Research Article

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Effects of resveratrol against scattered radiation-induced testicular damage in rats

[Saçılan radyasyonun sıçan testis dokusundaki hasarına karşı Resveratrol'ün etkilerinin değerlendirilmesi]

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Abstract

Objectives: To investigate the possible protective effects of resveratrol against oxidative testicular damage due to scattered radiation during pelvic ionizing radiation exposure in rats.

Methods: Rats were divided into 5 groups; control, radiation, and radiation + resveratrol therapy in early and late periods. Under anesthesia, 20 Gy ionizing radiation was applied to prostatic region. Resveratrol was administered (10 mg/kg/day) orally before ionizing radiation exposure. Animals were decapitated at the end of 1st and 10th weeks. Biochemical markers of oxidative stress; caspase-3 and sirtuin-1 protein expressions; testosterone levels were evaluated, histological examinations were performed.

Results: Significant increases in malondialdehyde, 8-hydroxy-deoxyguanosine levels, myeloperoxidase, and caspase-3 activities were observed after ionizing radiation exposure, also superoxide dismutase and glutathione activities were significantly decreased. Radiotherapy increased caspase-3 and decreased sirtuin-1 protein expressions. Resveratrol treatment significantly reversed these parameters and also reversed the decrease in testosterone levels back to control levels in late period.

Conclusion: Resveratrol showed antioxidant and sirtuin-activating properties against oxidative damage caused by scattered radiation to testis and provided hormonal protection. These results suggest that resveratrol may be an alternative protective agent on testicular tissues against the effects of scattered pelvic radiation.

Keywords: oxidative stress; radiotherapy; resveratrol; sirtuin-1; testis.

Öz

Giriş: Pelvik radyoterapi sırasında saçılan radyasyon testis dokularına zarar verebilir. Bu çalışmanın amacı, sıçanlarda, pelvik radyasyona maruz kalma sırasında saçılan radyasyona bağlı oksidatif testis hasarına karşı resveratrol'ün olası koruyucu etkilerini araştırmaktır.

Yöntem: Sıçanlar 5 gruba ayrıldı; kontrol grubu, erken ve geç dönemlerde radyasyon grupları ve erken ve geç dönemlerde radyasyon + resveratrol tedavisi grupları. Anestezi altında prostatik bölgeye 20 Gy iyonize radyasyon uygulandı. Resveratrol, radyasyona maruz kalmadan önce oral yoldan (10 mg/kg/gün) uygulandı. Birinci ve onuncu haftanın sonunda hayvanlar sakrifiye edildi. Oksidatif stresin biyokimyasal belirteçleri; kaspaz-3 ve sirtuin-1 protein ekspresyonları; testosteron seviyeleri değerlendirildi, histolojik incelemeler yapıldı.

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Bulgular: İyonizan radyasyona maruz kaldıktan sonra malondialdehit, 8-hidroksi-deoksiguanozin seviyeleri, miyeloperoksidaz ve kaspaz-3 aktivitelerinde önemli artışlar gözlenmiş, ayrıca süperoksit dismutaz ve glutatyon aktiviteleri önemli ölçüde azalmıştır. Radyoterapinin, kaspaz-3 ekpsresyonunu arttırdığı ve sirtuin-1 protein ekspresyonlarını azalttığı görüldü. Resveratrol tedavisi, bu parametreleri önemli ölçüde tersine çevirdi ve ayrıca testosteron seviyelerindeki düşüşü geç dönemde kontrol seviyelerine geri döndürdü.
Sonuç: Resveratrol'ün, testise saçılan radyasyonun neden olduğu oksidatif hasara karşı antioksidan ve sirtuin aktive edici özellikler gösterdiği ve hormonal koruma sağladığı görüldü. Bu sonuçlar, resveratrolün pelvik radyasyonun saçılan etkilerine karşı testis dokularında alternatif bir koruyucu ajan olabileceğini düşündürmektedir.

Anahtar sözcükler: Resveratrol; testis; radyoterapi; sirtuin-1; oksidatif stres.

Introduction

The mechanism of action of radiotherapy is based on two principles. The radiation applied to living tissue is divided into electrically charged ions, and when these ions pass through the cells, accumulated energy directly damages the cells. On the other hand, radiation exposure, through reactive oxygen species (ROS) generation also damages the cells indirectly [1].

While radiation to targeted organ destroys the tumor with high amounts of generated ROS, the scattered beam, although at a lower density, also causes damage to surrounding tissues, along with further creation of ROS. This scattered radiation phenomenon constitutes the mainstay of emergence of undesirable radiotherapy effects [1]. Although side effects of radiotherapy are dose-dependent, initial complaints begin soon after treatment. Fast-dividing cells are generally more susceptible to radiation than slow-dividing cells [2]. Many of these side effects impair the quality of life of patients undergoing radiotherapy for cancer treatment and reduce the patient's resistance to treatment [3, 4].

