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ORIGINAL ARTICLE

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# Ionizing radiation-induced testicular oxidative stress and apoptosis: the role of small GTPase RhoA

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**ABSTRACT**

**Purpose:** The effects of ionizing radiation on living organisms are mainly known as the generation of reactive oxygen species (ROS), apoptosis, and DNA damage. Small GTPases (RhoA, Rac1, Cdc42) are known to have roles in the regulation of oxidative stress and apoptosis. The aim of this study was to investigate the role of the RhoA molecule in testicular tissue damage due to oxidative stress and apoptosis induced by ionizing radiation.

**Material and method:** In this study, testicular tissues and blood samples obtained from our previous study were examined. In that study, rats were exposed to ionizing radiation at three different doses (0.02 Gy, 0.1 Gy, 5 Gy). Then tissue and blood samples were taken at three different times (2 hours, 24 hours, and 7 days) after irradiation. Immunohistochemical staining was performed to evaluate RhoA and cleaved caspase-3 expressions, while RhoA activity was assessed by G-LISA assay in testicular tissues. Serum malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity were analyzed to evaluate oxidative stress.

**Results:** The expression and activation of RhoA demonstrated a time-dependent increase across all levels of radiation doses. Similarly, the expression of cleaved caspase-3 also exhibited a time-dependent increase, consistent with the effects of radiation-induced damage observed in all experimental groups. After exposure to radiation, serum levels of MDA increased, while the activity of SOD decreased.

**Conclusion:** Our findings suggest that RhoA may contribute to radiation-induced testicular tissue damage by increasing oxidative stress and apoptosis.

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Apoptosis; RhoA; radiation-induced damage; rat; reactive oxygen species (ROS); testis

## Introduction

Although ionizing radiation has harmful effects on living beings, X-rays and gamma rays were frequently utilized for diagnostic and therapeutic purposes (Averbeck et al. 2020). The damaging consequences of ionizing radiation affect the whole organism, starting from the cellular level. The most important effects are DNA damage and the resulting uncontrolled cell proliferation, apoptosis, and the generation of free radicals (Hatzi et al. 2015; Pouget et al. 2018).

Apoptosis is a programmed cell death mechanism that eliminates damaged and senescent cells. It is induced in two different ways: either by extracellular stimuli or by mitochondria within the cell. In both mechanisms, caspase (caspase-3, caspase-6, and caspase-7) proteins are activated and initiate proteolytic processes, followed by DNA fragmentation, and thus, apoptosis occurs (Panganiban et al. 2013).

Small GTPases (RhoA, Rac1, Cdc42) play a role in various physiological and pathological processes in various tissues. In mammals, Rho/Rac/Cdc42 proteins are activated by extracellular signals and are involved in the reorganization of the cytoskeleton. In addition, they also play a role in regulating various cellular functions such as gene expression, cell polarity, morphogenesis, cell migration, apoptosis, and vesicle traffic (Bustelo et al. 2007). In general, Rac1 and RhoA are known to act in balance (either in the same or opposing directions) with each other (Velaithan et al. 2011). The balance between RhoA and Rac1 plays a decisive role in cell death or survival. Cells can trigger cell death by increasing Rac1 activity in conditions such as DNA damage or oxidative stress, while growth factors can increase RhoA activity and promote survival (Wang and Beier 2005; Heasman and Ridley 2008). RhoA and Rac1 GTPases play a role in ROS generation by NOX in various cells. RhoA differentially regulates ROS generation in platelets by inhibiting p47phox and Rac1 by inhibiting p67-phox phosphorylation (Akbar et al. 2016).

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RhoA activates the effector molecule ROCK (Rho-kinase) to increase actin-myosin contraction and contributes to the initiation of apoptosis (Coleman et al. 2001). RhoA activation inhibits the activation of caspases by promoting the formation of stress fibers in the cytoskeleton (Wang and Beier 2005). This effect of RhoA is mediated by regulating actin dynamics and increasing cell adhesion via ROCK (Rho-kinase). RhoA activates the PI3K/Akt pathway and stimulates signaling pathways that promote cell survival (Ashkenazi and Dixit 1998; Heasman and Ridley 2008).

In the literature, different in vivo studies have shown that ionizing radiation causes testicular damage by inducing apoptosis (Demyashkin et al. 2022; Lai et al. 2022). After exposure to ionizing radiation, the reproductive process in testicular tissue is affected and may cause infertility in the long term (De Felice et al. 2019; McBride and Schaue 2020). It has been shown that testicular damage, apoptosis, and oxidative stress increase in mice after thoracic region irradiation (Lai et al. 2022). During particle-type radiation application to testicular tissue, it was observed that Ki-67 and Bcl-2 expressions in germ cells decreased, and p53 and caspase 3 expressions increased (Demyashkin et al. 2022). There are very few studies in the literature investigating the role of RhoA in cellular signaling mechanisms induced by ionizing radiation. These studies show that ionizing radiation can activate RhoA, lead to the formation of actin stress fibers, and promote cell differentiation and survival in response to DNA damage (Guerra et al. 2008; Huang et al. 2021). RhoA activity increased after low-dose radiation in osteoblast cells, and osteoblast differentiation was induced through the RhoA/ROCK pathway (Huang et al. 2021).

