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Neuroprotective effect of *Myrtus communis* against ionizing radiation-induced brain injury: Insights from histopathological and biochemical analysis in rats: TROD-GROG 005

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ABSTRACT

Aim: To investigate the potential radioprotective effects of *Myrtus communis* on brain tissue.
Methods: Thirty female rats were divided into four groups. The control group (C) was applied with oral saline solution (SF) for four days. *Myrtus communis* (MC) groups started to receive MC (100 mg/kg, oral) either four days before (R + preMC) or immediately after (R + MC) irradiation for four days. Irradiation was applied 10 Gy in a single fraction. All rats were sacrificed on the fourth day of irradiation. Malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) levels, myeloperoxidase (MPO), superoxide dismutase (SOD), and tissue factor activities (TFa) were determined for biochemical analysis. Hematoxylin&Eosin staining was done for histopathological analyses, and electrophoretic analyses were performed.
Results: NO, MDA, and MPO levels were higher in all irradiated groups compared with the C group. MC administration decreased NO, MDA, and MPO levels in R + preMC and R + MC groups. MC administration increased GSH levels. TFa activity decreased in R groups but did not change with MC administration compared to the C group. Radiation-induced brain tissue injury decreased, and morphologically normal neurons were observed in both MC-added groups.
Conclusion: Myrtus communis has a potential neuroprotective effect on brain tissue, attributed to its antioxidative, anti-inflammatory, and anti-lipid peroxidative properties.

1. Introduction

Ionizing radiation (IR) is a therapeutic modality routinely used for the management of most tumors localized within the central nervous system (CNS) (van den Bent et al., 2023). Irradiation has the physical capability to form free radicals, which may cause direct or indirect DNA injury. Double-strand DNA breaks are the most well-documented mechanism of radiation-induced cell death. Due to radiation exposure, DNA injury results from oxidative stress caused by generating reactive oxygen species (ROS) in the tissue. The underlying mechanism of this phenomenon is based on the DNA base injury caused by ROS and the breakage of the DNA chain (Goldstein & Kastan, 2015; R.-X. Huang & Zhou, 2020). Unless the cell repairs this injury, apoptotic mechanisms become active, and cell death occurs (Averbeck & Rodriguez-Lafrasse,

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2021). The significant advantage of IR is that it focuses on cancerous tissue. However, radiation-induced ROS negatively affects the cancerous tissue and the adjacent healthy cells because of the increased oxidative stress (Krüger et al., 2015). Reactive oxygen species are generated under the radiation effect increase, particularly malondialdehyde (MDA) levels. At the same time, they weaken the antioxidant defense significantly by causing a severe decrease in glutathione (GSH) levels (Topcu et al., 2019). Even though IR is known to have a high efficacy in cells with high mitotic activity, it is known that the effectiveness of radiation is also high in normal mature CNS cells with limited mitotic activity (Gorbunov & Kiang, 2021; Q. Wang, Xie, et al., 2020). It has been documented that particularly late effects of radiation are permanent in the CNS, although depending on the dose (Barisano et al., 2018; Ye et al., 2022). The studies conducted on rodents have confirmed many histopathological changes, such as blood-brain barrier destruction, vascular damage, microglial activation, apoptosis, and increased proinflammatory mediators after irradiation of CNS. In vivo changes have been defined as functional changes in the neurons and impaired neurotransmission (X. Xu et al., 2021; Liao et al., 2020). Besides these, treating CNS tumors risks brain injuries such as focal cerebral necrosis, neurocognitive deficiency, and cerebrovascular disease (Goldstein & Kastan, 2015; R. X. Huang & Zhou, 2020). Despite advances in treatment techniques, potential brain injury is a severe side effect, and neuroprotective treatment options are limited (Gorbunov & Kiang, 2021; Q. Wang, Xie, et al., 2020; Kale et al., 2018).

Myrtus communis is an evergreen shrub from the Myrtaceae family, ranging from 1 to 5 m in length, and has cultural importance in the Mediterranean and Anatolia. *M. communis* is considered to originate from Iran and Afghanistan and is known to grow only in the Mediterranean Region. In our country, the genus *Myrtus* is represented by a single taxon (*Myrtus communis* L. subsp. *communis* L.) (bizimbitkiler.org.tr). The leaves, fruits, and flowers in the cane of this bush have medicinal properties. In our previous studies and studies of researchers from different countries, it has been reported that essential oil and extracts of *M. communis* leaves contain secondary metabolites such as flavonoids (Myricetin and quercetin-derived compounds), terpenes, phenolic acids (gallic acid, caffeic acid, ellagic acid and so on), coumarins, tannins (Arslan et al., 2022; Hennia et al., 2018; Romani et al., 1999; Sen et al., 2020).

Myrtus communis is used in the topical treatment of nasal mucosal inflammation by Herpes simplex Type 1 and Type 2. As well as its cultural importance, *M. communis* has medicinal use for various indications such as diarrhea, peptic ulcer, hemorrhoid, infection, hemorrhage, headache, palpitation, urethritis, conjunctivitis, excessive sweating, cough, pulmonary and skin diseases. Tretiakova et al. have discussed the apoptotic induction potential of *M. communis* in cancer cells. They have ascertained that *M. communis* induced the apoptosis of different cancer cell lines by activating caspase-3, -8, and -9, resulting in a cleavage of poly-(ADP-ribose)-polymerase. It has also been figured out that cytotoxicity mainly occurs due to mitochondrial membrane loss and induces apoptosis (Tretiakova et al., 2008). It has been stated in some in vitro studies that myrticola found in the leaves of *M. communis* has antioxidant, antibacterial, anti-inflammatory, and anticancer properties (Samareh Fekri et al., 2018; Tuberoso et al., 2010) and a neuroprotective effect (Aykac et al., 2019).