Scattered radiation effects after pelvic External beam radiation therapy (EBRT) has been widely studied and treatment modalities for these unwanted effects are being investigated. One of the main problems due to scattered radiation is the oxidative stress that is created in the testicular tissue due to close proximity of the organs to the pelvic area. The effects of this scattered radiation can be reflected by changes in oxidative stress biomarkers in testicular tissues. The anti-oxidative capacity of tissues is reflected by changes in glutathione levels and superoxide dismutase activity; lipid peroxidation is reflected by malondealdeyde levels and myeloperoxidase activity; oxidative DNA damage is reflected by 8-hydroxy-2′-deoxyguanosine levels. The apoptotic activity is reflected by caspase-3 protein expression. Sirtuin-1 is an inactivator of certain proapoptotic proteins, therefore changes in sirtuin-1 levels reflect the antiapoptotic activity of tissues. Due to all the changes in the aforementioned parameters, the 2 main functions of the testicular tissue, which are the spermatogenesis and hormone production, are both impaired after pelvic EBRT.

Seminiferous tubules are particularly radiosensitive and doses exceeding 1.5–2 Gy may lead to irreversible fertility loss [5]. Also, the radiation-induced damage to the Sertoli cells can impair sperm production [6]. Although Leydig cells are more radioresistant than Sertoli cells, damage to Leydig cells is also of utmost importance and can result in severe testosterone deficiency, even reaching castration-levels [2].

As a radiosensitive organ, testis protection is very important in patients undergoing pelvic radiotherapy. For this purpose, various pharmacological approaches have been studied before and after radiotherapy in rat models.

Resveratrol (RVT) (3,5,4′-trihydroxy-*trans*-stilbene) as a phenolic stilbene compound found in several plants, is a potent anti-cancer and anti-oxidant agent and is studied in various oxidative stress models [7–9]. Resveratrol increases NAD^+ concentration by stimulating the PARP1 protein expression therefore increases the sirtuin-1 activity, which in turn inactivates proapoptotic protein expressions [10]. Resveratrol has also been shown to decrease caspase-3 protein expression therefore decreasing the apoptotic activity in tissues [1]. However the effects of resveratrol has never been demonstrated in testicular damage following pelvic radiation. Resveratrol has also been shown to decrease oxidative stress in clinical trials including human subjects [11]. The fact that resveratrol can be supplied by many daily alimentation products and also as a daily over-the-counter supplement renders resveratrol to be a subject of interest.

Our aim is to evaluate the possible protective effects of resveratrol on testicular tissue of rats that were exposed to scattered radiation damage following pelvic radiotherapy.

Materials and methods

Animals and experimental design

The study was approved by Marmara University Animal Care and Use Committee (protocol number; 015.2019.mar.)

Sprague Dawley rats (300–350 g), obtained from Marmara University Application and Research Center for Experimantal Animals were divided into 5 groups, containing 10 rats. Control group (C) received only vehicle administration for 7 days; Radiotherapy + vehicle (early period) group received vehicle for 7 days prior to radiation and rats were sacrificed 1 week after radiation; Radiotherapy + vehicle (late period) group received vehicle for 7 days prior to radiation and rats were sacrificed 10 weeks after radiation; Radiotherapy + resveratrol (early period) group received resveratrol for 7 days prior to radiation and rats were sacrificed 1 week after radiation; Radiotherapy + resveratrol (late period) group received resveratrol for 7 days prior to radiation and rats were sacrificed 10 weeks after radiation.

Resveratrol was given as 10 mg/kg via oral route. Radiation was applied by the Radiation Oncology Team as a single dose of 20 Gy prostate-confined irradiation [12].

At the end of the experimental period, rats were sacrificed. Testicular tissues and blood samples were obtained for biochemical and histological analyses.

Biochemical analysis

Measurement of tissue glutathione (GSH) levels and superoxide dismutase (SOD) activity: Glutathione levels and superoxide dismutase activity reflect the anti-oxidative capacity of the tissues. GSH measurements were performed using a modified Ellman procedure and results are expressed in μmol GSH/g tissue [13]. SOD activity in tissue samples was measured according to the method described by Mylroie [14]. A standard curve was prepared routinely with bovine SOD (Sigma Chemical Co, Saint Louis, USA; S-2515, 3000 U) as reference. Absorbance readings were taken at 0 and 8 min of illumination and net absorbances were calculated.

Measurement of malondialdehyde (MDA) levels and myeloperoxidase (MPO) activity: MDA levels were assayed as products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation [15]. Myeloperoxidase activity in testicular tissues was measured by a procedure similar to that described by Hillegas [16]. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance, measured at 460 nm for 3 min.

