Current Eye Research

ISSN: 0271-3683 (Print) 1460-2202 (Online) Journal homepage: http://www.tandfonline.com/loi/icey20

# Effects of Molsidomine on Retinopathy and Oxidative Stress Induced by Radiotheraphy in Rat Eyes

Murat Atabey Özer, Nihat Polat, Serkan Özen, Hakan Parlakpınar, Kemal Ekici, Alaaddin Polat, Nigar Vardı, Kevser Tanbek & Azibe Yildiz

**To cite this article:** Murat Atabey Özer, Nihat Polat, Serkan Özen, Hakan Parlakpınar, Kemal Ekici, Alaaddin Polat, Nigar Vardı, Kevser Tanbek & Azibe Yildiz (2016): Effects of Molsidomine on Retinopathy and Oxidative Stress Induced by Radiotheraphy in Rat Eyes, Current Eye Research, DOI: 10.1080/02713683.2016.1238943

**To link to this article:** http://dx.doi.org/10.1080/02713683.2016.1238943

CURRENT EYE RESEARCH
http://dx.doi.org/10.1080/02713683.2016.1238943

Taylor & Francis
Taylor & Francis Group

# Effects of Molsidomine on Retinopathy and Oxidative Stress Induced by Radiotheraphy in Rat Eyes

Murat Atabey Özer [a], Nihat Polat[b], Serkan Özen[a], Hakan Parlakpınar[c], Kemal Ekici[d], Alaaddin Polat[e], Nigar Vardı[f], Kevser Tanbek[e], and Azibe Yildiz[f]

[a]Department of Ophthalmology, Medical Faculty, Giresun University, Giresun, Turkey; [b]Department of Ophthalmology, Medical Faculty, Inonu University, Malatya, Turkey; [c]Department of Pharmacology, Medical Faculty, Inonu University, Malatya, Turkey; [d]Department of Radiation Oncology, Medical Faculty, Inonu University, Malatya, Turkey; [e]Department of Physiology, Medical Faculty, Inonu University, Malatya, Turkey; [f]Department of Histology and Embryology, Medical Faculty, Inonu University, Malatya, Turkey

**ABSTRACT**

*Purpose*: To determine the role of Molsidomine in preventing radiation-induced retinopathy after head and neck region irradiation of rats with a single radiation dose of 15 Gy.

*Materials and Methods*: Male Wistar albino rats were randomly grouped into five as follows: (1) control group rats, which were applied through an intraperitoneal (i.p.) vehicle without radiotherapy (RT); (2) RT group rats received a single dose of 15 Gy irradiation and after daily 0.1 ml vehicle i.p. for 5 consecutive days; (3) molsidomine (MOL) group rats were treated for 5 consecutive days by i.p. with 4 mg/kg/day MOL; (4) irradiation plus MOL group (RT+MOL) rats received irradiation and after 10 days single daily i.p. dose of MOL for 5 consecutive days; and (5) MOL+RT group rats were treated for 5 consecutive days by i.p. with MOL before RT. At the end of the work the rats were sacrificed under high-dose anesthesia on the 16$^{th}$ day and then eye tissues were taken for histopathological, immunohistochemical (caspase-3), and biochemical analyses (superoxide dismutase [SOD], glutathione peroxidase [GSH], and malondialdehyde [MDA]).

*Results*: RT significantly decreased both the content of GSH and the activity of SOD, and significantly increased the production of MDA level in the rat eyes. MOL treatment significantly increased the SOD and GSH levels and significantly decreased the MDA production ($p < 0.0001$). In addition, RT significantly increased the number of ganglion cells (GCs; $p = 0.001$), whereas especially pretreatment with MOL improved ($p = 0.013$). RT led to significant retinopathy formation, and MOL therapy protected the retina from radiation-induced retinopathy ($p < 0.0001$).

*Conclusions*: We suggest that MOL is a powerful antioxidant and free radical scavenger that prevents the rat eyes from radiation-induced retinopathy and oxidative stress.

**ARTICLE HISTORY**
Received 10 May 2016
Revised 12 August 2016
Accepted 12 September 2016

**KEYWORDS**
Antioxidant; apoptosis; ionizing radiation; molsidomine; oxidative stress

## Introduction

Each year, 75,000 new cases of cranial region cancer are diagnosed in the United States, and 40% of these cases end in death.[1] Except for cancers of the salivary glands, bone, thyroid, and soft tissues, cancers in the cranial region are mostly squamous cell carcinoma. Radiation therapy (RT) with or without surgery has been used as a standard treatment modality for cranial region cancers,[2] and recently, orbital cancers such as uveal malignant melanoma have been treated with RT in addition to other treatments.[3]

It is known that ionizing radiation has some serious side effects such as dry eye, eyelash loss, cataracts, neovascular glaucoma, radiation retinopathy (RR), optic neuropathy, and secondary cancers.[4] Although the side effect mechanism of ionizing radiation on the eye has not been fully clarified, it is known that radiation generates oxidative stress on the eye tissues. Reactive oxygen species (ROS) are the main cause of oxidative stress. By the decomposition of the water superoxide radical ($O_2^-$), the hydroxyl radical ($OH^-$), and hydrogen peroxide ($H_2O_2$), ROS are produced. The harmful effects of ROS are balanced by the action of antioxidants, but insufficient antioxidants lead to the damage of cellular structures such as DNA, the cellular membrane, or intracellular molecules.[5] The use of antioxidant agents may protect against these effects of ROS.[6–8] In this study, we will be focusing on oxidative stress, apoptosis, and radiation-induced retinopathy.

