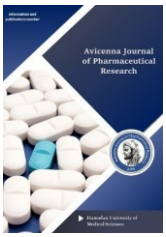
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Original Article

Antioxidant and Radioprotective Effects of Vitamin D and E Co-Administration on Bone Marrow in Gamma-Irradiated Male Rats

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Abstract

Background: The present study aimed to investigate the protective and therapeutic effects of vitamins E and D against gamma-radiation-induced damage in the bone marrow of male Wistar rats.

Methods: Forty-five male Wistar rats were randomly divided into 15 experimental groups, with three rats in each group. After the *in vivo* experiments and tissue processing, histopathological assessments were performed. Additionally, oxidative stress markers, including SOD, CAT, TAC, and GPx, were measured in the collected tissues.

Results: The results showed that exposure to 2 Gy of radiation significantly altered blood cell counts in the irradiated group, whereas these parameters improved in the treated groups. Histipathological examination of blood smears and bone marrow further supported the therapeutic effects of vitamins E and D against radiation-induced damage. In addition, biochemical analyses showed that TAC, SOD, GPx, and CAT levels were higher in the treatment groups than in the radiation group, suggesting the protective effects of these vitamins.

Conclusion: Our findings suggest that the combined administration of vitamins E and D is more effective than treatment with either vitamin alone in protecting against radiation-induced biochemical and histological changes in rat bone marrow.

Keywords: antioxidant, bone marrow, gamma radiation, radioprotection, vitamin D, vitamin E

Introduction

Ionizing radiation (IR) can elicit diverse cellular responses depending on the rate of exposure and dose [1]. The toxic effects of high-level IR on biological systems result from the excessive production of reactive oxygen species (ROS) which can overwhelm antioxidant defenses [2].

Radiation-induced hematopoietic dysfunction is a critical clinical challenge, frequently observed in both accidental radiation exposure and cancer

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patients undergoing radiotherapy. Maintaining hematopoietic homeostasis is essential for survival following radiation injury [3]. Patients receiving ionizing radiation (IR) or chemotherapy often experience acute and long-term bone marrow (BM) damage, which limits the efficacy of cancer treatment and significantly diminishes health-related quality of life [4]. Irradiation of normal tissues, whether due to unplanned exposure or therapeutic interventions can induce a broad spectrum of toxicities. Clinically managing these complications is highly challenging, as radiation triggers severe cellular dysfunction, including apoptosis and necrosis, particularly at higher doses [5, 6]. Due to the rapid turnover of their progenitor cell populations, the hematopoietic (HP) and gastrointestinal (GI) systems are highly radiosensitivity. The HP system is notably vulnerable to radiation doses exceeding 1 Gy. Consequently, HP syndrome is characterized by a precipitous depletion of circulating blood cells, culminating in severe clinical complications such as hemorrhage, neutropenia, and thrombocytopenia. Furthermore, the concurrent impairment of the adaptive immune system frequently leads to sepsis, primarily driven by opportunistic infections [7]. In the absence of therapeutic intervention, bone marrow failure is frequently lethal. However, given that a residual subpopulation of bone marrow stem cells may survive irradiation, the potential for hematopoietic regeneration remains. Consequently, protecting the hematopoietic (HP) system remains a fundamental strategy for enhancing survival following radiation injury [8]. It is well established that compounds with antioxidant and anti-inflammatory properties exert significant radioprotective effects, thereby preserving cellular integrity against oxidative stress-induced tissue damage [9].

A wide array of natural products has been investigated for the prophylaxis and treatment of various human diseases. Many of these agents have garnered specific patents and are classified as generally recognized as safe (GRAS) [10]. Increasingly, these compounds are acknowledged for their potent radioprotective potential coupled with minimal systemic toxicity. Vitamin E, a prominent family of fat-soluble nutrients, is highly regarded for its anti-inflammatory, neuroprotective, and antioxidant properties [11]. This compound effectively protects cells from oxidative damage triggered by radiation-induced free radicals [12]. Because the human body lacks the capacity to synthesize vitamin E endogenously, it must be acquired through dietary sources or exogenous supplementation. Currently, vitamin E is a collective term encompassing all tocopherols and their derivatives, with α-tocopherol being the most biologically active form. [13].