Measurement of 8-hydroxy-2′-deoxy-guanosine (8-OHdG) levels: 8-hydroxy-2′-deoxy-guanosine (Oxi Select™ Oxidative DNA Damage Elisa Kit, Cell Biolabs, US) levels, which reflect the oxidative DNA damage, were evaluated in testicular tissue homogenates using commercially available kits.

Western blot analysis for sirtuin-1 and caspase-3 protein expressions: Sirtuin-1 and caspase-3 protein expressions were measured by Western blotting. Samples were homogenized by cell lysis buffer and protein concentrations were determined using Bradford method [17]. Samples resolved by 4–12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis were transferred to polyvinylidene fluoride (PVDF) membrane, which was then blocked with bovine serum albumin. Membrane was incubated overnight with primary antibody (1:500 dilution anti-SIRT1 sc-15404, anti-caspase-3 sc-7148, anti-β-actin sc-47778 Santa Cruz Biotechnology, Heidelberg, Germany) and washed with TBST (Tris-buffered saline containing 0.1% Tween-20). The membrane was washed and incubated with horseradish peroxidase conjugated (HRP) secondary antibody for 2 h and afterwards the blot was developed with chemiluminescence reagents and exposed to film. Data were analyzed using "Image J Programme Optical Density Analysis Software" (NIH, USA). Signals were normalized with respect to β-actin.

Measurement of testosterone levels

Testosterone levels were measured in serum samples by Enzyme-Linked Immuno Sorbent Assay (ELISA), according to the manufacturer's guidelines of the commercial kit (MyBioSource Inc, San Diego, US).

Histological analysis: Histopathological analyses were performed after testicular tissues were fixed in 10% formalin solution and dehydrated in degraded ethanol series and cleared in toluene. Paraffin-embedded tissue samples were cut in 5 μm thickness by rotary microtome and stained with hematoxylin-and-eosin (H&E). Sections were evaluated according to the modified Johnsen scoring system and photographed under Olympus BX51 light microscope (Olympus Co., Ltd., Tokyo, Japan) (Table 1).

Statistical analysis: Statistical analyses were performed using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). All the data are expressed as mean ± standard deviation (S.D.) Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p<0.05$ were considered significant.

Results

Superoxide dismutase activity and glutathione levels were significantly decreased in both early and late radiation groups that received the vehicle compared to control levels ($p<0.01$ for SOD in both early and late periods; $p<0.001$ for GSH in both early and late periods). Resveratrol prevented this decrease, as the SOD activity and GSH levels were significantly higher than that of radiation groups that received the vehicle ($p<0.05$ for SOD in early period; $p<0.01$ for SOD in late period; $p<0.001$ for GSH in both early and late periods). Radiotherapy group that received resveratrol had more significantly increased the SOD activity in late period compared to radiotherapy group + resveratrol group in early period (Figure 1).

Myeloperoxidase activity, 8-hydroxydeoxyguanosine and malondialdehyde levels were all increased with radiotherapy, with late period having more pronounced effect on the results. However, treatment with resveratrol reversed this increase and returned all the parameters to control-like levels in a significant manner (Figure 2). The radiotherapy group that received vehicle has significantly increased levels of MPO ($p<0.01$ in early and $p<0.001$ in late period), 8-OHdG ($p<0.001$ in both early and late periods)

Table 1: Modified Johnsen scoring system.

Score	Histological finding
10	Full spermatogenesis observed
9	Spermatogenesis slighly impaired, frequent late spermatids, disorganized epithelium
8	<5 spermatozoa/tubule, few late spermatids
7	Absence of spermatozoa and late spermatids, many early spermatids
6	Absence of spermatozoa and late spermatids, few early spermatids
5	Absence of spermatozoa and spermatids, frequent spermatocytes
4	Absence of spermatozoa and spermatids, few spermatocytes
3	Only spermatogonia
2	Absence of germ cells, presence of only sertoli cells
1	Absence of seminiferous epithelium