Molsidomine (MOL) is a nitric oxide (NO) donor and widely used as a vasodilating, antianginal agent. MOL is a prodrug and transformed into the metabolite 3-morpholinosydnonimine (SIN-1), which spontaneously provides NO.[9] NO has a significant impact on tissue injury, inflammation, and cell defense, as well as on vasodilation. In addition, MOL has been shown to be a powerful antioxidant, antiapoptotic, and anti-inflammatory agent.[10] Based on these characteristics, MOL therapy may be helpful in eye tissue damage caused by ionizing radiation, which results in oxidative stress and the production of excessive reactive radicals.

In the literature, there is no investigation of MOL against RR caused by RT. This study was therefore designed to explore the possible protective and therapeutic effects of MOL on eye tissue damage caused by ionizing radiation.

**CONTACT** Murat Atabey Özer dratabeyozer@yandex.com Department of Ophthalmology, Medical Faculty, Giresun University, Giresun 28100, Turkey.
Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/icey.

## Materials and methods

All animal care procedures were approved by the Local Animal Experimentation Ethics Committee of Inonu University, and all experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. Male Wistar albino rats with an average age of 14–18 weeks (200–250 g) were housed under standard temperature of 20 ± 2°C and humidity of 55–60%, on a 12:12 hour light/dark cycle. The rats were randomly sorted into five groups (n = 8) as follows: 1) control group rats received an intraperitoneal (i.p.) vehicle applied without RT; 2) RT group rats received a single dose of 15 Gy irradiation, then a daily dose of 0.1 ml i.p. for five consecutive days; 3) MOL group rats were treated for five consecutive days by i.p. with 4 mg/kg/day MOL (molsidomine, Sigma Chemical Co., St Louis, MO, USA); 4) irradiation plus MOL group rats (RT+MOL) received irradiation and after 10 days, they received a single daily i.p. dose of MOL (4 mg/kg/day) for five consecutive days; and 5) MOL plus RT (MOL+RT) group rats were treated for five consecutive days by i.p. with 4 mg/kg/day MOL before irradiation.

The dose of MOL was chosen based on the previous dose-response studies that have been reported to affect the antioxidative effects in rats.[11,12]

### RT application

Before RT application, the rats were weighted and anesthetized with ketamine (30 mg/kg) and xylazine (5 mg/kg) i.p., and placed in a supine position. External whole-cranium irradiation was given with a 30 × 30 × 5 cm animal-fixing box, in which eight rats were kept for each irradiation, using 6 MV photons from the linear accelerator machine (Artiste®, Siemens Medical Solutions USA, Inc., Malvern, PA). To insure that the eyes and ears received a maximal dose, a wax bolus material of 1-cm thickness was placed over the rat cranium. The dose was calculated as Dmax dose at 1.5 cm depth. Irradiation was delivered for skin source distance (SSD) 100 cm technique and was given as a single dose of 15 Gy (Figure 1a and b).

The animals in each group were sacrificed under high-dose anesthesia on the 16th day, and then eye tissues were taken for histopathological and biochemical analyses at the end of the work.

### Biochemical analysis

Two hundred milligrams of frozen whole eye cup specimens, cut into pieces on dry ice, were homogenized in a 1.15% KCl buffer using a manual glass homogenizer for approximately 5 minutes, and flushed with centrifugation for approximately 10 seconds to remove large debris. The supernatant was used for analysis.

### Determination of eye tissue thiobarbituric acid-reactive substances representing malondialdehyde (MDA) content

The MDA contents of the homogenates were determined spectrophotometrically, by measuring the presence of thiobarbituric acid reactive substances (TBARS).[13] Three mL of 1% phosphoric acid and 1 mL 0.6% thiobarbituric acid solution were added to 0.5 mL of homogenate in a tube, for this reaction. This mixture was warmed in boiling water for 45 minutes. After the mixture had cooled, the colored part was extracted into 4 mL of n-butanol. The absorbance was measured using a spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan) at 532 nm. The amount of lipid peroxides was calculated as TBARSs of lipid peroxidation, and the results were given in nanomoles per gram tissue (nmol/g tissue) according to a prepared standard graph.

### Determination of SOD

SOD activity was determined with Sun et al.'s method[14] by inhibiting nitroblue tetrazolium (NBT) reduction with xanthine/xanthine oxidase used as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was expressed as units per milligram protein (U/mg protein).