To the best of our knowledge, our study is the first to examine the efficacy of *M. communis* on the ionizing radiation-induced effects on brain tissue. This research is crucial in expanding our understanding of the potential protective effects of *M. communis* extract against central nervous system damage induced by ionizing radiation in rats. Hence, this study aimed to investigate the possible protective effects of *M. communis* extract against central nervous system damage induced by ionizing radiation in rats.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained from Sigma-Aldrich (Missouri, ABD) and Merck (Darmstadt, Germany). All reagents and chemicals were of analytical grade or higher purity.

2.2. Animals

Animal experimentations were approved by our faculty Local Ethics Committee (Marmara University; May 17th, 2022, 33.2022mar), and the Directive 2010/63/EU was followed. Istanbul Health Sciences University Experimental Animals Application and Research Center and maintained at Marmara University Experimental Animals Application and Research Center (MU, DEHAMER). Thirty female Rattus species Norvecigus rats (200–250 g) were used in the study. The rats were kept in cages at a controlled temperature of 22 ± 2 °C and a humidity level of 63%–67%. Four rats were housed in each cage with 12-h light/12-h dark cycles. Feeding was performed ad libitum. Water and food were provided from the same center.

2.3. Collection and identification of plant material

Myrtus communis subsp. *communis* leaves were collected from the Turgutlu Region of Manisa, Türkiye, and defined by the botanist Dr. Gizem Bulut. Voucher specimens were kept in the Herbarium of the School of Pharmacy, Marmara University (MARE: No: 13,006).

2.4. Extraction

M. communis extract was prepared at Marmara University, Faculty of Pharmacy, Department of Pharmacognosy and was previously obtained under the same conditions (Sen et al., 2017). *M. communis* leaves (100 g) were dried in the shade at room temperature. The dried and powdered leaves were extracted with 96% ethanol using the Soxhlet device (Soxhlet extraction). After filtration, the extract was concentrated using a rotary evaporator. The MC powder obtained with a yield of 28.56% was kept in a dark glass bottle at 4 °C until treatment.

2.5. Experimental Design

Animals were divided into control (C) and three exposure groups. The control group (C) (n = 6) was applied with oral saline for only four days. IR exposure groups were assigned as “R" group (n = 8) given oral saline for four days beginning from the treatment day, MC treatment (R + MC) group (n = 8) treated with oral MC for four days starting from the treatment day of IR, and MC pretreatment (R + preMC) group (n = 8) treated with oral MC for totally eight days beginning four days before the treatment day. MC (dissolved in SF) was administered via oral gavage at 100 mg/kg in the morning. The dose of *M. communis* extract was decided according to our previous studies (Ozbeyli et al., 2020; Aykac et al., 2019).

2.6. Irradiation

Primarily, including the C group, all rats were administered anesthesia intraperitoneally using 60 mg/kg ketamine+10 mg/kg xylazine. Initially, 1-mm-thick slice simulation computed tomography (CT) images were obtained from four groups of rats. Rats were laid prone with taped tails and legs under anesthesia for three-dimensional planning (Varian Medical Systems, Palo Alto, CA, USA). While anesthesia is maintained, IR was applied to the whole body from a single field as a single fraction of 10 Gy with 6 MV photon energy and an acceleration dose of 500 MU/min in the linear accelerator device (Clinac, Varian Medical Systems, Palo Alto, CA, USA). According to previous studies, a radiation dose of 10 Gy was applied in single fraction schemes for whole-body irradiation (Chargari et al., 2013; Lee et al., 2011; Parente et al., 2020). Following the treatment of IR, the rats were placed in cages. The rats were sacrificed on the fourth day after irradiation. The brain tissues were removed in all groups.

2.7. Brain tissue homogenate preparation

10% brain homogenates in physiological saline were prepared using a mechanical homogenizer. After homogenization, brain homogenates were centrifuged for 10 min at 3000×*g*. The supernatants were used for biochemical analysis.

2.7.1. Determination of NO level

The method for assessing brain nitric oxide (NO) was previously used in the literature (Miranda et al., 2001). Nitrate is reduced with vanadium (III) chloride to nitrite. The reaction of N-(1-Naphthyl) ethylene diamine dihydrochloride with nitrite in the presence of sulfanilamide affords the resulting complex diazonium compound. The absorbance of the resulting-colored complex is measured spectrophotometrically at 540 nm (Miranda et al., 2001).

2.7.2. Determination of MDA level

Brain MDA level was assayed for lipid peroxidation products by monitoring thiobarbituric acid reactive substance formation as described previously. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56 × 105 M−1 cm−1, and results are expressed as nmol MDA/g tissue (Ledwożyw et al., 1986).

2.7.3. Determination of MPO activity

Brain MPO activity was measured using a procedure described by Hillegas et al. (Hillefass et al., 1990). Brain tissue samples were homogenized in 50 mM potassium phosphate buffer (PB), with pH 6.0, and centrifuged at 41,400×*g* for 10 min. The pellets were then suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400×*g* for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H2O2 solution. 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. MPO activity was expressed as U/g tissue (Hillefass et al., 1990).