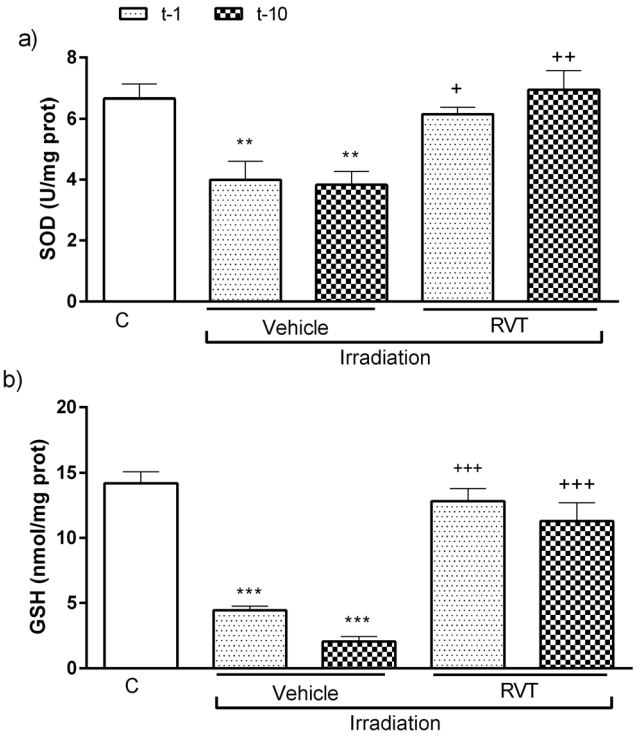

Figure 1: Superoxide dismutase (SOD) activity and glutathione (GSH) levels. (*p<0.05, **p<0.01, ***p<0.001 compared to control group; +p<0.05, ++ p<0.01, +++ p<0.001, radiation + resveratrol group compared to radiation + vehicle group – comparisons have been made separately for both early and late periods).

and MDA (p<0.001 in both early late periods). These MPO activities, 8-OHdG and MDA levels were also higher in late period compared to early period in radiation groups that received vehicle (p<0.001 for all parameters). Resveratrol didn't decrease the MPO activity significantly when early period radiation + vehicle group was compared with early period radiation + resveratrol group (p>0.05), however, it did significantly decrease the MPO activity in late period when radiation + vehicle and radiation + resveratrol groups were compared (p<0.001). The activity of MPO in late period radiation + resveratrol group was similar to that of control group. The levels of 8-OHdG significantly increase with radiation groups that received vehicle in both early and late periods (p<0.001). The levels were even significantly higher in late period compared with early period (p<0.001). Resveratrol successfully decreased the 8-OHdG levels in late period in radiation + resveratrol group compared to radiation + vehicle group (p<0.001), however in early period, resveratrol didn't achieve a significant decrease and 8-OHdG levels remained significantly higher that of control group (p<0.01 for early period; p<0.001 for late period). Malondialdehyde levels significantly increase in radiation + vehicle groups in both early and late periods (p<0.001). The latter increased even more, compared to early group (p<0.001). Resveratrol treatment significantly reduced the MDA levels in both early and late periods (p<0.001), but the levels remained still significantly higher than that of control group in late period (p<0.001).

Sirtuin-1 protein expression has been decreased significantly in the radiation groups, with the late period being more pronounced than the early period (p<0.05 in early period; p<0.001 in late period compared to control group). Resveratrol given prior to radiation in both early and late period groups provided a significant increase compared to radiation + vehicle groups as the values have significantly improved to control-like levels (p<0.01 in early period; p<0.001 in late period compared to radiation + vehicle groups). Caspase-3 protein expression has increased significantly in radiation groups due to an increase in apoptosis in tissues (p<0.001). Resveratrol caused a significant decrease in caspase-3 protein expressions in both early and late periods (p<0.001 for both early and late periods compared to radiation + vehicle groups), however, levels still remained higher than that of control group (p<0.001 for early period; p<0.01 for late period) (Figure 3.).

Testosterone levels were similar to control levels in the early period in both radiation + vehicle and radiation + resveratrol groups. However in radiation + vehicle group in the long period, the levels were significantly decreased when compared with control group (p<0.001) and radiation + resveratrol group (p<0.001). The levels in radiation + resveratrol group in the late period were similar to control levels and the levels of radiation + resveratrol group in early period; and the levels were significantly higher than that of radiation + vehicle group in the late period (p<0.001) (Table 2).