Recently, significant attention has been directed toward the radioprotective properties of vitamin D, the synthesis of which dependent on specific wavelengths of light [14]. Although vitamin D is an endogenous compound, its deficiency has become increasingly prevalent in modern societies [15]. Beyond its established role in bone homeostasis, vitamin D exerts diverse regulatory functions, particularly by influencing the systemic oxidant-antioxidant balance [16, 17].

Accordingly, this study aimed to evaluate the efficacy of vitamin E and vitamin D in mitigating oxidative stress following exposure to ionizing radiation.

Materials and Methods

Experimental animals

Male Wistar albino rats (200–250 g) obtained from the National Center for Radiation Research and Technology (NCRRT), Hamadan, Iran, were housed in specially designed cages and kept under standard conditions of temperature (25˚C ± 5˚C), humidity, and light-controlled room (12:12 h light: dark cycle), and were provided with a standard laboratory diet and water ad libitum. All experiments were conducted in accordance with the National Institutes of Health guide for the Care and Use of Laboratory Animals (NIH).

Publications No. 8023, revised 1978) and approved by the Medical Ethics Review Board of Hamadan University of Medical Sciences (Approval No. IR.UMSHA.REC.1402.169).

Reagents and chemicals

Vitamin D and vitamin E powders were purchased from Sigma-Aldrich. Following irradiation, vitamin D and E were administered at doses of 5000

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IU/kg/day and 100 IU/kg/day, respectively, for 14 consecutive days. Absorbance was measured using an ELISA microplate reader (BioTek ELx800™). The CAT, SOD, GSH, and TAC assay kits were obtained from KIAZIST Co. (Iran). All other chemicals were of the highest available analytical grade and were supplied by Sigma-Aldrich (USA) or Merck (Germany).

Radiation facility

The animals were housed in specially designed, well-ventilated acrylic cages and exposed to whole-body irradiation at a total dose of 2 Gy using a biological irradiator equipped with the specified radioactive source (Tc-99m sodium pertechnetate).

Experimental design

The animals were randomly allocated into fifteen experimental groups (n = 3 per group) : (1) control, (2) gamma-ray, (3) vitamin D + E, (4) vitamin E, (5) vitamin D, (6) gamma-ray + vitamin D + E, (7) gamma-ray + vitamin E, (8) gamma-ray + vitamin D, (9) pre-treatment vitamin D + E, (10) pre-treatment vitamin E, (11) pre-treatment vitamin D, (12) post-treatment vitamin D + E, (13) post-treatment vitamin E, (14) post-treatment vitamin D, and (15) olive oil.

Vitamin D and vitamin E were administered intraperitoneally at doses of 5000 IU/kg/day and 100 IU/kg/day, respectively, according to the treatment schedule for each group. Phosphate-buffered saline (PBS; pH 8.0) was used as the vehicle for the control group, whereas olive oil served as the vehicle for vitamin D and vitamin E administration.

Sample Preparation

At the end of the experimental period (day 15), rats were fasted for 12 h and subsequently euthanized. Bone marrow tissue was rapidly excised and homogenized in accordance with the manufacturer's instructions for the assay kits. Additionally, blood samples were collected via cardiac puncture using sterile syringes.

Histopathological and Hematological Analysis

Bone marrow tissue specimens were collected and fixed in 10% neutral buffered formalin. Following fixation, samples underwent standard processing: dehydration, clearing, and embedding in paraffin wax. Paraffin sections (5 µm thick) were prepared, stained with hematoxylin and eosin (H&E), and examined using light microscopy at 40× magnification.

For hematological assessment, the peripheral blood smears were prepared using the wedge technique, air-dried, fixed in methanol, and stained with Wright-Giemsa stain. The slides were examined under a light microscope at 100× magnification using oil immersion [18].

Measurement of oxidant and antioxidant markers

TAC and the activities of SOD, GPx, and CAT were measured using commercial assay kits (KIAZIST, Iran) according to the manufacturer's instructions. TAC was determined based on the reduction of Cu^{2+} to Cu^{+}, with absorbance measured at 450 nm. For the SOD and GPx assays, tissue samples were homogenized in chilled phosphate-buffered saline and centrifuged at 12,000 rpm for 15 min at 4°C. The resulting supernatants were used for subsequent analyses. SOD and GPx activities were measured at 570 and 340 nm, respectively. CAT activity was assessed in tissue homogenates using a Purpald-based assay kit at 540 nm and normalized to the total protein content determined by the Bradford method.