The molecular mechanisms of infertility caused by ionizing radiation have not yet been elucidated. To develop effective treatment methods in this regard, the acute and late effects of different doses need to be investigated. In our previous study (Manisaligil et al. 2022), we showed that Rac1 activity increased in testicular tissue in rats due to radiation and that intracellular ROS levels also increased with testicular damage. Due to the balance between Rac1 and RhoA proteins, we investigated the effect of radiation exposure on RhoA activity and/or expression levels. Our study will guide research on the prevention of infertility that may occur due to ionizing radiation.

## Methods

### Animals

In this study, we used testicular tissue and serum samples obtained from irradiated rats in our previous study (Manisaligil et al. 2022). Adult, male Wistar albino rats (n = 60) weighing 250 ± 20 g were used in that study. The Ethics Committee of the Research of Laboratory Animals in Dokuz Eylul University (45/2016) approved the study. All procedures performed by the National Institute of Health are according to the Principles of Laboratory Animal Care. All subjects were kept in standardized temperature (22 ± 2 °C) and illumination (12:12 light/dark), and cages with mesh bottoms provided free access to tap water and pelleted food.

### Experimental design

A study was conducted on three groups of rats that received low (0.02 Gy, n = 18), medium (0.1 Gy, n = 18), and high (5 Gy, n = 18) doses of radiation in addition to a control group (n = 6) which is not exposed to radiation. Following the radiation exposure, each group of rats was anesthetized with ether in the 2nd hour (n = 6), 24th hour (n = 6), and 7th day (n = 6) of irradiation. Blood samples were taken from the vena cava caudalis before the sacrification process. Testicular tissues were removed quickly right after the sacrification. Histological and immunohistochemical analyses were performed to evaluate the damage and apoptosis. On the other hand, RhoA activation analysis was performed to evaluate RhoA activity in testicular tissue.

### Radiation exposure

Rats were administered low, medium, and high doses of radiation corresponding to 0.02 Gy, 0.1 Gy, and 5 Gy, respectively. A digital Roentgen device (PHILIPS, Tele Diagnost, Amsterdam, Netherlands) in the Department of Radiology, Dokuz Eylul University, was used for low-dose exposures. A linear accelerator (Siemens Primus, Erlangen, Germany) available in Dokuz Eylul University, Radiation Oncology Department, was used for medium and high dose exposures. Following a series of measurements using an ionization chamber (RTI, Model R100, Mölndal, Sweden), a low-dose setup was achieved with a tube potential of 133 kV, a tube current of 300 mA, and an exposure time of 0.5 s, to give 0.02 Gy. Medium and high dose exposures were achieved using 6 MV photon beams with a dose rate of 300 Monitor Unit (MU)/min. Irradiation setups with 10 MU and 500 MU provided 0.1 Gy and 5 Gy radiation doses, respectively. Each rat was kept in a 13x18x10 cm plastic container through which X-rays can penetrate easily. The irradiation field was set to $13 \times 18\ cm^2$ at a skin-to-source distance (SSD) of 83 cm for all exposures (Said et al. 2019; Zhang et al. 2020).

### Histomorphological examination

A total of 60 paraffin-embedded testicular tissue samples were sliced by a rotary microtome (Leica RM-2255, Köln, Germany) into 5 µm-thick sections and stained with hematoxylin-eosin before examination for histological changes, which was made under a light microscope (Olympus BH2, Tokyo, Japan). Following the microscopic examination, tissue was photographed with a digital camera (DP71, Olympus, Japan). Johnsen Testicular Biopsy Score was used for the quantitative evaluation of spermatogenesis. In addition, tubular biopsy scores were determined for each subject by obtaining the tubular diameter as the mean value of approximately 30 measurements taken from different points of the section (Kaya et al. 2019). Each slice was evaluated by an experienced histologist who acted as a blind observer.

### Immunohistochemical analysis

For the evaluation of RhoA and Cleaved Caspase-3 expressions, sections of testicular tissue were stained using primary antibodies at a 1:1000 dilution, RhoA (NB100-91273, Novus, USA) and Cleaved Caspase-3 (NB100-56112, Novus, USA) primary antibodies according to the previously described (Sylow et al. 2014). Immunohistochemical staining intensity was assessed with H-Score, which can be between 0 and 3. Immunostaining intensity was evaluated as 0 (No Staining), 1 (Poor Staining), 2 (Moderate Staining), and 3 (Intense Staining). The H-score was calculated with the following equation (Ozbilgin et al. 2015).

$$H-score = \sum Pi\,(i+1)$$

Where Pi; was defined as the percentage value of each staining intensity from 0% to 100%, which is then rescaled to staining intensity as i: 0, 1, 2, 3. Five different regions for each preparation were evaluated microscopically, and the mean score was obtained for each preparation.

### Biochemical analysis

To analyze active RhoA-GTP levels, assays were performed using a G-LISA RhoA Activation kit (Cytoskeleton, BK124, UK) according to the manufacturer's recommendations.

MDA and SOD analyses were performed in serum samples to evaluate oxidant/antioxidant capacity. The High Performance Liquid Chromatography (HPLC) method was used to determine the MDA level. A colorimetric-based Superoxide Dismutase activity kit (Biovision, K335-100, USA) was used to determine SOD activity.