2.7.4. Determination of SOD activity

Brain SOD activity was measured by the method of Mylorie et al. (Mylroie et al., 1986). This method measures the ability of SOD to increase the effect of riboflavin (Sigma-Aldrich, R9504) sensitized photo-oxidation of o-dianisidine (Sigma-Aldrich, D3252). The activity of SOD is generated by illuminating the reaction mixture that contains o-dianisidine dihydrochloride and riboflavin with light from a fluorescent lamp. The oxidation of o-dianisidine, which is sensitized by riboflavin, is enhanced by superoxide dismutase, and the increase in the absorbance is linearly dependent on superoxide dismutase concentration. The absorbance of the resulting-colored product is evaluated spectrophotometrically at 460 nm (Mylroie et al., 1986).

2.7.5. Determination of GSH level

Brain GSH level was determined by the Beutler method (Weissman, 1976). The colored product resulting from the reaction of the sulfhydryl groups with Ellmann's reagent, 5,5′-Dithiobis (2-nitrobenzoic acid) (Merck, D8130), was evaluated spectrophotometrically. The results were calculated using an extinction coefficient of 13,600 M–1cm–1 (Weissman, 1976).

2.7.6. Determination of TFa

According to Quick's one-stage method, brain tissue factor activity (TFa) was evaluated using normal plasma. This was performed by mixing 0.1 ml brain homogenate with 0.1 ml of 0.02 M CaCl2; the clotting reaction started after adding 0.1 ml of plasma. All reagents were brought to the reaction temperature (37 °C) before mixing. Tissue factor activity was expressed as seconds (Ingram & Hills, 1976).

2.8. SDS-polyacrylamide gel electrophoresis

The basic concept of Laemmli SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to examine brain tissue electrophoretically. The BIO-RAD mini protean precast II dual slab gel apparatus was used for SDS-PAGE (BIO-RAD, USA). For protein electrophoresis, mini-PAGE gels (Any kD precast polyacrylamide gel, 8.6 × 6.7 cm [W x L], Catalog Number: 4569033, BIO-RAD, USA) were used (Laemmli, 1970).

2.9. Histopathological preparation

Brain tissue was followed by a routine paraffin embedding technique after fixation with 10% formaldehyde for light microscopic examination. For histopathological examination, paraffin sections taken from the brain were stained with cresyl violet. The stained sections were evaluated by specialist histologists blind to the groups using a photomicroscope (Olympus BX51, Tokyo, Japan), and their photographs were taken.

2.10. Statistical analysis

The statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). Descriptive statistics were used for categorical and continuous variables in the study. Homogeneity of variances, a prerequisite for parametric tests, was checked with the "Levene" test. The assumption of normality was checked with the "Shapiro-Wilk" test. The statistical analysis of the parameters was carried out by one-way variance analysis (ANOVA). In corrections, the Bonferroni test, one of the multiple comparison tests, was used. The variances were accepted as statistically significant for a $p < 0.05$ value and expressed as mean ± S.E.M.

3. Results

3.1. Biochemical results

In the brain tissue, a significant increase was found in the NO level of the R group compared with the C group. NO level was also higher in the R + preMC and R + MC groups than in the C group. However, the NO level was lower in the R + preMC and R + MC groups than in the R group (Fig. 1a). The MDA level and MPO activities significantly increased in the R group compared to the C group. However, MC administration in the R groups significantly decreased MDA levels and MPO activity compared to the R group. MPO activity was lower in the R + preMC group than the R group but similar to the R + MC group (Fig. 1b and c).

SOD activity significantly decreased in the R group compared to the C group. In contrast, it increased dramatically in the R + preMC group. It did not significantly change in the R + MC group compared to the R group (Fig. 2a). GSH level significantly decreased in the R group compared with the C group. Besides this, GSH levels of both R + MC and R + preMC groups were almost equal to the C group. In contrast, those values were significantly higher than the R group (Fig. 2b). In the TFa test, prolonged clot formation time indicates decreased TF activity. Therefore, TFa decreased in the R groups compared to the C group. MC administration to the R group did not change the prolonged clot formation time (Fig. 2c). The results are summarized in Table 1.

3.2. Electrophoretic examination of brain tissue

Fig. 3 shows the electrophoretic patterns of brain tissue in all groups.