Statistical analysis

Data were analyzed statistically using SPSS. Differences among the experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. The data are presented as mean ± standard deviation (SD). Statistical significance was considered at $p < 0.05$.

Results

Histopathological and Hematological Findings

Figure 1 ilustrates the microscopic findings of blood smears from the experimental groups. In the control group, lymphocytes were identified, and the erythrocytes exhibited normal morphology, with no obvious cellular alterations or signs of damage (Fig. 1A). Similar findings were observed in the olive-oil-treated group, in which leukocytes were present and no remarkable morphological abnormalities were

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detected (Fig. 1B). In contrast, the irradiated group showed prominent platelet aggregation, whereas lymphocytes were not readily identifiable in the examined microscopic field, suggesting radiation-associated alterations in the blood smear (Fig. 1C).

In the group treated with the combination of vitamins E and D, a leukocyte morphologically consistent with a monocyte was observed, while the erythrocytes appeared intact, with no obvious morphological abnormalities (Fig. 1D). Similarly, in the vitamin E-treated group, lymphocytes were present, and the erythrocytes exhibited normal morphology and a relatively uniform distribution, suggesting a possible protective effect of the treatment (Fig. 1E). In the vitamin D-treated group, cells morphologically consistent with lymphocytes were identified, and the erythrocytes appeared normal and uniformly distributed (Fig. 1F).

In the group receiving the combined vitamins D and E during irradiation, intact lymphocytes were observed among the erythrocytes, with no obvious erythrocyte abnormalities, suggesting a possible protective effect against radiation-associated damage (Fig. 1G). In the irradiated group treated with vitamin E, leukocytes were also observed; however, platelet aggregation persisted, suggesting partial protection with residual morphological alterations (Fig. 1H).

In the gamma-irradiation-plus-vitamin-D group, prominent platelet aggregation and morphological alterations were observed, with no obvious protective effect in the examined microscopic field (Fig. 1I). In the post-irradiation vitamin E-treatment group, marked platelet accumulation was evident, whereas lymphocytes were not readily identifiable in the examined field, indicating persistent radiation-associated alterations (Fig. 1J). Similarly, in the post-irradiation vitamin D-treatment group, few white blood cells were identifiable, and the field was predominantly occupied by erythrocytes, suggesting marked radiation-associated morphological alterations (Fig. 1K).

In the post-irradiation vitamin D + E-treatment group, microscopic analysis revealed the presence of platelet aggregation (indicated by arrow 1), rather than intact mononuclear leukocytes (Fig. 1L).

Furthermore, in the vitamin D + E pretreatment group, morphologically intact leukocytes were observed. Specifically, white arrow 1 indicates a mononuclear cell consistent with a lymphocyte, whereas white arrow 2 identifies a polymorphonuclear cell with a multi-lobed nucleus, characteristic of a neutrophil (Fig. 1M).

In the vitamin E pretreatment group, lymphocytes were readily identifiable, suggesting preservation of these cells following radiation exposure (Fig. 1N). Similarly, in the vitamin D pretreatment group, leukocytes morphologically consistent with neutrophils and lymphocytes were identified, suggesting a possible protective effect of the pretreatment protocol (Fig. 1O).