### Determination of reduced glutathione content

The GSH content in the eye tissue was analyzed following a previously described method.[15] An enzymatic reaction in a tube containing NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase was initiated by the addition of $H_2O_2$, and the change in absorbance at 340 nm was observed by a spectrophotometer. Activity was given in units per gram protein (μmol/g tissue).

### Histological evaluation

Enucleated eyes were fixed in 10% formalin and embedded in paraffin. Paraffin-embedded specimens were cut into 4-μm-thick sections as horizontal sections through the optic disk of the eye, and mounted on slides. The sections were stained with hematoxylin and eosin. The measurements of the retina thickness and various retinal layers for each eye were performed in the same topographic region of the retina. Thicknesses of the inner plexiform layer to the inner limiting membrane (IPL-ILM, a measure of inner retinal thickness), the inner nuclear layer (INL), and the outer nuclear layer (ONL) were measured for each eye. An additional count of GCs was performed. Averages for these measurements of the thickness and cell counts taken in five adjacent areas within 1–2 mm of the optic disk were calculated. We carried out all of the morphometrical analyses in a blind fashion.

For immunohistochemical analyses, thick sections were taken onto polylysine-coated slides. After rehydrating, the samples were transferred to a citrate buffer (pH 7.6) and heated in a microwave oven for 20 minutes. After cooling for 20 minutes at room temperature, the sections were washed with phosphate buffer saline (PBS). Then, the sections were kept in 3% $H_2O_2$ for 7 minutes and afterward washed with PBS. After that, the sections were incubated with primary rabbit-polyclonal cysteine aspartate specific proteases (caspase-3) (Neomarker), then rinsed in PBS, and incubated with biotinylated goat anti-polyvalent for 10 minutes and streptavidin peroxidase for 10 minutes at room temperature. The staining procedure was completed with chromogen+substrate for 15 minutes, and the slides were counterstained with Mayer's hematoxylin for 1 minute. Caspase-3 was used according to the manufacturer's

instructions except for one minor revision. GCs stained with caspase-3 were counted. Five areas for each specimen were examined under 40× magnification. For histological evaluation (layer measurements and cell counts), a LeicaDFC280 light microscope by X40 objective (Leica Micros Imaging Solutions Ltd, Cambridge, UK) was used.

### Statistical analysis

For detecting even minor effects, the required sample sizes used in this experiment were identified using statistical power analysis. The sample sizes necessary for a power of 0.80 were estimated using NCSS software. All data were analysed with a commercially available statistical software package (SPSS for Windows v. 15.0, Chicago, Ill., USA). Distributions of the groups were analysed with the Kolmogorov–Smirnov test, and all groups showed a normal distribution. For all parameters, one-way ANOVA was performed with Tukey's post hoc test. Results were presented as mean±SD, and $p < 0.05$ was regarded as statistically significant in all data.

## Results

### Biochemical results

In brief, as shown in Table 1, the MDA level was significantly higher in the RT group when compared to the control, MOL, MOL+RT, and RT+MOL groups ($p < 0.0001$). In the eye tissue, SOD and GSH levels were significantly lower in the RT group when compared with the other groups ($p < 0.0001$).

### Histological results

Layers of the retina were observed as regular and intact in the control and MOL groups (Figure 2a and b).However, the RT group showed mild disorganization of the INL and IPL. The overall retinal thickness was significantly decreased in the RT group (152.26 ± 24.32) compared to the control group (174.80 ± 15.97) due to loss of cell layers ($p<0.05$). In addition, there was a more marked loss of cell bodies within the ganglion cell layer (GCL) in the RAD group (17.60 ± 7.12) than in the control group (25.70 ± 5.02) (Figure 3a) ($p < 0.05$). Also, the thicknesses of IPL-ILM (41.42 ± 8.97) and INL (25.89 ± 5.18) were significantly lower than the control group (70.07 ± 8.36–32.28 ± 4.19, respectively) ($p < 0.05$).

On the other hand, the administration of MOL significantly protected the thickness of the overall retina against radiation damage. Despite that the thinness of INL was improved by MOL treatment, there was no statistically significant difference between the RT and MOL treatment groups ($p > 0.05$). Treatment with MOL after radiation application was more effective than treatment with MOL before RT application with regard to protection of the thickness of IPL-ILM. A normal pattern of organization in the ONL was observed in all experimental groups. Likewise, the thickness of the ONL did not change with the application of radiation. The total number of GCs was higher in the MOL treatment groups than in the RT group, but no histologic differences were found among these groups ($p>0.05$) (Figure 3b and c).