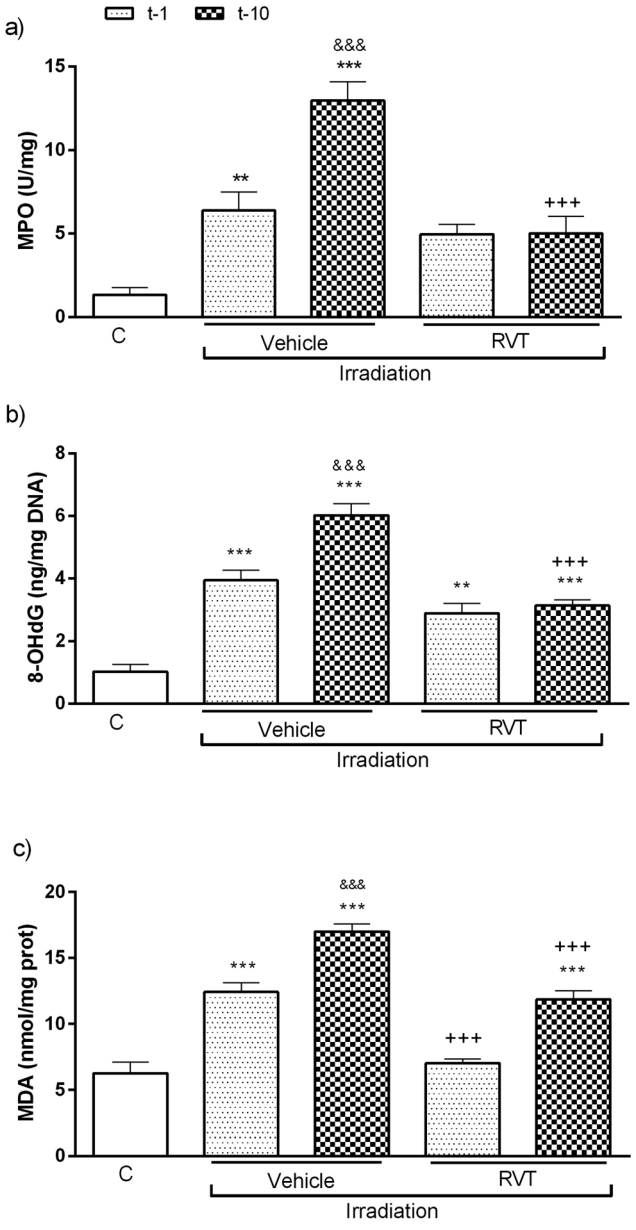

Figure 2: Myeloperoxidase (MPO) activity, 8-OHdG (8-hydroxydeoxyguanosine) and malondialdehyde (MDA) levels. (*p<0.05, **p<0.01, ***p<0.001 compared to control group; +p<0.05, ++ p<0.01, +++ p<0.001, radiation + resveratrol group compared to radiation + vehicle group – comparisons have been made separately for both early and late periods).

Histological examination revealed severe tissue damage in groups subjected to radiotherapy; late period effects were more pronounced than early period effects of radiation (Figure 4a, b). A small number of tubular cell proliferations have been observed in radiotherapy + resveratrol group in the early period (Figure 4c). A relatively larger number of tubular proliferations with germ cells have been observed in radiotherapy + resveratrol group in the late period (Figure 4d). The data obtained from Johnsen scoring system coherent with these morphological findings. Radiotherapy applied groups had very low scores on Johnsen Scoring system, which indicated presence of degenerative seminiferous tubules. Resveratrol treated groups, even though the levels didn't reach back to control levels, showed significant improvement compared to radiation only groups in both early and late periods (Figure 5).

Discussion

Pelvic radiotherapy is used for many decades for the treatment of various cancers [18]. In clinical practice, especially in younger population, scattered radiation that has detrimental effects on testis is a major concern for both fertility and hormone production. One of the major protective methods against scattered radiation exposure to testis is the use of shielding. However, there is reflex contraction of cremaster muscle decreases the distance between the treatment area and the testis, when these shields are used. A 2 cm distance difference is responsible for a 37% increase in total accumulated dose [18]. So, additional protection against the effects that occurs due to oxidative processes caused by scattered radiation is needed. Radiotherapy has both direct and indirect effects on the target field. When radiation beams are focused on a specific area, a direct DNA oxidation occurs that leads to cell lysis and apoptosis. The indirect effects are caused by reactive oxygen species that are generated on both target area and the areas effected by scattered radiation. This secondary and rather delayed indirect effect, both due to ROS generated at the target site and also the scattered radiation effect, is the main perpetrator of adjacent tissue damage as well as a generalized inflammatory response in subjects undergoing pelvic radiotherapy [1, 19, 20].

The out-of-field effect of irradiation leads to molecular and cellular damage in distant non-irradiated tissues where oxidative stress is the most important underlying mechanism. Najafi demonstrated that the radiation applied to pelvic area of rats caused a significant inflammatory response in lung tissue, as demonstrated by the increased MDA and decreased GSH levels with decreased SOD activity [21]. When Mohye El-Din irradiated the cranial area of rats with 2 Gy X-ray, authors observed a significant increase in GSH levels, SOD and catalase activities in spleen tissues. They also demonstrated an increased apoptosis by showing a decrease in Bcl-2; increase of p53, Bax, caspase-3 and caspase-8 in spleen cells [22].