### Statistical analysis

All values were expressed as the mean ± standard error (mean ± SEM). Statistical comparisons among groups regarding biochemical parameters and RhoA activities were analyzed using two-way ANOVA and a post-hoc Bonferroni test. The Kruskal-Wallis and Mann–Whitney U-test were used to compare RhoA immunostaining intensity values among groups. All statistical procedures were performed using SPSS 15.0 software for Windows (Chicago, IL, USA). A value of $p < .05$ was considered significant.

## Results

### The effects of radiation on apoptosis in testicular tissue

It has been found that apoptosis in the experimental groups increased over time in all groups given radiation ($p < .05$) (Figure 1, Figure 2). When the early effects of radiation were examined (2nd hour), it was observed that low-dose radiation did not affect apoptosis. Still, medium and high-dose radiation significantly increased apoptosis in a dose-dependent manner ($p < .05$) (Figure 2). However, the expression levels of the medium and high dose groups at 24th hours and 7th days were significantly higher than the expression levels of the low dose group at 24th hours and 7th days ($p < .05$) (Figure 2).

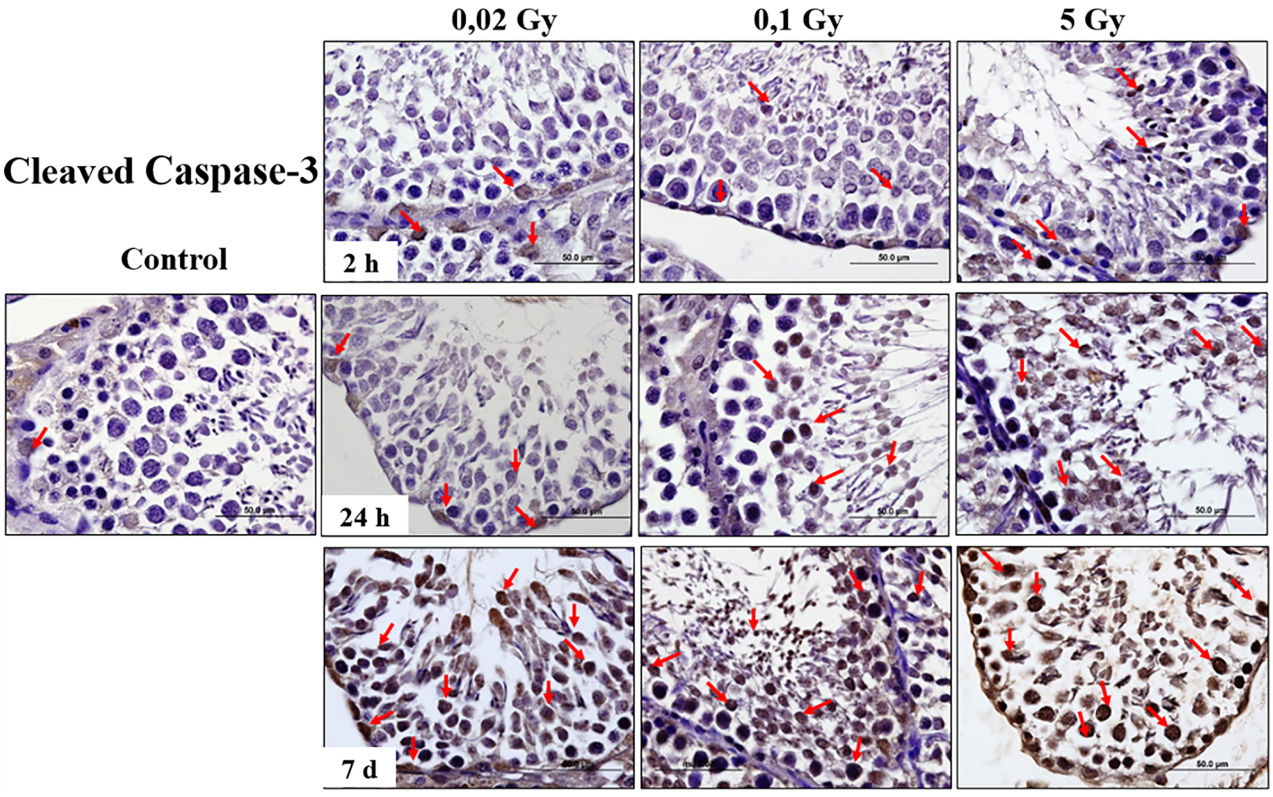



Figure 1. Representative immunohistochemical cleaved caspase-3 expressions images (x100) of experimental groups. The arrows show immunopositive cells.