In the caspase-3 examination, the control and MOL groups showed a few caspase-3-positive cells (Figure 4a and b). In contrast, caspase-3-positive cells in the GCL were found to be significantly increased in the RT group

**Table 1.** The comparison of the eye-tissue lipid peroxidation parameter and antioxidant contents among the study groups.

| Parameters | | Control | RT | MOL | MOL+RT | RT+MOL |
|---|---|---|---|---|---|---|
| MDA | nmol/g tissue | 14.38 ± 4.28 | 27.47 ± 3.49[a] | 14.86 ± 4.06[b] | 14.89 ± 1.66[b] | 16.18 ± 3.12[b] |
| SOD | U/mg protein | 0.60 ± 0.05 | 0.42 ± 0.01[c] | 0.58 ± 0.05[d] | 0.57 ± 0.03[d] | 0.59 ± 0.06[d] |
| GSH | µmol/g tissue | 29.63 ± 2.47 | 15.49 ± 2.99[c] | 27.20 ± 3.49[d] | 28.86 ± 3.82[d] | 26.93 ± 2.24[d] |

Data are presented as mean ± SD.
[a]Significant increase versus control group ($p < 0.0001$).
[b]Significant decrease versus RT group ($p < 0.0001$).
[c]Significant decrease versus control group ($p < 0.0001$).
[d]Significant increase versus RT group ($p < 0.0001$).

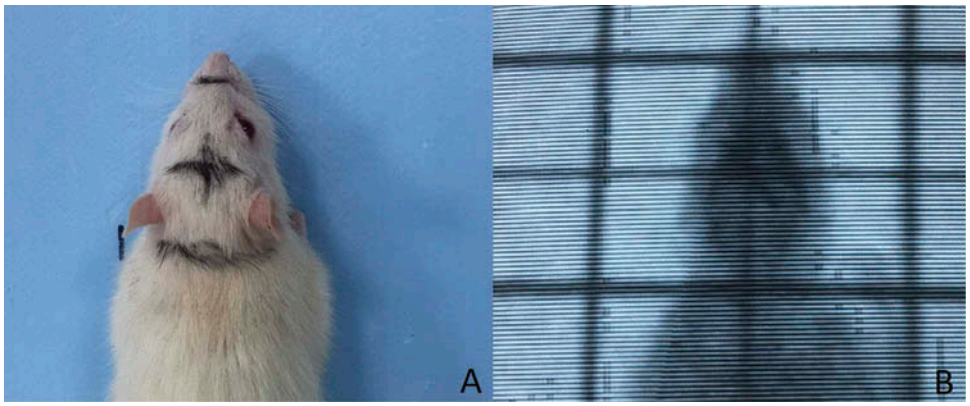


**Figure 1.** Irradiation field (a), and simulation radiograph (b).

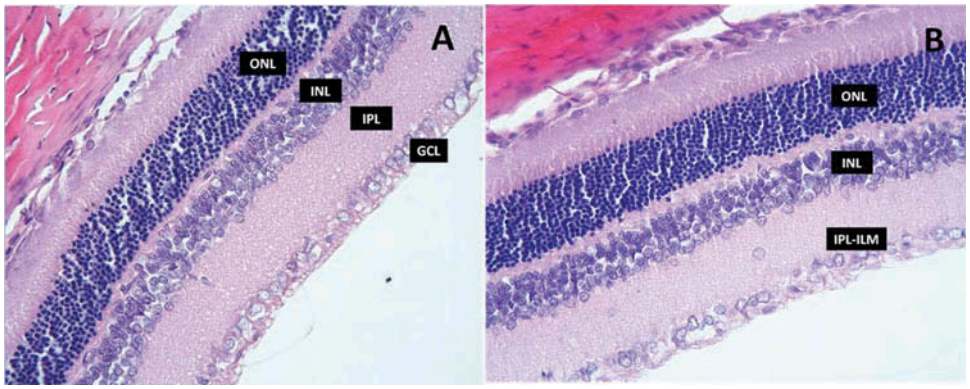


**Figure 2.** (a) Control and (B) MOL groups; layers of retina are regular. H-E X40. ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, ILM: inner limiting membrane, GCL: ganglion cell layer.

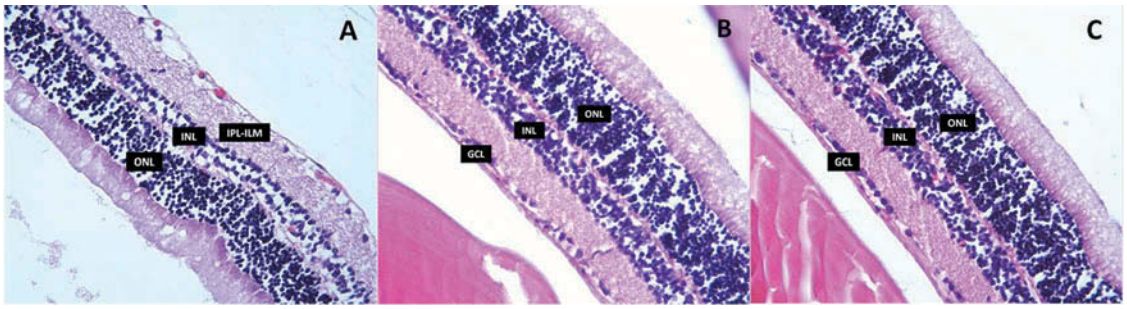


**Figure 3.** (a) RAD group; mild disorganization of INL and its thinness are evident than the control group. (b) RAD+MOL and (c) MOL+RAD groups; the histologic appearance of the groups is nearly similar to each other. H-E X40. ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer, ILM: inner limiting membrane, GCL: ganglion cell layer.