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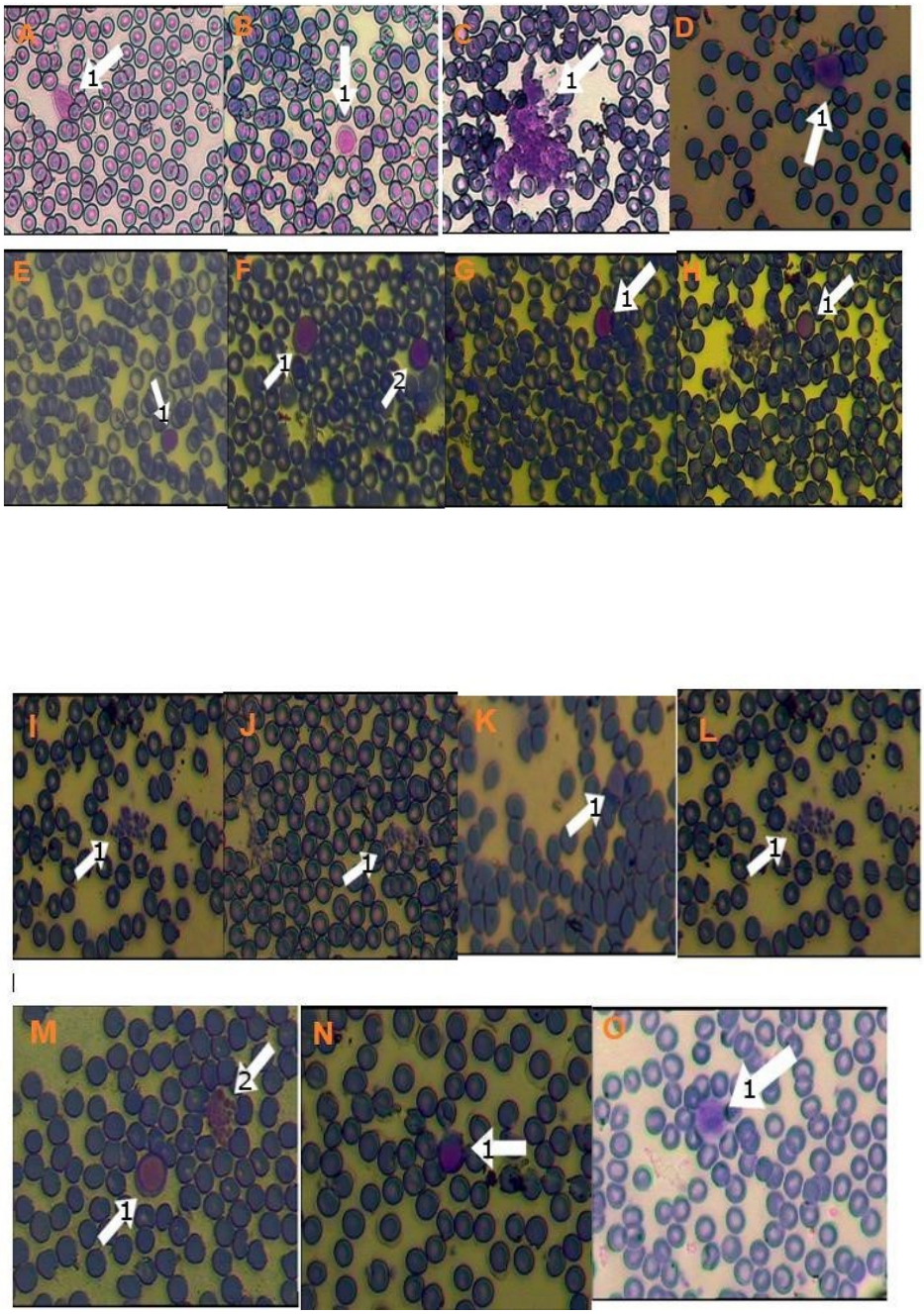


Figure 1. Representative photomicrographs of Wright–Giemsa-stained blood smears from the different experimental groups. (A) Control group; (B) olive oil-treated group; (C) gamma-irradiated group; (D) vitamin E + vitamin D group; (E) vitamin E group; (F) vitamin D group; (G) gamma irradiation + vitamin E + vitamin D group; (H) gamma irradiation + vitamin E group; (I) gamma irradiation + vitamin D group; (J) post-irradiation vitamin E-treated group; (K) post-irradiation vitamin D-treated group; (L) post-irradiation vitamin E + vitamin D-treated group; (M) vitamin E + vitamin D pretreatment group; (N) vitamin E pretreatment group; and (O) vitamin D pretreatment group. Original magnification: ×400.