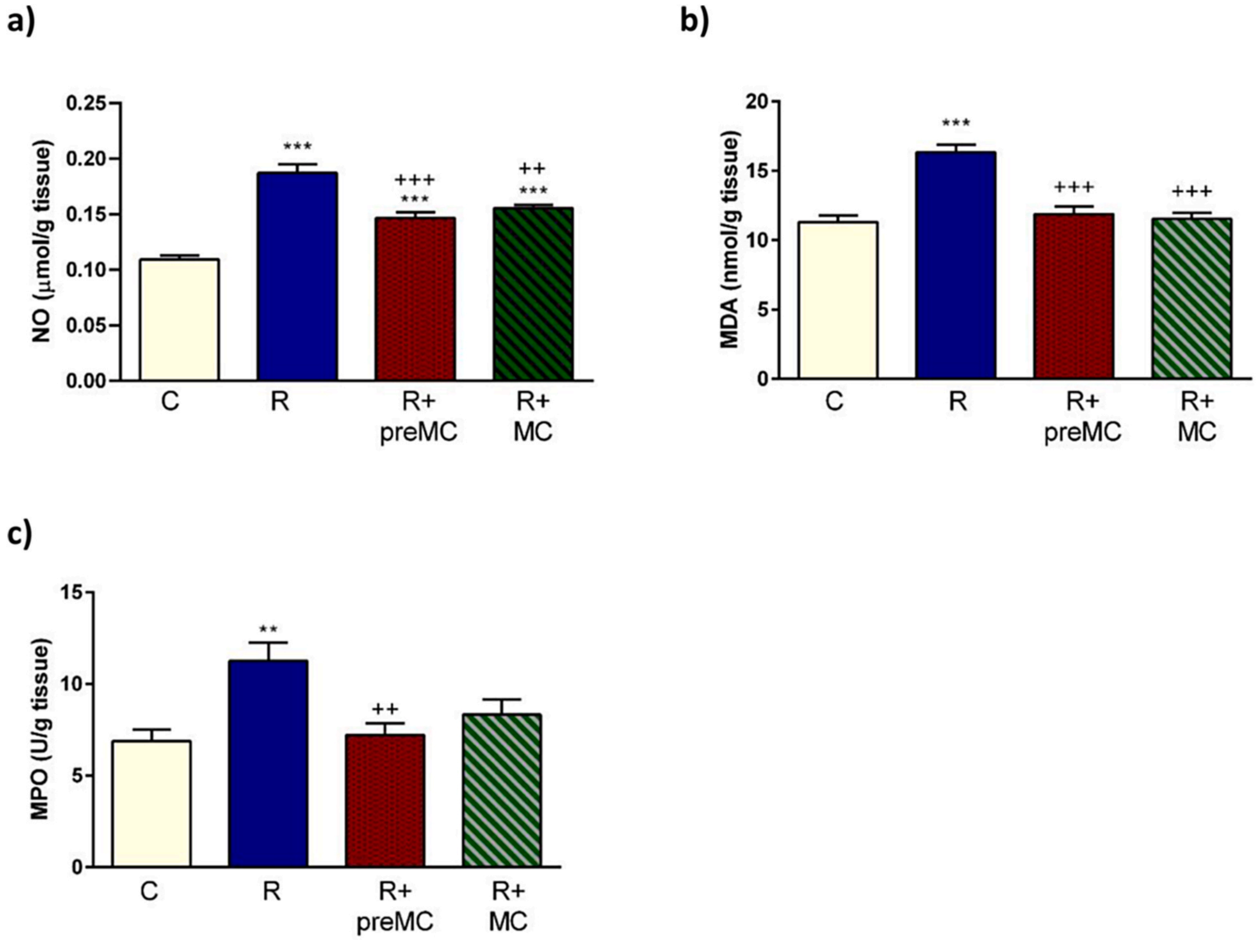

Fig. 1. (a) NO level, (b) MDA level, (c) MPO activity in the brain tissues of control (C), irradiated (R), R + preMC and R + MC groups. Each group consists of eight animals. The range bars show the SEM. *p < 0.05 **p < 0.01 and ****p < 0.001 compared with control group; +p < 0.05, ++p < 0.01 and +++p < 0.001 compared with R group. Group definitions: C: control; R: irradiation; R + preMC: *Myrtus communis* treatment before and after irradiation; R + MC: *Myrtus communis* treatment after irradiation.

In the visual examination of SDS-PAGE results, some differences were detected in the protein profiles of the brain tissues between the groups.

In the R group, an increase was detected in the 72 kDa, 52 kDa, and 46 kDa protein bands and a decrease in the 23 kDa and 17 kDa protein bands compared with the control group. While a decrease was detected in the 72 kDa, 52 kDa, 46 kDa, and 17 kDa protein bands in the R + preMC group, an increase was observed in the 23 kDa protein band. In the R + MC group, there were increases in 23 kDa and 17 kDa protein bands. The appearance of the other protein bands was similar to the protein bands of the R groups (Fig. 2).

3.3. Histopathological results

The histological examination of the brain showed cerebral cortex structure with a regular morphology in the C group, increased injured neurons as well as morphologically normal neurons in the R group, and decreased injured neurons as well as morphologically normal neurons in both R + MC and R + preMC groups (Fig. 4).

4. Discussion

Ionizing radiation is commonly used in the treatment of primary brain tumors and metastases (Scaringi et al., 2018). However, exposure to IR may cause short-term and long-term complications. These complications manifest as brain injury, inflammation, and fibrosis. But their mechanisms have yet to be well known. (Buthut et al., 2018; Topcu et al., 2019). Studies on antioxidant-based radioprotective agents in preventing or reducing injury due to IR are currently being carried out (El-Missiry et al., 2021; Zhang et al., 2016). This is the first study to report that MC decreases oxidative stress and inflammation biochemically and histopathologically in the irradiated rat brain through its antioxidant properties.

It is known that IR produces ROS by reacting with DNA, protein, and membrane lipids in the cell. ROS production is essential for IR-related tissue injury (Aras et al., 2021). Excessive production of oxygen radicals impairs the balance between prooxidants and antioxidants. Oxidative balance shifts to the prooxidant side while the intracellular concentration of antioxidants decreases (Srinivasan et al., 2007). Elevated free radical compounds in the neuronal cells accompany increased lipid peroxidation in the membrane lipids. Neural membranes in the brain are affected by free radicals more quickly since they are more enriched, particularly for unsaturated fatty acids. Therefore, free radicals' formation influences the neurons' structure and function. Oxidative stress emerges when ROS accumulates, resulting from excessive production or inadequate neutralization and damage to proteins, lipids, and DNA (Blokhina et al., 2003; Gehrmann et al., 2015). Also, mitochondria significantly contribute to cellular ROS.