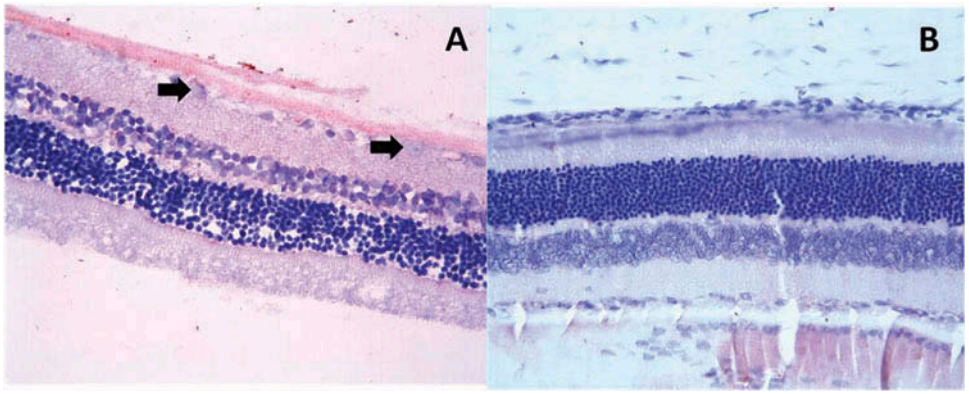


**Figure 4.** (a) Control group; the views of normal retina, ganglion cells are seen as caspase-3 negative (thick arrow). (b) MOL group; the aspect of caspase-3 (–) staining is close to the control group. Caspase-3 immunostaining X40.

(10.00 ± 1.58) when compared with the control group (0.20 0.45) ($p < 0.05$) (Figure 5a). Treatment with MOL before radiation application (2.40 ± 2.70) was found to be more effective than treatment with MOL after radiation application (8.80 ± 6.06) in terms of preventing the apoptosis in the GC (Figure 5b and c).

Thickness of the overall retina, retinal layers, total number of the GCs, and results of the immunohistochemical staining are given Tables 2 and 3.

## Discussion

RT is widely used to treat and prevent the recurrence of many tumors in the cranial region.[2] Ionizing radiation causes cellular damage by several mechanisms, with the major mechanism being the DNA strand breaking in the cells. Shortly, radiation-induced ionizations may act directly on the cells or indirectly on water molecules, causing water-derived radicals. Radicals react with molecules, resulting in the breakage of chemical bonds or in

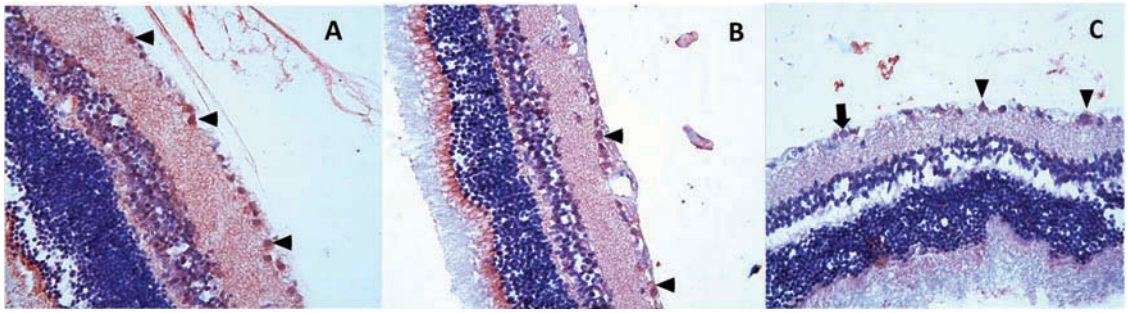


**Figure 5.** (a) RAD group; abundant caspase-3 staining cells within GCL are observed (arrow heads). (b) RAD+MOL group; there are obvious caspase-3 (+) cells in the GCL (arrow heads). (c) MOL+RAD group; a prominent reduction of caspase-3 (+) staining cells (arrow heads) is seen in the GCL according to the RAD+MOL group (thick arrow indicates caspase (–) ganglion cell). Caspase-3 immunostaining X40.