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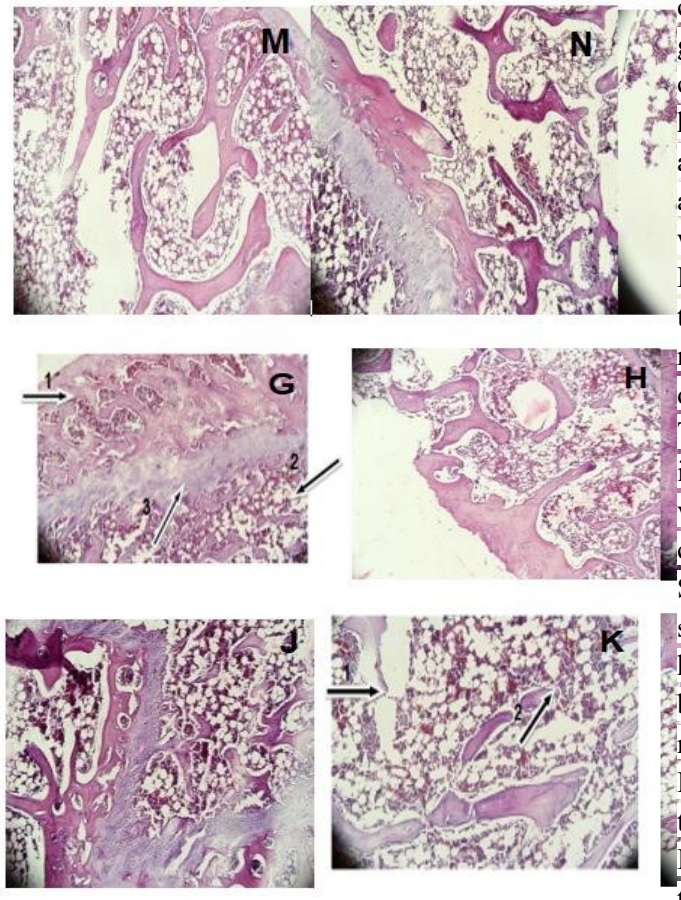


Figure 2 presents representative photomicrographs of bone marrow sections from the different experimental groups.

Fig. 2A depicts the bone marrow biopsy of the normal (control) group. As observed, the visualized tissue represents the subcortical region of the bone marrow. The overall trabecular network and marrow architecture were well preserved, exhibiting no signs of pathological damage. Furthermore, the proportion of adipocytes relative to bone and marrow cells was notably low.

In the olive oil-treated group, the marrow was located immediately beneath the cortical bone. Although cellularity in this region appeared to be slightly lower, the overall organization of the trabecular bone and bone marrow remained intact. Hematopoietic cells and adipocytes were distributed in a relatively normal honeycomb-like pattern (Fig. 2B).

In the radiation-exposed group, histological alterations are evident in specific tissue regions, notably the bone marrow and cartilage. Conversely, the skeletal muscle tissue remains unaffected due to its inherently low mitotic activity. Within the cartilaginous tissue, specifically encompassing the growth plate, the regular columnar arrangement of chondrocytes was markedly disrupted. These cells have lost their typical columnar alignment, adopting a multinucleated morphology accompanied by an altered staining affinity within the cartilage matrix, which is indicative of acute degeneration. Furthermore, the overall honeycomb architecture of the bone marrow was severely disorganized as a result of radiation-induced injury and the subsequent destruction of hematopoietic cells (Fig. 2C).

The bone marrow architecture was well maintained in the group treated with the combination of vitamins D and E, and no obvious pathological changes were identified (Fig. 2D).

Similarly, the vitamin D-treated group showed no specific pathological alterations. Both the honeycomb-like marrow pattern and the trabecular bone structure were largely preserved (Fig. 2E). No marked abnormalities were detected in the vitamin E-treated group either, and the overall architecture of the bone and bone marrow remained intact (Fig. 2F).

In the group exposed to gamma irradiation and treated with the combination of vitamins D and E, vascular congestion was present in the cortical region. The growth-plate cartilage beneath the cortex also showed degenerative features, including nuclear crowding and disruption of the normal columnar arrangement of chondrocytes. Although the trabecular bone remained relatively intact, the marrow contained fewer hematopoietic cells and a greater proportion of adipocytes than normal marrow (Fig. 2G).

