose of radiation are among the risk factors of radiation-induced gastrointestinal injury [23]. It was demonstrated in rats that the height of intestinal villi decreased at a radiation dose of 4 Gy; the villi were enlarged, flattened and inverted at a dose of 5 Gy; and the villi structure was damaged, the villi were ruptured and smashed, and the recess structures were missing when the radiation dose was increased to 6 Gy [24]. Radiation is lethal at the dose of 9 Gy [25]. In the present study, radiation was applied to the abdominal region of rats at a dose of 8 Gy, and a decrease was observed in the height and number of villi in the examinations performed on day 3 and day 7.

Protective effects of melatonin against radiation-induced intestinal injury have been investigated in experimental studies. It was reported that melatonin, used at pharmacological doses, has alleviating effects on organ damage (liver, lungs, colon and small intestine) that occur after whole body irradiation (800 cGy) in rats [26]. Various doses of melatonin (1, 5, 10, 20 mg/mL) prevented dose-dependently intestinal injury in rats receiving whole body irradiation at various doses (7-21 Gy). No threatening side effects except for sleeping for hours were determined in rats even receiving melatonin at the highest dose [27]. In another study, mucosal ulceration in the small intestine, epithelial cell necrosis, decreased villus height and decreased number of villi and decreased villus height to crypt depth ratio were observed in rats exposed to whole body radiation at a dose of 8 Gy. However, intestinal mucosal structure was preserved in the rats that were protected in advance with melatonin given at a dose of 100 mg/kg via intraperitoneal route 1 h before radiation [28]. It was demonstrated

that intestinal structural defects occurred and the height of villi and crypts were decreased in the rats receiving whole-body radiation at a dose of 8 Gy and that these intestinal changes could be prevented with intraperitoneal melatonin given in advance at a dose of 15 mg/kg [29]. In another study, the effects of oxidative stress in the small intestine that indirectly occurs against oral radiation and the therapeutic effects of melatonin were investigated in rats subjected to irradiation of their tongues at a dose of 7.5 Gy/day for 5 days and melatonin gel was applied locally into their mouths at a dose of 45 mg/day for 21 days. Reduction of mucosal dysfunction, improvement in intestinal mucosal healing, and decreased intestinal apoptosis were observed in the rats treated with melatonin gel. Shortened and degenerated villi healed completely with melatonin [30]. Radioprotective effects of melatonin administered at the doses of 50 and 100 mg/kg via intraperitoneal route were demonstrated in rats receiving radiation to abdominal and pelvic fields at a dose of 8 Gy. Biochemical analyses revealed that melatonin affects the intestinal tissue by reducing oxidative stress and increasing antioxidant enzymes and that it is more effective at high doses [31]. In the present study, when villus height in the ileum was considered, melatonin was effective at a dose of 10 mg/kg, whereas radioprotective effects were inadequate at a dose of 5 mg/kg. In the jejunum, villus height significantly improved on day 7, if not on day 3, with 5 mg/kg melatonin. However, the height of villi significantly improved on both day 3 and day 7 in those receiving melatonin at a dose of 10 mg/kg. Regarding the number of villi, it was observed that decrease in the number of villi due to radiation improved with both doses of melatonin and reached to the level of the control group on both day 3 and day 7.

Despite the different designs (subject characteristics, site of radiation, melatonin dose and route of administration, time of post-radiation evaluation, biochemical vs. histological examination for evaluation, etc.) of the above-mentioned experimental trials, the common outcome for all is the radioprotective effects of melatonin on the intestine. Zetner et al [32] performed a systematic literature review and reported that the studies dem-

onstrating radioprotective effects of melatonin are experimental animal studies and that clinical human studies are required. Vijayalaxmi et al [33] as well reviewed laboratory investigations exhibiting antioxidant effects of melatonin, and they summarized the evidence that could be used in designing clinical studies to show the radioprotective properties of melatonin.

It has been suggested that melatonin affects the response to radiation in many different ways. Melatonin, a natural body hormone, is a strong antioxidant and antiinflammatory agent showing certain anticancer characteristics [34]. That is to say, melatonin directly neutralizes free radicals, upregulates antioxidant enzymes, and suppresses pro-oxidant enzymes. In addition, it is a strong stimulator of DNA-repair response [34]. Owing to its antiinflammatory characteristics, melatonin plays a protective role against unfavorable effects of radiotherapy also over immunoregulatory mechanisms [34,35]. In the last two decades, clinical studies have demonstrated the role of melatonin as an auxiliary agent in the treatment of numerous diseases. Moreover, most of the studies reported very low toxicity within a wide range of doses (from 0.1 to 300 mg); besides, the doses used in the clinical studies are lower than that used in the experimental trials [36].

In conclusion, the present study found that melatonin applied at two different doses before radiation has radioprotective effects against radiation-induced intestinal injury. Low-dose melatonin was adequate for the protection of radiation-induced injury to villus height in the jejunum and the number of villi in the ileum and jejunum, but the dose of melatonin needs to be increased to maintain villus height in the ileum. In the light of the information given above and based on the results of our experimental trial, we may conclude that the probability of confirming the protective/therapeutic effects of melatonin for radiation-induced enteritis in clinical studies should be considered.

## Conflict of interests

The authors declare no conflict of interests.

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