**Table 2.** Thickness of the retinal layers in the all groups (μm).

| Groups | The overall retina | IPL- ILM | INL | ONT |
|---|---|---|---|---|
| Control | 174.80 ± 15.97 | 70.07 ± 8.36 | 32.28 ± 4.19 | 58.57 ± 3.81 |
| MOL | 172.15 ± 19.14 | 65.03 ± 11.61 | 33.01 ± 3.95 | 58.92 ± 9.85 |
| RT | 152.26 ± 24.32 | 41.42 ± 8.97 | 25.89 ± 5.18 | 57.41 ± 9.49 |
| MOL+RT | 177.08 ± 21.61 | 47.62 ± 9.21 | 29.03 ± 3.14 | 63.02 ± 8.10 |
| RT+MOL | 174.92 ± 16.20 | 48.63 ± 6.81 | 28.86 ± 4.58 | 59.46 ± 7.87 |
| *p*-Values | The overall retina | IPL- ILM | INL | ONT |
| Control-MOL | 1.000 | 0.1 | 1.000 | 1.0 |
| Control-RT | *0.001* | *0.0001* | *0.0001* | 1.000 |
| RT-MOL+RT | *0.001* | 0.102 | 0.063 | 0.156 |
| RT-RT+MOL | *0.001* | 0.009 | 0.200 | 0.989 |
| MOL+RT-RT+MOL | 1.000 | 1.000 | 1.000 | 0.609 |

*Note.* Italicized 0.001 and 0.0001 values of *p* are assumed as statistically significant.

**Table 3.** The effect of MOL on the number of total ganglion cells and caspase-3 expression in the ganglion cell layer of RAD-applied rats.

| Groups | Casp (+) GC | Total number of GC |
|---|---|---|
| Control | 0.20 ± 0.45 | 25.70 ± 5.02 |
| MOL | 0.20 ± 0.45 | 30.77 ± 6.37 |
| RT | 10.00 ± 1.58 | 17.60 ± 7.12 |
| MOL+RT | 2.40 ± 2.70 | 22,93 ± 7.30 |
| RT+MOL | 8.80 ± 6.06 | 19.47 ± 6.38 |
| *p*-Values | Casp (+) GC | Total number of GC |
| Control-RT | *0.001* | *0.0001* |
| RT-MOL+RT | *0.013* | 0.057 |
| RT-RT+MOL | 1.000 | 0.967 |
| MOL+RT-RT+MOL | 0.557 | 0.432 |

GC: Ganglion cell.
*Note.* Italicized <0.05, 0.001 and 0.0001 values of *p* are assumed as statistically significant.

oxidation (addition of oxygen atoms) of the affected molecules. Consequently, the cell develops apoptosis and cell death due to this DNA strand breakage.[16, 17] In the current study, we established a retinopathy model by a 15 Gy single dose of radiation to the whole head and neck region in the rats.[5]

We evaluated the biochemical parameters (MDA, SOD, GSH) for analyzing oxidative stress. The MDA levels served as an index of cellular damage by free radicals. MDA is a by-product of oxidant-induced lipid peroxidation and protein oxidation, and leads to irreversible cell damage.[15] SOD and GSH are part of the antioxidant defense system, and these molecules protect cells against oxidative damage. An imbalance between TBARS and the antioxidant defense system leads to oxidative stress. The most sensitive cellular components in ROS formation are membrane lipids, proteins, nucleic acids, and DNA molecules.[18,19] In the present study, we observed that RT exposure led to lipid peroxidation by increasing the MDA levels, yet the MDA levels almost returned to control levels with MOL treatment. There were lower GSH and SOD levels in the RT group and the control group, and treatment with MOL reduced the RT damage by reducing oxidative stress and increasing antioxidant activity. This reduction occurs because MOL is a strong, direct, free radical scavenger and an indirect antioxidant through the induction of antioxidant enzymes.[8,10]

RR is a chronic and progressive illness that may result from the exposure to radiation.[20,21] RR may be from unavoidable exposure to excessive radiation from the treatment of head and neck region malignancies, or secondary to the treatment of intraocular tumours.[22–24]

If enough radiation is absorbed, it will begin a series of reactions that lead to cell death, which stimulates division and migration of cells to repair the discontinuity in the vessel wall. Migratory cells that are sensitive to radiation initiate a vicious cycle that might trigger the clotting cascade due to the incompetence of the vascular endothelium, leading to clinically observable RR.[25–27]

In 1987, Irvine and Wood were the first to experimentally model radiation-induced retinopathy.[28] Thereafter, prominent retinal changes in rats following a single dose of 15–20 Gy were demonstrated by Amoaku.[29] RR was histologically characterized by apoptosis, leukocyte adhesion and stasis, vessel occlusion, retinal endothelial cell death, hypoxia, occlusive changes, microaneurysmal changes in capillaries,

parenchymal inflammation, presence of necrotic and gliotic regions, glial hypertrophy, neuronal swelling, and degeneration.[30,31] It is known that MOL, which is an NO donor, has a beneficial effect on microangiopathy and occlusive changes. Because NO is a powerful vasodilator, it plays a crucial role in maintaining tissue blood flow and perfusion. Furthermore, NO blocks leukocyte adhesion and platelet activation, and prevents smooth muscle cell proliferation.[32] Because of these characteristics, we thought that MOL treatment might exert beneficial effects against RR generation.