ROS produced in the mitochondria, also by targeting the electron-carrying chain, causes a cycle involving increased ROS production and subsequently causes depletion of ATP and cell death. In light of this evidence, the definition of new compounds that can resist oxidative stress and become potentially therapeutic has recently attracted

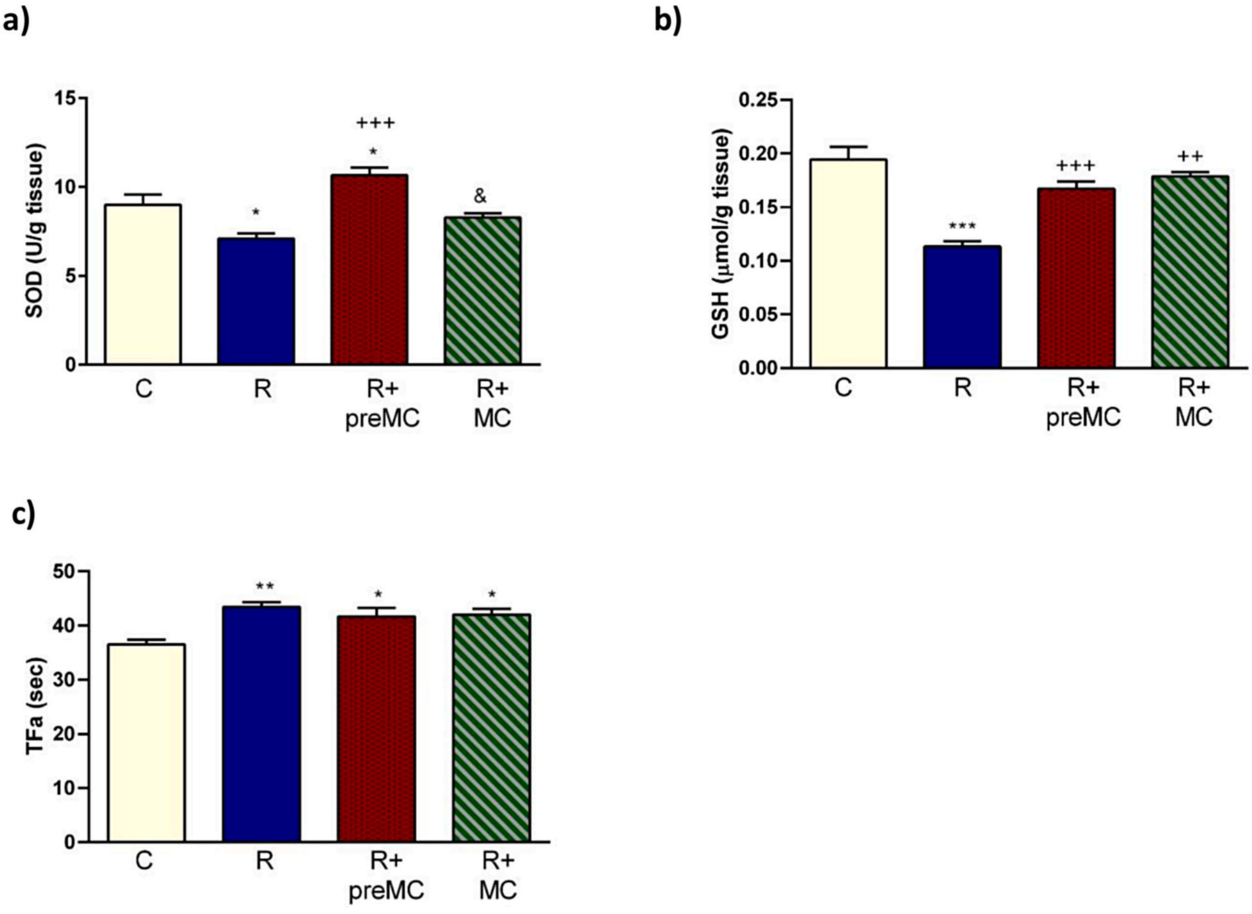

Fig. 2. (a) SOD activity, (b) GSH level, (c) TF activity in the brain tissues of control (C), irradiated (R), R + preMC, and R + MC groups. Each group consists of eight animals. The range bars show the SEM. *$p < 0.05$ **$p < 0.01$ and ****$p < 0.001$ compared with control group; +$p < 0.05$, ++$p < 0.01$ and +++$p < 0.001$ compared with R group & $p < 0.05$ compared with R + preMC group. Group definitions: C: control; R: irradiation; R + preMC: *Myrtus communis* treatment before and after irradiation; R + MC: *Myrtus communis* treatment after irradiation.

Table 1
(a) NO level, (b) GSH level (c) MDA level (d) MPO activity (e) SOD activity and (f) TF aktivity in the brain tissues of control (C), irradiated (R), R + preMC and R + MC groups.

	R group compared with C group	preMC group compared with C group	MC group compared with C group	preMC group compared with R group	MC group compared with R group
NO (µmol/g)	$\uparrow ^{***}$	$\uparrow^{***}$	$\uparrow^{***}$	$\downarrow^{+++}$	$\downarrow^{++}$
GSH (µmol/g)	$\downarrow^{***}$	↔	↔	$\uparrow^{+++}$	$\uparrow^{++}$
MDA (nmol/g)	$\uparrow^{***}$	↔	↔	$\downarrow^{+++}$	$\downarrow^{+++}$
MPO (U/g)	$\uparrow^{**}$	↔	↔	$\downarrow^{++}$	↔
SOD (U/g)	$\downarrow^{*}$	$\uparrow^{*}$	↔	$\uparrow^{+++}$	↔
TFa (sec)	$\uparrow^{**}$	$\uparrow^{*}$	$\uparrow^{*}$	↔	↔

The symbols indicating the statistically significant difference compared with C group.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
The symbols indicating the statistically significant difference compared with R group.
$^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$.
↑: statistically significant increase.
↓: statistically significant decrease.
↔: no significant difference is present ($p > 0.05$).

significant attention (Menendez-Montes et al., 2021). Since oxidative stress plays a vital role in IR-related neuronal injury, many antioxidants have been researched regarding applicability as radioprotectors. Chrysin has been used for its low toxicity in IR-related injury (Mansour et al., 2017). Curcumin, one of the agents penetrating the blood-brain barrier, has also been recommended for neuroprotection (Kostova et al., 2023). These studies have stated that antioxidant agents can reduce DNA injury and injury-related ROS activity. Studies on MC have denoted that it may inhibit ROS generated during inflammation, including superoxide formation produced through NADPH oxidase and hydrogen peroxide produced by superoxide dismutase activity (Yaşar et al., 2021).