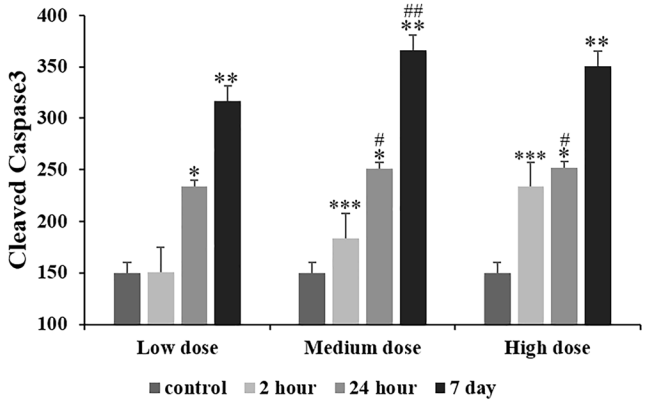



**Figure 2.** Graph bar of cleaved caspase-3 expressions. Results are mean (±S.E.M.). $p < .05$ was considered statistically significant. Low dose: 0.02 Gy, Medium dose: 0.1 Gy, high dose: 5 Gy. *: low, Medium and high dose; 24th hour groups increased *vs* control and 2nd hour groups ($p < .05$), **: low, Medium and high dose; 7th day groups increased *vs* control 2nd hour and 24th hour groups ($p < .05$), ***: Medium and high dose; 2nd hour groups increased *vs* control groups ($p < .05$), #: Medium and high dose; 24th hour groups increased *vs* low dose 24th hour group ($p < .05$), ##: Medium dose; 7th day increased *vs* low and high dose 7th day groups ($p < .05$).

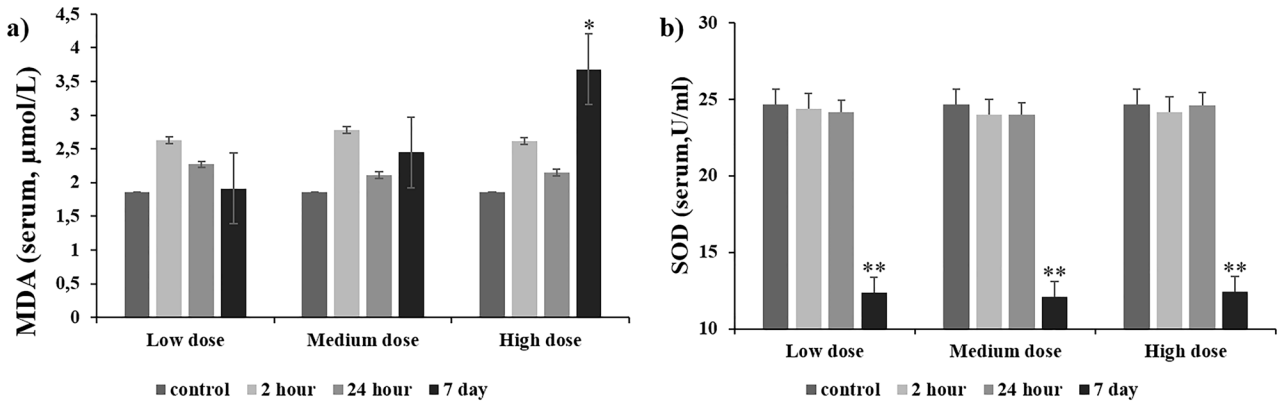



**Figure 3.** Graph bars of testis MDA (a) and SOD (b) levels of experimental groups. Results are mean (±S.E.M.). p<.05 was considered statistically significant. Low dose: 0.02 Gy, Medium dose: 0.1 Gy, high dose: 5 Gy. *: high dose group, 7th day group increased *vs* control and 24th hour group and low dose 7th day group ($p < .05$). **: all dose groups 7th day decreased *vs* control, 2nd and 24th hour groups ($p < .05$).

### *The effects of radiation on the oxidant/antioxidant balance in testicular tissue*

Serum MDA levels in all irradiated groups increased compared to the control group; significance was detected only in the high-dose 7th day group.

It was also observed that radiation exposure increased oxidative stress in all groups, even as early as the 2nd hour (Figure 3a). Serum SOD activity showed similar characteristics for all irradiated groups. For each of the low, medium, and high dose groups, while no change was observed in serum SOD activity in the early periods (2nd and 24th hours), a significant decrease was detected on the 7th day ($p < .05$) (Figure 3b).

### *The effects of radiation on testicular tissue*

In our previous study, we demonstrated that radiation-induced damage to testicular tissue. We observed irregularities in the seminiferous tubule epithelium, shedding of epithelial cells, an increased number of atrophic tubules, and a reduction in seminiferous tubule diameters when compared to the control group. (Manisaligil et al. 2022).

### *The effects of radiation on RhoA expression and RhoA activation*

RhoA expression in low, medium, and high radiation dose groups increased significantly over time ($p < .05$). RhoA expression increased at 2 hours in all radiation doses compared to the control group. However, the highest increase was on day 7 (Figure 4, Figure 5a).

Additionally, an indication of higher 2nd hour and 7th day expression levels of medium and high radiation dose groups compared to the low radiation dose group supports that the increase was dose-dependent ($p < .05$) (Figure 5a).

Alterations in RhoA activity, demonstrated by the G-LISA assay and RhoA-GTP values, showed a similar pattern in all irradiated groups and were increased compared to the control group. Similar to the increase in RhoA expression, the highest activation was observed on day 7, and this increase was significant in the low and medium-dose groups but not in the high-dose group.

These results may indicate that RhoA activation increases over time, with the increase at high doses being less than at low and medium doses. Therefore, the increase in RhoA activity may be dose-independent (Figure 5b).