This oxidative response created with scattered radiation beams can be reversed with anti-oxidant agents. In

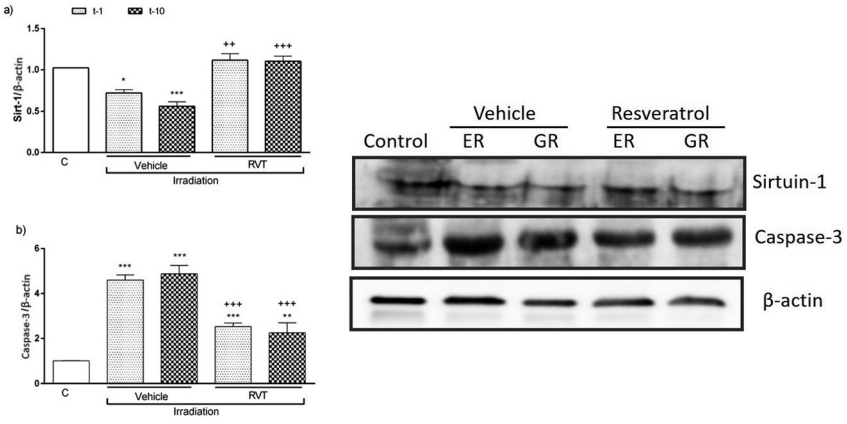

Figure 3: a) Sirtuin-1/B-actin ratio; b) Caspase-3/B-actin ratio; c) Sirtuin-1, Caspase-3, and B-Actin protein expressions on western blot. (*p<0.05, **p<0.01, ***p<0.001 compared to control group; +p<0.05, ++ p<0.01, +++ p<0.001, radiation + resveratrol group compared to radiation + vehicle group – comparisons have been made separately for both early and late periods).

Table 2: Testosterone levels.

Group	Control	Radiation + Vehicle		Radiation + Resveratrol	
		Early period	Late period	Early period	Late period
Testosteron levels, ng/mL	4.1 ± 0.21	3.8 ± 0.14	2.3 ± 0.12$^{***, \&\&\&}$	3.8 ± 0.11	3.6 ± 0.25^{+++}

***p<0.001: vs. control; $^{+++}$ p<0.001: vs. vehicle treated late radiation period; $^{\&\&\&}$ p<0.001: vs. resveratrol treated late radiation period.

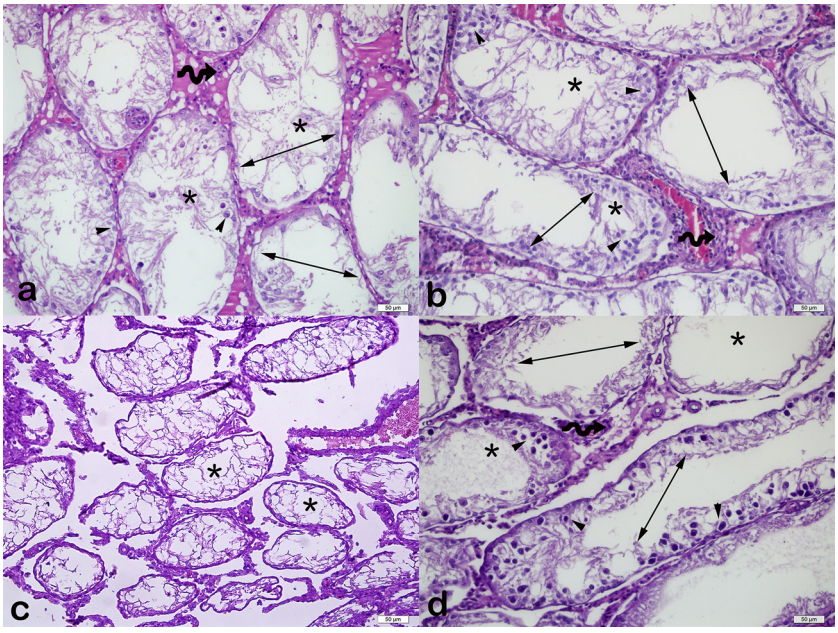

Figure 4: Histological examination; a) Radiotherapy group in early period: Tissue damage in tubular content (double-headed arrows), gaps in lumen (*), small number of germ cells (arrowheads), increased distance between tubules (S-shaped arrow); b) Radiotherapy group in late period: empty tubules showing severe tissue damage (*), only a couple of germ cells were observed in basal membrane; c) Radiotherapy + resveratrol group in early period: although tissue damage was observed in tubular content (double-headed arrows) and lumen (*) increase in germ cells was observed (arrowheads), reduction of intratubular space (S-shaped arrow); d) Radiotherapy + resveratrol group in late period: although tissue damage was observed in tubular content (double-headed arrows) and lumen (*) increase in germ cells was observed (arrowheads) on basal membrane, reduction of intratubular space (S-shaped arrow) was prominent.