In this study, the MDA level was tested as an essential indicator of lipid peroxidation. MDA levels significantly increased in the R group

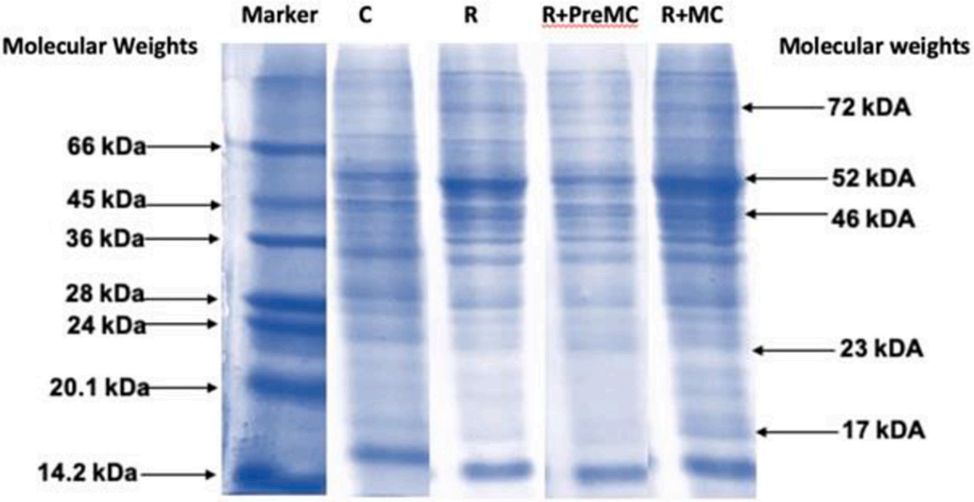

Fig. 3. Protein profiles of the brain tissues among C, R, R + PreMC, and R + MC groups. Group definitions: C: control; R: irradiation; R + preMC: *Myrtus communis* treatment before and after irradiation; R + MC: *Myrtus communis* treatment after irradiation.

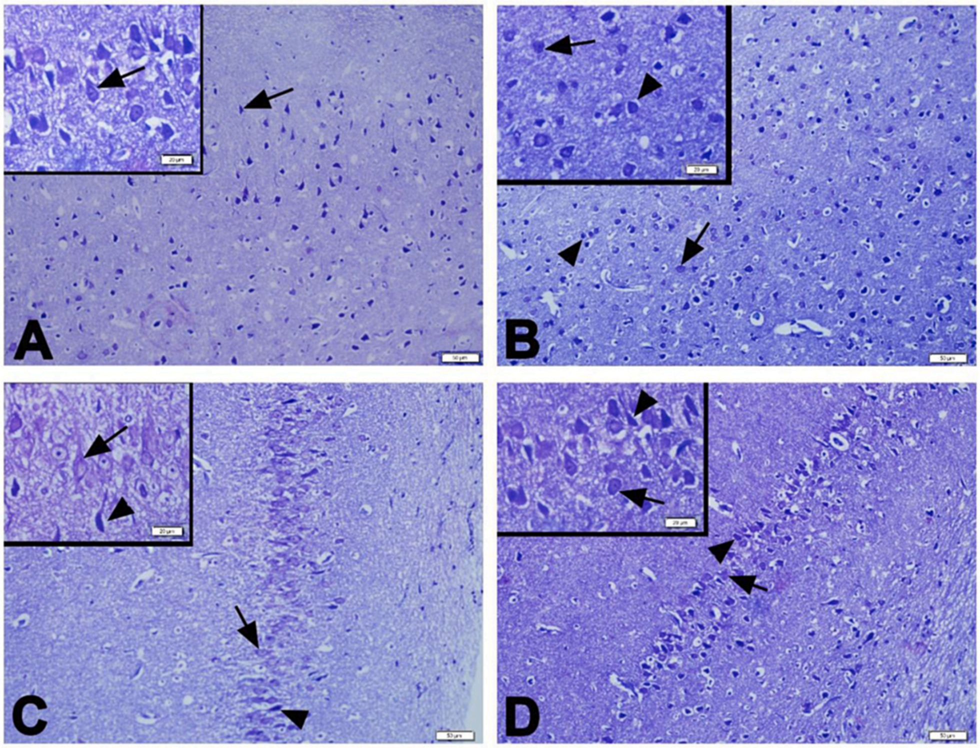

Fig. 4. The representative microphotographs of the brain in the experimental groups. The neurons with regular morphology in the control group (A) (arrow); the neurons with regular morphology (arrow) and increased injured neurons (arrowhead) in the irradiation group (B); the neurons with regular morphology (arrow) in the pretreatment (R + preMC) (C) and treatment (R + MC) (D) groups as well as decreased injured neurons (arrowhead) are represented. Cresyl violet stain. Scale bars: 50 μm, insets: 20. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

after treatment of IR, whereas MDA levels decreased in the preMC + R and MC + R compared with the R group. Conversely, a more dramatic decrease was discovered in the preMC + R group compared with the MC + R group. MDA level was also evaluated in a study investigating the effect of alpha-lipoic acid on oxidative damage and apoptosis due to ionizing irradiation in the brains of rats. At 24 h after irradiation, MDA levels in irradiated rats, pretreated with alpha-lipoic acid, were significantly higher than in the control group ($p < 0.001$); however, it resulted in a significant reduction compared to the irradiated group ($p < 0.05$) (J. Xu et al., 2023). Another study examined the protective effectiveness of 5, 7-Dihydroxyflavone (DHF) acrylamide in neuronal damage against irradiation and found that MDA levels increased significantly in the irradiated group. On the other hand, a significant decrease in MDA levels was detected after DHF application (Mansour et al., 2017).