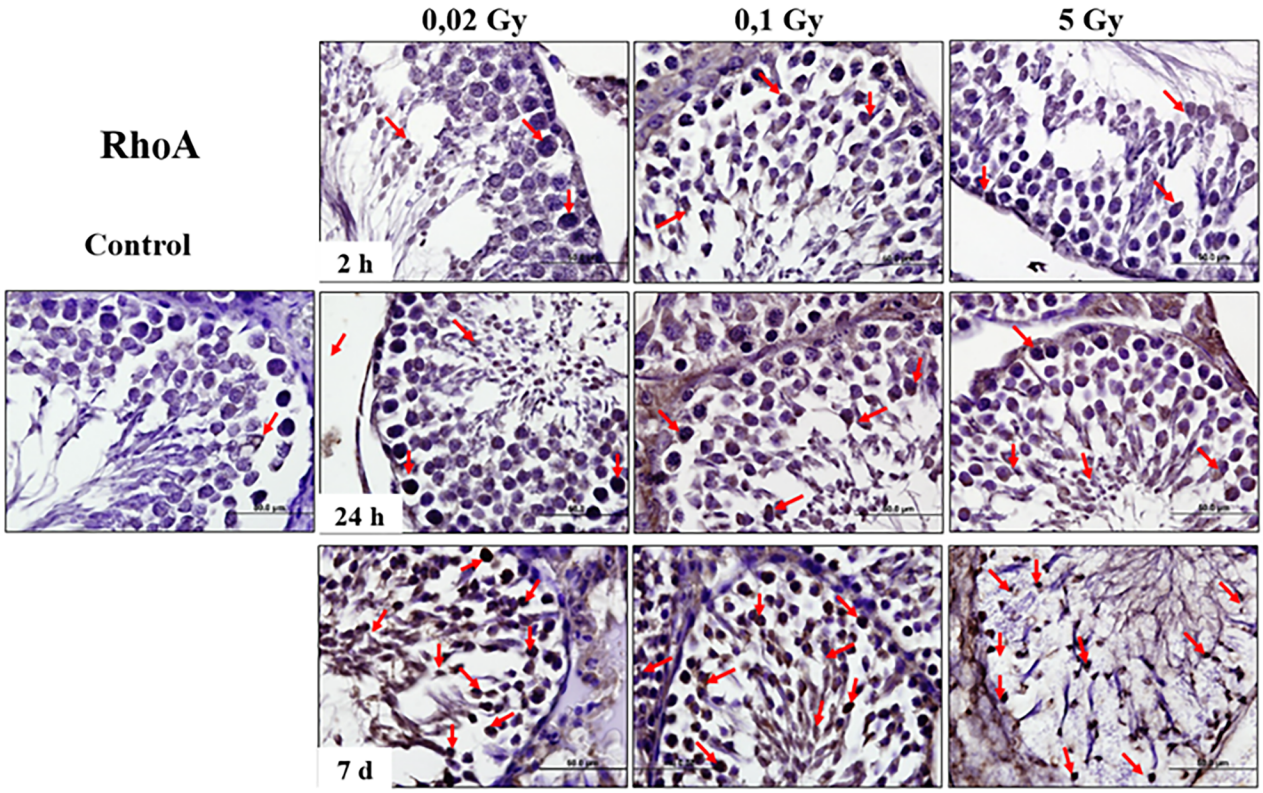



**Figure 4.** Representative immunohistochemical RhoA expressions images (x100) of experimental groups. The arrows show immunopositive cells.