GCs are very important for visual function; as is known, rods and cones transform light energy into an electrical signal and convey information through retinal ganglion cells (RGCs) the only neurons in the retina that send axonal projections to the brain.[33] It is well established that caspases are vital mediators of apoptosis. Among these caspases, caspase-3 is known as an effector caspase and an initiator of the death cascade and, thus, an important marker of the cell's entry point into the apoptotic signaling pathway.[34] For this reason, in our study, caspase-3 positive GC and total number of GC in the GCL were calculated. RT led to apoptosis and notable histological changes such as mild disorganization of INL and IPL, and a reduced overall retinal thickness and a lower number of cell bodies within the GCL were observed. In the immunohistochemical examination, RT led to a significant increase in caspase-3-positive cells in the GCL.

In the case reports, the beneficial effects of certain drugs such as systemic corticosteroids and pentoxifylline on RR have been observed by Shields CL et al.[35] and Gupta P et al.,[36] respectively. In some experimental RR models, different antioxidant molecules such as vitamin-E, L-carnitine, and amifostine were tested and successful results were obtained.[5,37] We also believe that powerful antioxidants may reduce the effects of ionizing radiation. In this study, we observed that MOL showed positive effects on radiation-induced retinopathy. However, we found pretreatment with MOL more effective than posttreatment with MOL in terms of preventing the apoptosis in the GC.

There are several studies about the effects of irradiation on the retina and/or other eye tissues and treatment of these adverse effects in the literature. In these works, only apoptosis or biochemical parameters were investigated.[37,38] Akkus Yıldırım et al.[37] studied the prevention of radiation-induced retinopathy with amifostine in rats. In this study, retinopathy was evaluated only by using apoptotic markers. Similarly, Ertekin et al.[38] studied a cataract model by using biochemical parameters. However, in our study, we analyzed oxidative stress markers, apoptosis with caspase cascade, and retinal layer thickness. From this standpoint, we believe that our work is more comprehensive and enlightening. Even though there are recent studies examining the effects of MOL on other organs and tissues such as the brain, kidney, and heart, its effects on radiation-induced retinopathy and other ocular pathologies have not yet been studied.

In conclusion, we suggest that when RT is applied to the head and neck region, it triggers oxidative stress and leads to radiation-induced retinopathy. MOL seems to prevent oxidative stress and radiation-induced retinopathy at least partially in rats. In this regard, this study forms a basis for such preclinical experiments and calls for clinical studies, which may soon verify useful results. Although the results of our study are promising, there are several limitations. First, the level of inducible nitric oxide synthase (iNOS) expression is valuable to determine the retinal status and necessary for supporting our results. In addition, RR should be confirmed by the fundus photography. However, because of limited facilities, we could not assay it unfortunately. According to our results, we believed that widespread use of systemic antioxidants and anti-inflammatories such as MOL will be seen in the near future. However, further experimental and clinical studies are required to confirm these findings before conducting clinical applications for preventing and treating radiation-induced retinopathy.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## ORCID

Murat Atabey Özer http://orcid.org/0000-0003-1807-6911

## References

1. Davies L, Welch HG. Epidemiology of head and neck cancer in the United States. Otolaryngol Head Neck Surg 2006;135:451–457.
2. Chen JH, Yen YC, Liu SH, Lee FP, Lin KC, Lai MT, et al. Dementia risk in ırradiated patients with head and neck cancer. Medicine (Baltimore) 2015;94(45):1983.
3. Weis E, Salopek TG, McKinnon JG, Larocque MP, Temple-Oberle C, Cheng T, et al. Management of uveal melanoma: a consensus-based provincial clinical practice guideline. Curr Oncol 2016;23 (1):57–64.
4. Reichstein D. Current treatments and preventive strategies for radiation retinopathy. Curr Opin Ophthalmol 2015;26(3):157–166.
5. Sezen O, Ertekin MV, Demircan B, Karslioğlu I, Erdoğan F, Koçer I, et al. Vitamin E and L-carnitine, separately or in combination, in the prevention of radiation-induced brain and retinal damages. Neurosurg Rev 2008;31(2):205–213.
6. Acar DE, Acar U, Yumusak N, Korkmaz M, Acar M, Atilgan HI, et al. Reducing the histopathological changes of radioiodine to the lacrimal glands by a popular anti-oxidant: lycopene. Curr Eye Res 2014;39(7):659–665.
7. Acar U, Atilgan HI, Acar DE, Yalniz-Akkaya Z, Yumusak N, Korkmaz M, et al. The effect of short-term vitamin E against radioiodine-induced early lacrimal gland damage. Ann Nucl Med 2014;27(10):886–891.
8. Karakoc HT, Altintas R, Parlakpinar H, Polat A, Samdanci E, Sagir M, et al. Protective Effects Of Molsidomine Against Cisplatin-Induced Nephrotoxicity. Adv Clin Exp Med 2015;24 (4):585–593.
9. Kalouda T, Pitsikas N. The nitric oxide donor molsidomine induces anxiolytic-like behaviour in two different rat models of anxiety. Pharmacol Biochem Behav 2015;138:111–116.
10. Toplu Y, Parlakpinar H, Sapmaz E, Karatas E, Polat A, Kizilay A. The protective role of molsidomine on the Cisplatin-induced ototoxicity. Indian J Otolaryngol Head Neck Surg 2014;66(3):314–319.
11. Ozturk K, Ozyurt H, Somay A, Karaca C. The effects of nitric oxide donor molsidomine on skeletal muscle damage in a rat hind limb model of ischemia-reperfusion. Eur Surg Res 2009;42:71–77.
12. Bentli R, Parlakpinar H, Polat A, Samdanci E, Sarihan ME, Sagir M. Molsidomine Prevents Cisplatin-induced Hepatotoxicity. Arch Med Res 2013;4(7):521–528.

13. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by tiobarbituric acid test. Anal Biochem 1978;86:271–278.
14. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1998;34(3):497–500.
15. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–70.
16. Ward JF. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 1988;35:95–125.
17. Bardak Y, Cekic O,Totan Y, Cengiz M. Effect of verapamil on lenticular calcium, magnesium and iron in radiation exposed rats. Int Ophthalmol 1998;22:285–288.
18. Ozansoy G, Akin B, Aktan F, Karasu C. Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. Mol Cell Biochem 2001;216:59–63.
19. Ceylan A, Karasu C, Aktan F, Guven C, Can B, Ozansoy G. Effects of simvastatin treatment on oxidant/antioxidant state and ultrastructure of diabetic rat myocardium. Gen Physiol Biophys 2003;22:535–547.
20. Krema H, Somani S, Sahgal A, Xu W, Heydarian M, Payne D, et al. Stereotactic radiotherapy for treatment of juxtapapillary choroidal melanoma: 3-year follow-up. Br J Ophthalmol 2009;93:1172–1176.
21. Seibel I, Hager A, Riechardt AI, Davids AM, Böker A, Joussen AM. Antiangiogenic or corticosteroid treatment in patients with radiation maculopathy after proton beam therapy for uveal melanoma. Am J Ophthalmol 2016;168:31–39.
22. Bianciotto C, Shields CL, Pirondini C, Mashayekhi A, Furuta M, Shields JA. Proliferative radiation retinopathy after plaque radiotherapy for uveal melanoma. Ophthalmology 2010;117 (5):1005–1012.
23. Stannard C, Sauerwein W, Maree G, Lecuona K. Radiotherapy for ocular tumours. Eye (Lond) 2013;27(2):119–127.
24. Amoaku WM, Archer DB. Cephalic radiation and retinal vasculopathy. Eye (Lond) 1990;4(1):195–203.
25. Archer DB, Amoaku WM, Gardiner TA. Radiation retinopathy – clinical, histopathological, ultrastructural and experimental correlations. Eye (Lond) 1991;5(2):239–251.
26. Irvine AR, Alvarado JA, Wara WM, Morris BW, Wood IS. Radiation retinopathy: an experimental model for the ischemic – proliferative retinopathies. Trans Am Ophthalmol Soc 1981;79:103–22.
27. Giuliari GP, Sadaka A, Hinkle DM, Simpson ER. Current treatments for radiation retinopathy. Acta Oncol 2011;50(1):6–13.
28. Irvine AR, Wood IS. Radiation retinopathy as an experimental model for ischemic proliferative retinopathy and rubeosis iridis. Am J Ophthalmol 1987;103:790–797.
29. Amoaku WMK. The effects of ionizing radiation on the retina: a clinical and experimental study [thesis]. Belfast Ireland, Queen's University 1975.
30. Archer DB, Amoaku WM, Gardiner TA. Radiation retinopathy—clinical, histopathological, ultrastructural and experimental correlations. Eye (Lond) 1991;5:239–251.
31. Hiroshiba N, Ogura Y, Sasai K, Nishiwaki H, Miyamoto K, Hamada M, et al. Radiation-induced leukocyte entrapment in the rat retinal microcirculation. Invest Ophthalmol Vis Sci 1999;40:1217–1222.
32. Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 2003;54(4):469–487.
33. Fahy ET, Chrysostomou V, Crowston JG. Mini-Review: Impaired Axonal Transport and Glaucoma. Curr Eye Res 2016;41 (3):273–283.
34. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ 1999;6(2):99–104.
35. Shields CL, Demirci H, Marr BP, Mashayekhi A, Dai VV, Materin MA, et al. Intravitreal triamcinolone acetonide for acute radiation papillopathy. Retina 2006;26:537–544.
36. Gupta P, Meisenberg B, Amin P, Pomeranz HD. Radiation retinopathy: the role of pentoxifylline. Retina 2001;21:545–547.
37. Akkus Yildirim B, Çetin E, Topkan E, Ozyigit G, Cengiz M, Surucu S, et al. Prevention of radiation-induced retinopathy with amifostine in wistar albino rats. Retina 2015;35 (7):1458–1464.
38. Ertekin MV, Koçer I, Karslioğlu I, Taysi S, Gepdiremen A, Sezen O, et al. Effects of oral Ginkgo biloba supplementation on cataract formation and oxidative stress occurring in lenses of rats exposed to total cranium radiotherapy. Jpn J Ophthalmol 2004;48(5):499–502.