In a previous study conducted with wogonin, obtained from the root of Scutellaria baicalensis Georgi, it was determined that the level of GSH, functioning as a free radical scavenger, reducing peroxide, and playing a central role in the antioxidant mechanism, decreases in the brain and blood after IR. It is considered that this reduction increases the level of free radicals (L. Wang, Li, et al., 2020). In this study, although

GSH levels decreased after exposure to IR, GSH levels were increased with wogonin administration. However, the remarkable aspect of the study is that the pretreatment group was superior to the post-treatment group. Similar to this study, MC treatment increased GSH levels in the irradiated rats. Besides, the elevation in the GSH level of the preMC + R group was higher than that in the MC + R group. This result could be attributed to MC's antioxidant and ROS scavenging effect. Radiation-induced ROS production can be neutralized by MC treatment. In the literature, there was a lack of studies that determines MPO and SOD activities in case of exposure to IR in brain tissue. MPO, a member of the heme peroxidase-cyclooxygenase superfamily, is expressed in neutrophils, monocytes, and some tissue macrophages, and it produces hypochlorous acid (HOCl) in the case of inflammation. Hypochlorous acid is a potent antioxidant. Ramos et al. have demonstrated that MPO activity is elevated after exposure to IR (Ramos et al., 2022). Özyurt et al. investigated the possible protective effects of quercetin against IR-induced bladder and kidney damage in rats. In the study, irradiation caused a significant increase in MPO activity in both kidney and bladder tissues compared to MPO activity measured in tissues of control rats. On the other hand, a significant decrease in MPO activity was observed in irradiated rats treated with quercetin. It even approached the values found in control rats (Özyurt et al., 2014). In another study examining Punica granatum peel extract's effect, MPO activity was observed in irradiated rats. However, MPO activity was depressed in the group that received Punica granatum before irradiation (Toklu et al., 2009). In our study, MPO activity increased in the R group, similar to these studies. It was also found that MC treatment depressed MPO activity after irradiation exposure. The striking result of the study is that MC treatment before radiation exposure was more effective in reducing oxidative stress than post-radiation treatment.

The studies related to SOD are also very conflicting. SOD is an important enzyme which eliminates superoxide and provides the primary defense against oxidative damage. Motallebzadeh et al. conducted a study to determine the effects of melatonin on irradiation-induced oxidative stress in the brainstem region of Wistar rats. One of the groups was injected with melatonin 1 h before irradiation. Irradiated rats exhibited a significant reduction in SOD levels compared to the control group. It was found that the activity of SOD was significantly increased in pretreatment with the melatonin group in comparison to the irradiated group (Motallebzadeh et al., 2022). Another study found that application of curcumin before and after irradiation prevented oxidative damage caused by irradiation by significantly increasing the activities of antioxidant enzymes such as SOD (Shabeeb et al., 2020). In our study, consistently with these studies, SOD activity decreased after exposure to IR. However, we figured out that MC treatment before IR provided an elevation in SOD activity, whereas MC treatment after IR showed no contribution to SOD activity. Contrary to our study, Otsuka et al. (Otsuka et al., 2006) and Zhu et al. (Zhu et al., 2007) have demonstrated that SOD activity increased after IR. These inconsistencies between the studies may be related to the differences between the radiation-fraction doses used in each study.

IR also increases NO and its products, the final product of lipid peroxidation caused by ROS in the brain. ROS reacts with unsaturated fatty acids in the first stage during lipid peroxidation (Ahlatci et al., 2014). Although there is a small number of studies related to IR, it is known that NO levels increase oxidative stress (Ping et al., 2020). Shirazi et al. used different radiation schemes in their study investigating the effects of radiation on peripheral blood and the radioprotective effects of melatonin. Exposure to varying irradiation doses was found to cause a dose-dependent decrease in antioxidant enzyme activity and an increase in serum NO levels. In the study, pretreatment with melatonin was shown to have a radioprotective effect by reducing NO levels in all dosage schedules (Shirazi et al., 2013). In our study, the NO level increased after IR. MC added to IR resulted in a sharp decrease in NO. However, more importantly, it was found that MC treatment before exposure to IR decreased the NO level more effectively than the MC treatment after the IR exposure. NO level in the preMC + R group was almost equal to the C group.

Another significant result of our study was that decreased TFa following IR. Studies have suggested that IR causes endothelial cell apoptosis and reduces vascular thromboresistance by increasing the expression of TF (J. Wang et al., 2004; Goldin-Lang et al., 2007). Goldin-Lang et al. have ascertained that IR elevated the procoagulant activity of TF. As in lung and placenta tissue, the brain is one of the tissues richest in tissue factor. Since tissue factor is a protein and radiation can cause denaturation, brain TF activity was found to be decreased after radiation exposure in this study (Goldin-Lang et al., 2007). In our study, even though TF secretion increased due to the damage in the brain tissue after radiation, MC application could not provide renaturation because the protein structure was denatured.