Ghobadi's study, they showed that the inflammatory responses created by scattered radiation from the pelvic area were reversed with melatonin, a strong anti-oxidant agent, in the lung tissues of the rats. Melatonin administration before irradiation caused a significant reduction in glutathione peroxidase and MDA levels in both target and non-target tissues [23]. Similar results were obtained in our study. Decrease in GSH and SOD, anti-oxidant enzymes, due to depletion; increase in oxidative parameters; MPO as a marker of lipid peroxidation and MDA as a marker of neutrophil activation have been observed with radiation in both early (1 week after radiation) and late (10 weeks after radiation) periods. Preventive resveratrol treatment, given 1 week before radiation returned these parameters to

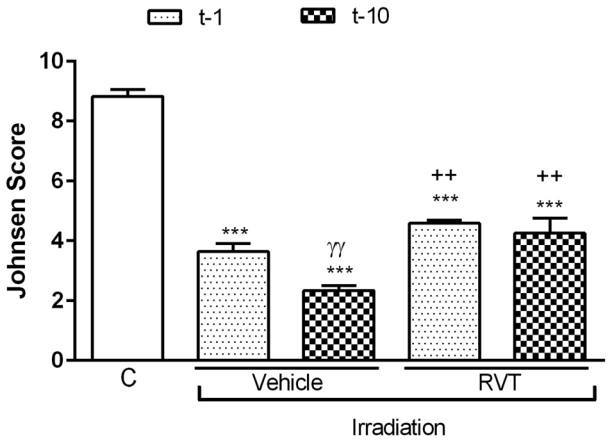

Figure 5: Johnsen scores. (***p<0.001: vs control; $^{++}$ p<0.001: vs. vehicle treated radiation periods; γ γ p<0.001: vs. vehicle treated early period).

control-like levels, showing a profound protective effect, especially in period groups, proving its strong anti-oxidant capabilities.

Radiotherapy increases 8-OHdG which indicates DNA oxidative damage. In Oscarsson's study, rats subjected to pelvic irradiation of 20 Gy demonstrated increased 8-OHdG levels in bladder tissues, which was normalized with Hyperbaric Oxygen Therapy [24]. Scattered radiation had also effects on 8-OHdG levels on lung tissues of pelvis-irradiated rats. Fardid demonstrated that after 3 Gy pelvic irradiation, 8-OHdG levels were significantly increased, showing DNA oxidation in non-target tissues. They also demonstrated that these effects were reversed with pre-radiotherapy treatment with the anti-oxidant melatonin [25]. In our study, resveratrol had protective effect on DNA oxidation, as evidenced by a profound amelioration of 8-OHdG levels in treatment groups compared to radiation-only groups on both 1-week and 10-weeks periods. Even though there is a significant therapeutic effect on DNA oxidation, the 8-OHdG levels remained significantly higher in the 10-week period despite resveratrol treatment; pointing out both the serious damage that occurs due to scattered radiation in long-term and the actual need for preventive measures in cases of pelvic radiation as DNA damage may cause serious secondary complications.

Sirtuin-1 inactivates the proapoptotic p53 protein through a NAD^+ dependent pathway. Resveratrol stimulates the PARP1 protein expression and ultimately increases NAD^+ concentration therefore increasing the sirtuin-1 activity. Therefore resveratrol is an indirect stimulator of sirtuin-1 activity [10]. It was also proven by Howitz that resveratrol slows down the aging process and increases cell lifespan by 70% by activation of Sirtuin-1 [26]. Also the inhibition of phosphodiesterases, which are the main targets of resveratrol, causes an intracellular cAMP increase and through a cascade of metabolic reactions, leads to activation of Sirtuin-1, which is another pathway for resveratrol to stimulate sirtuin-1 activity [27]. It was also reported in another study by our group that resveratrol had an important role in maintaining Sirtuin-1 levels and therefore protecting the nNOS and eNOS protein expressions in scattered radiation-exposed cavernosal tissues of rats [1]. As Sirtuin-1 expression has a very important role in cell survival, the restoration of Sirtuin-1 protein expression on Western Blotting in our study with the resveratrol treatment before radiotherapy may represent a major preventive option.

Caspase-3 protein expression is the marker of apoptosis in tissues [28]. In cases of oxidative response to various conditions, the levels of caspase-3 protein expression have been shown to be increased in tissues [28, 29]. Various antioxidant treatments have been shown to be effective against this inflammation-induced increase in caspase-3 protein expression [30]. In our study, radiotherapy and the scattered radiation effect on testicular tissues caused a major oxidative stress as evidenced by previously mentioned biomarkers and also increased the caspase-3 protein expression in testicular tissues, implicating a major apoptotic process in tissues. Resveratrol has been successful in decreasing the caspase-3 protein expression significantly, however, levels still remained significantly higher than control levels, showing that the scattered radiotherapy effect on tissues is a great threat to tissue integrity and cell survival.