In the group receiving gamma irradiation and vitamin D, the general marrow architecture was largely preserved. The relative distribution of hematopoietic cells and adipocytes was also closer to that observed in the control group, as shown more clearly in the corresponding photomicrograph (Fig. 2H).

By contrast, the gamma-irradiated group treated with vitamin E showed a greater proportion of adipocytes relative to hematopoietic cells, indicating an apparent reduction in marrow cellularity. These features were highlighted in the figure, where marker 1 points to a residual pocket of hematopoietic tissue confined by encroaching adipose cells. Marker 2 indicates a large area of

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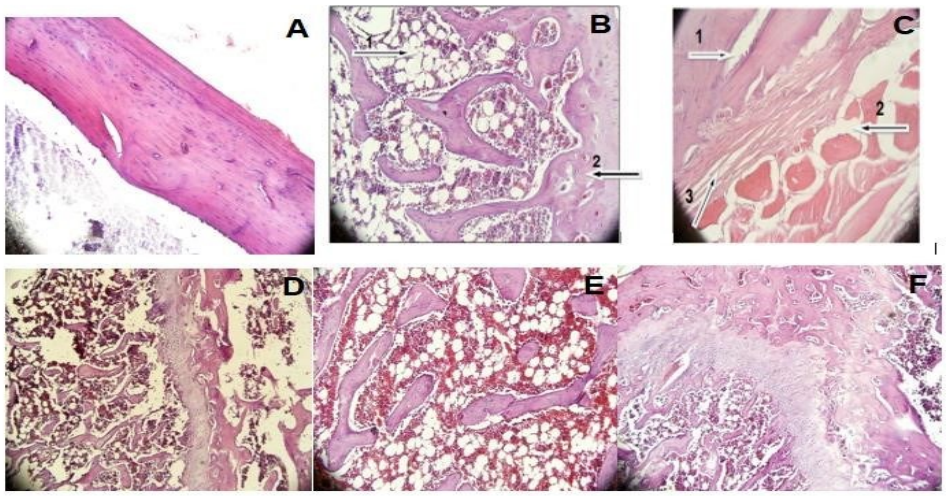
marrow adiposity, illustrating the extensive replacement of blood-forming elements with fat, which contributed to the overall hypocellular state of the marrow (Fig. 2I).

In the group pretreated with vitamins D and E, the relative proportion of adipocytes and hematopoietic cells was largely maintained, while the cartilage retained a near-normal appearance (Fig. 2J). In the vitamin E pretreatment group, adipocytes were somewhat more abundant than hematopoietic cells; however, no marked structural abnormalities were identified (Fig. 2K). In the vitamin D pretreatment group, the adipocyte-to-hematopoietic-cell ratio suggested a reduction in hematopoietic cellularity after gamma irradiation (Fig. 2L).

The group receiving combined vitamin D and vitamin E post-treatment showed radiation-associated changes in both the growth-plate cartilage and the bone marrow adjacent to the trabecular bone. These changes were characterized primarily by a reduction in the number of hematopoietic cells (Fig. 2M).

Marked radiation-associated alterations were also evident in the bone marrow of the vitamin E post-treatment group. These included disorganization of the marrow architecture and an increased proportion of adipocytes relative to hematopoietic cells. However, cartilage abnormalities were not prominent in this group (Fig. 2N).

Finally, in the vitamin D post-treatment group, the marrow architecture appeared disorganized, with adipocytes representing a greater proportion of the tissue than hematopoietic cells. The normal honeycomb-like pattern was partially lost, suggesting that the protective effect of this treatment, if present, was incomplete (Fig. 2O).