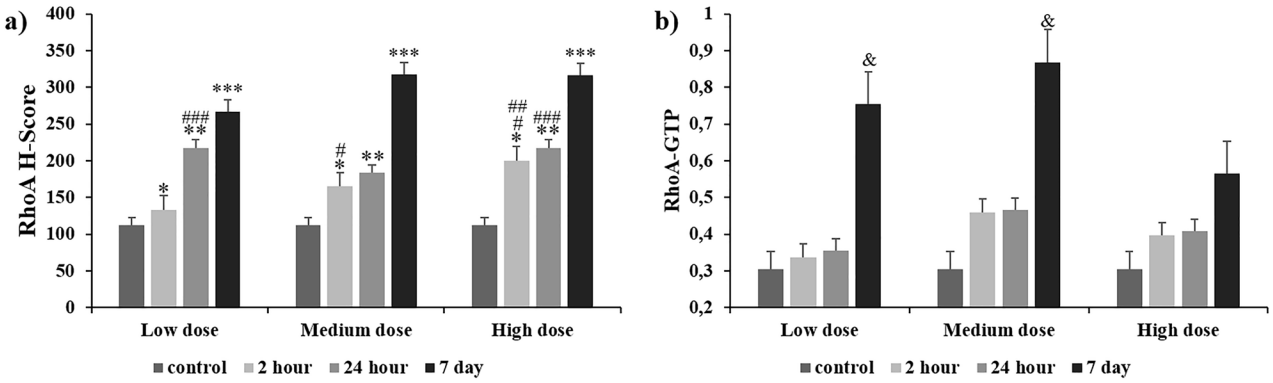



**Figure 5.** Graph bar of RhoA expressions (a) and RhoA activation (RhoA-GTP) levels (b). Graph bar of RhoA expressions. Results are mean (±S.E.M.). $p < .05$ was considered statistically significant. Low dose: 0.02 Gy, Medium dose: 0.1 Gy, high dose: 5 Gy. *: 2nd hour groups increased *vs* control groups ($p < .05$), **: 24th hour groups increased *vs* control and 2nd hour groups ($p < .05$), ***: 7th day groups increased *vs* control, 2nd hour and 24th hour groups ($p < .05$), #: Medium and high dose; 2nd hour and 7th day groups increased *vs* low dose 2nd hour and 7th day groups ($p < .05$), ##: high dose 2nd hour group increased *vs* low and medium dose 2nd hour groups ($p < .05$), ###: low and high dose; 24th hour groups increased *vs* medium 24th hour group ($p < .05$). &: low and medium dose; 7th day groups increased *vs* control, 2nd hour and 24th hour groups ($p < .05$).

## Discussion

The damage to tissues caused by ionizing radiation, whose molecular mechanisms are not yet fully known, has been shown in various studies (Hatzi et al. 2015; Shimura and Kojima 2018). In diagnostic and therapeutic procedures involving ionizing radiation to the pelvic region, testicular tissue is damaged either directly or by scattered radiation. Ionizing radiation affects the reproductive process in testicular tissue and may cause infertility in the long term (De Felice et al. 2019; McBride and Schaue 2020).

One of the cellular events that develops due to radiation is apoptosis. It has been shown that oxidative stress and apoptosis in germ cells exposed to radiation cause infertility. (Ko et al. 2014). It has been shown that X rays between 25 and 200 mGy applied to mice increase apoptosis by increasing p53 expression in testicular tissue, and the highest increase was at 75 mGy irradiation and at the 12th and 18th hours (Liu et al. 2006). Wang et al. reported that the rate of apoptotic cells in testicular tissue increased compared to the control group 7 days after 5 Gy irradiation in mice (Wang et al. 2020). In another study, it was reported that active

caspase-3 expression increased in rat testicular tissue 5 days after irradiation with 10 Gy compared to the control group. (Said et al. 2019). Our study's results, consistent with these studies, showed that apoptosis at all doses (low, medium, and high) increased compared to the control group, even in the early stages.

It is known that radiation increases oxidative stress and causes tissue damage. It has been shown in the analyses of various tissues and serum samples that oxidative stress increases after radiation application (Rezaeyan et al. 2016; Shaban et al. 2017; Wang et al. 2020). In the study conducted by Fahmy et al., it was reported that MDA levels increased and SOD levels decreased in brain, kidney and testicular tissues in 24 hours after 0.3 Gy whole body irradiation in rats (HA et al. 2013). There are studies showing that MDA levels increased and SOD levels decreased similarly at doses of 8 Gy and 5 Gy in rats 7 days after whole body irradiation. (Shaban et al. 2017; El-Mesallamy et al. 2018). In fact, in another study, it was reported that MDA levels increased and SOD levels decreased in testicular tissue 24 hours after 18 Gy irradiation to the thoracic region of mice. (Rezaeyan et al. 2016). In our study, it was observed that radiation increased oxidative stress in all groups, even in the early period of 2 hours. When serum MDA and SOD levels were evaluated together, it was observed that MDA levels increased significantly only on the 7th day of the high dose, while SOD levels decreased significantly on the 7th day in all groups. In studies evaluating oxidant and antioxidant capacity, it was preferred to study the values of these parameters in the tissue because serum values are affected by many factors. Although these analyses are considered a limitation of our study, our previous study has shown that oxidative stress is increased in testicular tissue (Manisaligil et al. 2022). In this study, we also wanted to show whether the changes in MDA and SOD caused by oxidative stress are reflected in serum values. In our previous study, while MDA values in testicular tissue appeared to be correlated with serum values, it was determined that although the decrease in testicular tissue SOD values occurred from the 2$^{nd}$ hour onwards, it was reflected in serum SOD values on the 7$^{th}$ day, and these results were found to be consistent with our study results.

Since many factors may be effective in increasing apoptosis and oxidative stress in radiation-induced damage, the role of RhoA, one of the small GTPases, in these processes was evaluated in our study. Therefore, it was found that the increase in RhoA activation and expression started as early as 2 hours after radiation exposure and continued to increase up to 7 days. It was seen that these increases were positively correlated with the increase in apoptotic and oxidative stress. Since it is known that Rac1 and RhoA act in opposite directions, our previous study demonstrated an increase in Rac1 expression and activity. In contrast, in this study, a decrease in RhoA expression and activity was shown in the literature (Wang and Beier 2005; Ko et al. 2014; Said et al. 2019).

Studies conducted on rodents have reported that small GTPases cause apoptosis, oxidative stress, and tissue damage in various tissues such as the heart, retina, kidney, and diabetic or non-diabetic animal models (Wang and Beier 2005; Du et al. 2016; Wang et al. 2023). It is not known whether RhoA has such an effect on radiation-induced testicular tissue damage. In endothelial cells, it has been reported that the RhoA/ROCK pathway has no effect on radiation-induced apoptosis 18 hours after irradiation with 15 Gy (Rousseau et al. 2011). Although the 5 Gy dose in the high-dose group in our study was much lower than in this study, RhoA activation was considered less than the activation in low and medium doses, which is consistent with this study.