Testosterone level decrease and even hypogonadism is an important problem after pelvic irradiation. In patients undergoing pelvic EBRT, 15.5% hypogonadism was reported after approximately 14 months post-radiotherapy and 2-year hypogonadism rate was 17.6%. Also, in the same series, 27% of the irradiated patients had low testosterone levels compared with 10% in non-irradiated patients. The most pronounced decrease was observed in the first 6 months following EBRT [6]. Pompe evaluated 248 patients who received EBRT for prostate cancer for testosterone changes. 186 patients (75%) had any kind of decrease from baseline, with 44.8% having at least 25% decrease, 13.3% having at least 50% decrease. 43.4% of patients had a decrease below 8 nmol/L, which indicates hypogonadism. 38.1% of these patients didn't recover from decreased testosterone levels. The authors claimed that this testosterone decrease is multifactorial and is due to scattered radiation and also psychological stress induced decrease [2]. Although rare in literature, testosterone levels have been studied in rats following pelvic RT. In Ekici's

study, time interval from RT (800 cGy) to testosterone assessment is 10 days. Authors claimed that the initial effects of RT arise in the first 3 days and in first 2 weeks, the concurrent effects occur [31]. They reported in this study that the testosterone level decreased in the radiotherapy group and was normalized back to control levels in rats that received the therapeuric agent before RT in a protective manner [31]. Kimura evaluated the testosterone level 2, 4, and 9 weeks after RT. Authors found no significant difference between the RT (single 20 Gy fraction) and sham groups. However, although not statistically significant, the numerical values are lower in RT group compared with sham groups (4 vs. 7) in the 2nd week following RT [32]. Biological half-life of testosterone ranges from 30 to 60 min (mean 45 min). When bilateral orchiectomy was performed, Lin reported that testosterone levels reached to castration levels in three to 12 h (mean 8.6 h) [33]. However, radiotherapy effect on testosterone is very different and effects occur in a much slower fashion compared to castration. The time period when testosterone assessment should be done after pelvic radiotherapy is vague. In our study, after radiotherapy, we found no decrease in the early period but observed a significant decrease in testosterone levels in the late period. Resveratrol, given in a protective manner before RT, prevented the decrease in the late period as observed by similar testosterone levels between the control group and the late period treatment group.

In previous studies, it has been shown that radiation exposure has detrimental effects on spermiogenesis reflected by histological examinations. In the study by Najafi, the authors have demonstrated significant spermatogenic arrest, atrophy of seminiferous tubules, thickening of basal lamina, Leydig cell hyperplasia, edema, epididymis vacuolation and reduction in Johnsen Scores in rats after exposure to radiotherapy. They also demonstrated that these effects can be prevented and ameliorated to some extent with antioxidants resveratrol, Q10 and alpha-lipoic acid. In antioxidant-treated groups increased Johnsen Scores and protected basal lamina structures were observed; however these agents couldn't protect from radiation induced edema, seminiferous tubule atrophy and Leydig cell hyperplasia [34]. In our study, histological examination has shown severe tissue damage in both early and late periods of radiotherapy, with late period showing a more pronounced effect, which is due to the late detrimental effects of radiotherapy with severe inflammatory response. Resveratrol treatment before radiotherapy could prevent the damage and germ cells could have been observed in testicular tissues, showing the beneficial effects of treatment. These effects are also reflected on the Johnsen Scores of groups. Resveratrol treatment couldn't restore the scores back to control levels however in the radiation groups that received resveratrol treatment, scores are significantly higher than that of radiation groups that didn't receive resveratrol treatment, in both early and late periods.

Lack of FSH and LH measurements is a limitation of the study. The evidence of Leydig cell damage could be demonstrated with the ratio of testosterone to LH as higher ratios mean low testosterone with a compensatory increase in LH due to Leydig cell dysfunction. Compensatory increases in FSH can be used as a marker of Sertoli cell damage [6]. However due to technical deficiencies, the evaluation of FSH and LH is planned to be performed in a future study.

Conclusion

Radiotherapy and the scattered radiation beams cause significant tissue damage both in the target area and the adjacent tissues. The protective mechanisms against the unwanted out-of-field-effects rely mostly on preventing the oxidative stress and the inflammatory process with antioxidants. In our study, resveratrol has been shown to prevent and ameliorate the tissue damage caused by pelvic radiation and scattered radiation effects in rat testicular tissues.

Resveratrol can be provided as a daily over-the-counter agent and with its strong antioxidant and anti-inflammatory effects along with its high safety profile, resveratrol can provide a new prophylactic approach against the bystander effects of radiotherapy.

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