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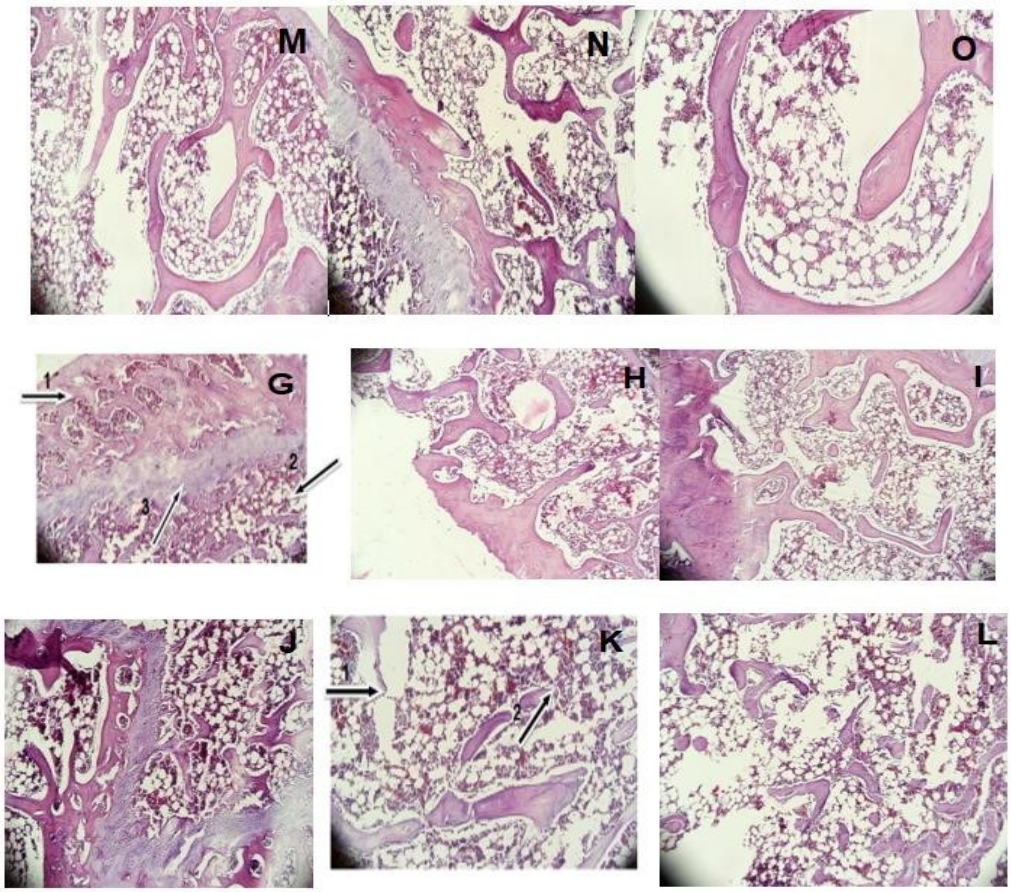
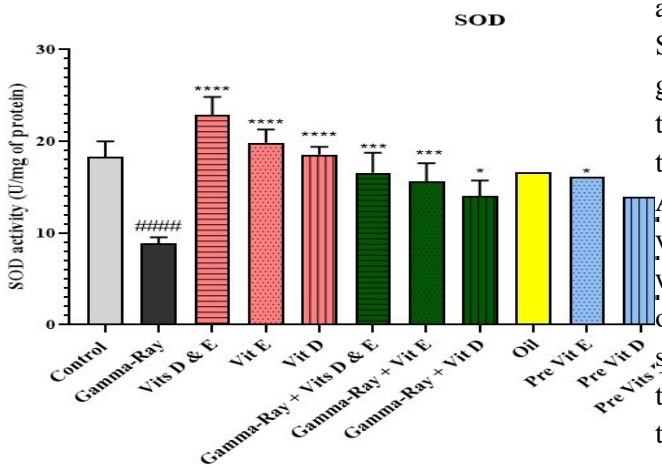


Figure 2. Representative photomicrographs of bone marrow sections from rats in the different experimental groups. (A) Control group; (B) olive-oil-treated group; (C) gamma-irradiated group; (D) vitamin E + vitamin D-treated group; (E) vitamin E-treated group; (F) vitamin D-treated group; (G) gamma-irradiated group treated with vitamin E + vitamin D; (H) gamma-irradiated group treated with vitamin D; (I) gamma-irradiated group treated with vitamin E; (J) vitamin E + vitamin D pretreatment group; (K) vitamin E pretreatment group; (L) vitamin D pretreatment group; (M) vitamin E + vitamin D post-treatment group; (N) vitamin E post-treatment group; and (O) vitamin D post-treatment group. Hematoxylin and eosin (H&E) staining; original magnification: ×400.