These results also support that the role of small GTPase RhoA in radiation-induced testicular damage may vary depending on the radiation dose. However, the inability to evaluate whether there is an improvement in testicular damage following RhoA inhibition can be considered a limitation of our study. These issues will be prioritized in our next studies.

## Conclusion

The data obtained in our study suggest that RhoA activation and expression, apoptosis, and oxidative stress increase over time at all radiation doses; RhoA may play a role in these processes. In our previous study, we also demonstrated that the activity of Rac1, a small GTPase, is in the opposite direction to RhoA activity and that changes in the activity of these molecules can occur in the low dose ranges, such as 0.002-0.1 Gy, which are used in current radiological applications. Especially in young male patients, treatments should be developed to prevent infertility that may occur due to testicular damage caused by exposure to low doses of radiation. Our study supports the idea that treatments targeting small GTPases may hold promise in radiation-induced testicular damage.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## References

Akbar H, Duan X, Saleem S, Davis AK, Zheng Y. 2016. RhoA and Rac1 GTPases differentially regulate agonist-receptor mediated reactive oxygen species generation in platelets. PLoS One. 11(9):e0163227. doi:10.1371/journal.pone.0163227.

Ashkenazi A, Dixit VM. 1998. Death receptors: signaling and modulation. Science. 281(5381):1305–1308. doi:10.1126/science.281.5381.1305.

Averbeck D, Candéias S, Chandna S, Foray N, Friedl AA, Haghdoost S, Jeggo PA, Lumniczky K, Paris F, Quintens R, et al. 2020. Establishing mechanisms affecting the individual response to ionizing radiation. Int J Radiat Biol. 96(3):297–323. doi:10.1080/09553002.2019.1704908.

Bustelo XR, Sauzeau V, Berenjeno IM. 2007. GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo. Bioessays. 29(4):356–370. doi:10.1002/bies.20558.

Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, Olson MF. 2001. Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat Cell Biol. 3(4):339–345. doi:10.1038/35070009.

De Felice F, Marchetti C, Marampon F, Cascialli G, Muzii L, Tombolini V. 2019. Radiation effects on male fertility. Andrology. 7(1):2–7. doi:10.1111/andr.12562.

Demyashkin G, Koryakin S, Moiseev A, Saburov V, Zatsepina M, Epifanova M, Stepanova Y, Shchekin V, Vadyukhin M, Shegay P, et al. 2022. Assessment of proliferation and apoptosis in testes of rats after experimental localized electron irradiation. Curr Issues Mol Biol. 44(11):5768–5777. doi:10.3390/cimb44110391.

Du R, Wang JL, Wang YL. 2016. Role of RhoA/MERK1/ERK1/2/iNOS signaling in ocular ischemic syndrome. Graefes Arch Clin Exp Ophthalmol. 254(11):2217–2226. doi:10.1007/s00417-016-3456-1.

El-Mesallamy HO, Gawish RA, Sallam AM, Fahmy HA, Nada AS. 2018. Ferulic acid protects against radiation-induced testicular damage in male rats: impact on SIRT1 and PARP1. Environ Sci Pollut Res Int. 25(7):6218–6227. doi:10.1007/s11356-017-0873-6.

Guerra L, Carr HS, Richter-Dahlfors A, Masucci MG, Thelestam M, Frost JA, Frisan T. 2008. A bacterial cytotoxin identifies the RhoA exchange factor Net1 as a key effector in the response to DNA damage. PLoS One. 3(5):e2254. doi:10.1371/journal.pone.0002254.

Ha F, ASh AE-A, Gharib O. 2013. Possible ameliorative role of low dose of radiation against cisplatin induced oxidative stress and tissue damage in male rats. Eur J Biol Med Sci Res. 1(4):10–18.

Hatzi VI, Laskaratou DA, Mavragani IV, Nikitaki Z, Mangelis A, Panayiotidis MI, Pantelias GE, Terzoudi GI, Georgakilas AG. 2015. Non-targeted radiation effects in vivo: a critical glance of the future in radiobiology. Cancer Lett. 356(1):34–42. doi:10.1016/j.canlet.2013.11.018.

Heasman SJ, Ridley AJ. 2008. Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol. 9(9):690–701. doi:10.1038/nrm2476.

Huang Q, Chai H, Wang S, Sun Y, Xu W. 2021. 0.5-Gy X-ray irradiation induces reorganization of cytoskeleton and differentiation of osteoblasts. Mol Med Rep. 23(5):1–1. doi:10.3892/mmr.2021.12018.

Kaya C, Barbaros Baseskioglu A, Yigitaslan S, Yasemin Ozatik F, Ozatik O, Uslu S. 2019. The therapeutic potential of amifostine on cyclophosphamide-induced testicular dysfunction in rats: an experimental study. Int J Reprod Biomed. 17(4):245–252. doi:10.18502/ijrm.v17i4.4549.

Ko EY, Sabanegh ES, Jr., Agarwal A. 2014. Male infertility testing: reactive oxygen species and antioxidant capacity. Fertil Steril. 102(6):1518–1527. doi:10.1016/j.fertnstert.2014.10.020.