According to the results of histopathological examinations, it has been found that the treatment of MC can reduce tissue injury caused by IR. Histopathological evaluation of brain tissue was also performed. The neurons were evaluated concerning hypertrophy, inflammatory reaction, edema, and injury. Brain tissues in the control tissues were normal, whereas inflammatory reaction, edema, and injury were encountered in the neurons of the R group, consistent with the literature (Zhang et al., 2016). In our study, a decreased number of injured and edematous neurons and the neurons with normal morphology in the groups applied with MC were observed.

In the brain electrophoretic examination, 72 kDa, 52 kDa, 46 kDa, 23 kDa, and 17 kDa protein bands were altered in the groups. The brain protein alterations detected in this study were consistent with the radiation-induced protein changes in the literature (Kishimoto et al., 1989; Sawaya et al., 1994; Siman et al., 2004; Venter, 1983). In this study, regarding the changed protein bands of brain tissue, it was determined by literature research that 72 kDa, 52 kDa, 46 kDa, 23 kDa, and 17 kDa proteins could be brain type-IV collagenase (Sawaya et al., 1994) protein, tubulin-associated unit (tau) protein (Siman et al., 2004) or smooth muscle muscarinic receptor (Venter, 1983), the catalytic domain of protein kinase C (Kishimoto et al., 1989), smooth muscle muscarinic receptor (Siman et al., 2004) and the smaller immunoreactive proteolytic fragment of tau protein (Siman et al., 2004), respectively.

Our previous studies revealed that MC has phenolic compounds such as caffeic acid derivative, ellagic acid, myricetin, myricetin hexoside, myricetin rhamnoside, quercetin rhamnoside, sinapinic acid derivative, trihydroxy cinnamic acid derivative with a high phenolic and flavonoid content (Arslan et al., 2022; Ozbeyli et al., 2020; Sen et al., 2016). Also, Romani et al. found phenolic compounds (flavonoids such as myricetin and catechin derivatives and phenolic acids such as gallic acid, caffeic acid, and ellagic acid) in MC (Romani et al., 1999). In addition, our research team reported in previous studies that MC has antioxidant and anti-inflammatory activity in vitro and in vivo bioactivity studies (Ozbeyli et al., 2020; Sen et al., 2016, 2017). It also revealed that the total phenol (368.68 mg/g extract) and flavonoid (111.35 mg/g extract) content of the extract was quite high (Sen et al., 2017). Phenolic compounds are generally known for their antioxidant and anti-inflammatory activities (Haminiuk et al., 2012; Lin et al., 2016). It has also been suggested that phenolic compounds such as phenolic acids and flavonoids have radioprotective effects (Akbar Boojar, 2020; Mun et al., 2018). However, the mechanism of action of phenolic acids on the brain has yet to be discovered. Phenolic acids may be responsible for inhibiting lipopolysaccharide-induced microglial inflammation, inhibition of TNF-α, IL-6, IL-1β, and NO production, and reduction of mRNA and protein levels of COX-2 and iNOS. They may exert anti-inflammatory effects through these mechanisms (WU et al., 2015). The activity of phenolic acids in the brain has been discussed only regarding biochemical and enzyme activity changes. Although the transfer of polyphenols across the blood-brain barrier is assumed to be limited, a few studies have indicated that phenolic acid compounds may be absorbed into the brain. These studies stated that phenolic acids could accumulate in the brain at nanomolar or micromolar concentrations (Tarozzi et al., 2007; Wu et al., 2016). Therefore, the radioprotective effect of MC may be due to its high content of phenolic compounds, which provide it with significant antioxidant and anti-inflammatory activity.

4.1. Limitations

The study had several limitations. The first limitation of the study was that only specific biochemical and histopathological parameters were analyzed. Secondly, we did not perform neurocognitive tests to investigate the effects of *Myrtus communis* on cognitive functions after radiation exposure. However, we are planning for a follow-up experiment to investigate the long-term impact of MC on cognitive functions after radiation exposure. Another limitation of the study is that although its strength is that it is the first study to evaluate the effect of *Myrtus communis* on radiation-induced brain injury, the lack of other studies in this field limits the generalizability of the effect of *Myrtus communis*.

5. Conclusion

Our study showed that the administration of Myrtus communis effectively mitigated numerous biochemical and histopathological alterations in brain tissue associated with ionizing radiation. NO, MDA and MPO levels increased after irradiation and decreased after Myrtus communis application. Additionally, GSH and SOD levels, which decreased after irradiation, also decreased with the addition of Myrtus communis. Another critical finding is that although satisfactory results were shown in both the pre-treatment and post-treatment groups, A striking effect was seen in the pre-treatment group. Although previous studies stated that Myrtus communis may have anti-inflammatory and anticancer properties, the present study first manifested that Myrtus communis is a neuroprotective agent in the brain and prevents oxidative stress after radiation exposure. The neuroprotective efficacy of Myrtus communis can be ascribed to its antioxidative, anti-inflammatory, and anti-lipid peroxidative properties. This study serves as an in vivo pilot investigation, examining the potential of Myrtus communis as a novel radioprotective agent in clinical applications, and is poised to provide valuable insights for future research in this field.

CRediT authorship contribution statement

Dicle Aslan: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Burcu Alan:** Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nagehan Özyılmaz Yay:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sümeyye Yılmaz Karaoğlu:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Büşra Ertaş:** Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis. **Ali Şen:** Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Cemile Ceylan:** Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Tuğba Tunalı Akbay:** Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Feriha Ercan:** Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Beste M. Atasoy:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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