Lai PP, Jing YT, Guo L, Qin TZ, Xue YZ, Zhang ZW, Wang X, Miao X, Zhang W, Ding GR. 2022. Abscopal effects of thoracic X-ray radiation on spermatogenesis in mice. Front Physiol. 13:984429. doi:10.3389/fphys.2022.984429.

Liu G, Gong P, Zhao H, Wang Z, Gong S, Cai L. 2006. Effect of low-level radiation on the death of male germ cells. Radiat Res. 165(4):379–389. doi:10.1667/rr3528.1.

Manisaligil YA, Gumustekin M, Micili SC, Ural C, Cavdar Z, Sisman G, Yurt A. 2022. The role of small GTPase Rac1 in ionizing radiation-induced testicular damage. Int J Radiat Biol. 98(1):41–49. doi:10.1080/09553002.2021.1988752.

McBride WH, Schaue D. 2020. Radiation-induced tissue damage and response. J Pathol. 250(5):647–655. doi:10.1002/path.5389.

Ozbilgin K, Karaca F, Turan A, Köse C, Vatansever S, Ozcakir T. 2015. The higher heparin-binding epidermal growth factor (HB-EGF) in missed abortion. Taiwan J Obstet Gynecol. 54(1):13–18 (Eng). doi:10.1016/j.tjog.2013.08.011.

Panganiban RA, Snow AL, Day RM. 2013. Mechanisms of radiation toxicity in transformed and non-transformed cells. Int J Mol Sci. 14(8):15931–15958. doi:10.3390/ijms140815931.

Pouget JP, Georgakilas AG, Ravanat JL. 2018. Targeted and off-target (Bystander and Abscopal) effects of radiation therapy: redox mechanisms and risk/benefit analysis. Antioxid Redox Signal. 29(15):1447–1487. doi:10.1089/ars.2017.7267.

Rezaeyan A, Haddadi GH, Hosseinzadeh M, Moradi M, Najafi M. 2016. Radioprotective effects of hesperidin on oxidative damages and histopathological changes induced by X-irradiation in rats heart tissue. J Med Phys. 41(3):182–191. doi:10.4103/0971-6203.189482.

Rousseau M, Gaugler MH, Rodallec A, Bonnaud S, Paris F, Corre I. 2011. RhoA GTPase regulates radiation-induced alterations in endothelial cell adhesion and migration. Biochem Biophys Res Commun. 414(4):750–755. doi:10.1016/j.bbrc.2011.09.150.

Said RS, Mohamed HA, Kamal MM. 2019. Coenzyme Q10 mitigates ionizing radiation-induced testicular damage in rats through inhibition of oxidative stress and mitochondria-mediated apoptotic cell death. Toxicol Appl Pharmacol. 383:114780. doi:10.1016/j.taap.2019.114780.

Shaban NZ, Ahmed Zahran AM, El-Rashidy FH, Abdo Kodous AS. 2017. Protective role of hesperidin against gamma-radiation-induced oxidative stress and apoptosis in rat testis. J Biol Res (Thessalon). 24:5. doi:10.1186/s40709-017-0059-x.

Shimura N, Kojima S. 2018. The lowest radiation dose having molecular changes in the living body. Dose-Response. 16(2). doi:10.1177/1559325818777326.

Sylow L, Kleinert M, Pehmøller C, Prats C, Chiu TT, Klip A, Richter EA, Jensen TE. 2014. Akt and Rac1 signaling are jointly required for insulin-stimulated glucose uptake in skeletal muscle and downregulated in insulin resistance. Cell Signal. 26(2):323–331. doi:10.1016/j.cellsig.2013.11.007.

Velaithan R, Kang J, Hirpara JL, Loh T, Goh BC, Le Bras M, Brenner C, Clement MV, Pervaiz S. 2011. The small GTPase Rac1 is a novel binding partner of Bcl-2 and stabilizes its antiapoptotic activity. Blood. 117(23):6214–6226. doi:10.1182/blood-2010-08-301283.

Wang A, Wang L, Lu X, Wang Y, Chen X, Shi Z, Li P, He C, Zhang S, Gao Y, et al. 2020. A Chinese herbal prescription Yiqi Jiedu decoction attenuates irradiation induced testis injury in mice. Biomed Pharmacother. 123:109804. doi:10.1016/j.biopha.2019.109804.

Wang G, Beier F. 2005. Rac1/Cdc42 and RhoA GTPases antagonistically regulate chondrocyte proliferation, hypertrophy, and apoptosis. J Bone Miner Res. 20(6):1022–1031. doi:10.1359/JBMR.050113.

Wang T, Li N, Yuan L, Zhao M, Li G, Chen Y, Zhou H. 2023. MALAT1/miR-185-5p mediated high glucose-induced oxidative stress, mitochondrial injury and cardiomyocyte apoptosis via the RhoA/ROCK pathway. J Cell Mol Med. 27(17):2495–2506. doi:10.1111/jcmm.17835.

Zhang L, Huang B, Tang H, Ye X, Yao Y, Gong P, Tang H. 2020. Amifostine inhibited the differentiation of RAW264.7 cells into osteoclasts by reducing the production of ROS under 2 Gy radiation. J Cell Biochem. 121(1):497–507. doi:10.1002/jcb.29247.