Evaluation of superoxide dismutase (SOD) activity in bone marrow tissue

As shown in Figure 3, gamma irradiation markedly reduced SOD activity compared with the control group ($p < 0.001$). SOD activity was higher in the non-irradiated groups treated with vitamins E and D, either alone or in combination, than in the gamma-irradiated group. Among the irradiated groups, the combination of vitamins E and D and vitamin E alone was associated with a significant increase in SOD activity compared with the gamma-irradiated group. A significant increase was also observed in the gamma irradiation + vitamin D group, although the difference was less pronounced.



Among the pretreatment groups, vitamin E and the vitamin E + vitamin D combination were associated with significantly higher SOD activity than that observed in the gamma-irradiated group, whereas no statistically significant difference was observed for the vitamin D pretreatment group. In the post-treatment groups, the vitamin E + vitamin D combination showed a significant increase in SOD activity, while no significant difference was indicated for vitamin E or vitamin D alone.

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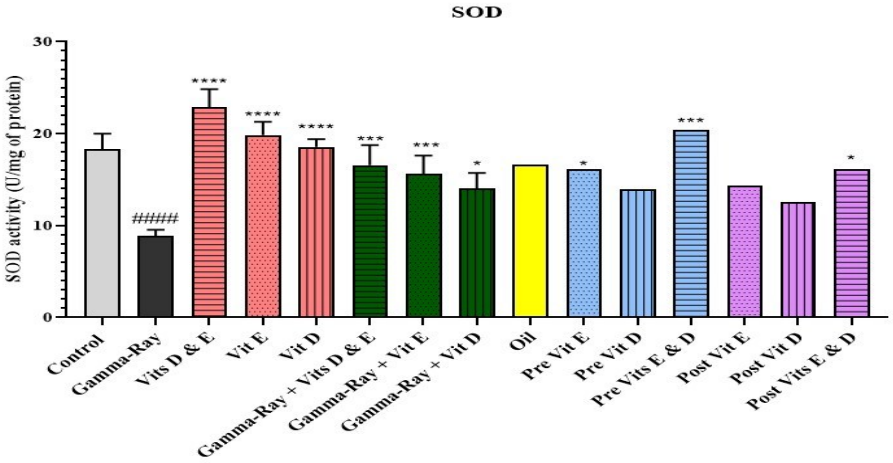


Figure 3 Comparison of superoxide dismutase (SOD) activity among the experimental groups. The effects of vitamins E and D, administered alone or in combination, on bone marrow SOD activity in rats exposed to gamma irradiation are shown. Data are presented as mean ± SEM (n = 3). $^{\#\#\#\#}p < 0.001$ indicates a significant difference between the gamma-irradiated and control groups. $^{*}p < 0.05$, $^{***}p < 0.005$, and $^{**}p < 0.001$ indicate significant differences between the respective treatment groups and the gamma-irradiated group.

Evaluation of total antioxidant capacity (TAC) in bone marrow tissue

As illustrated in

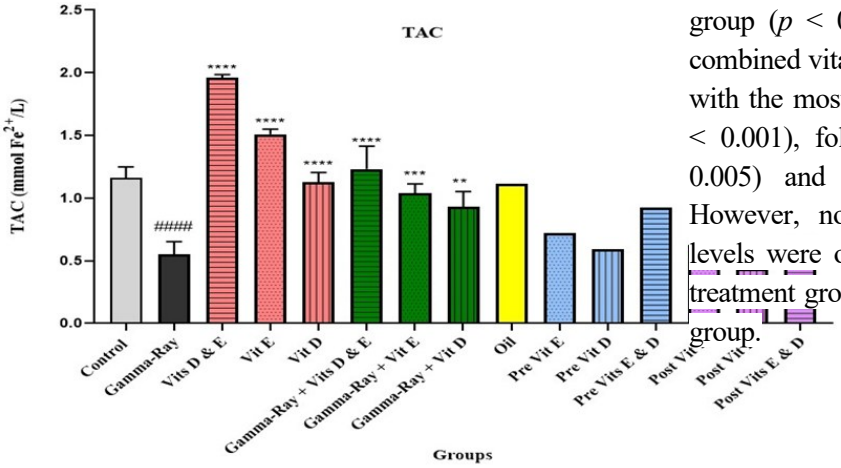
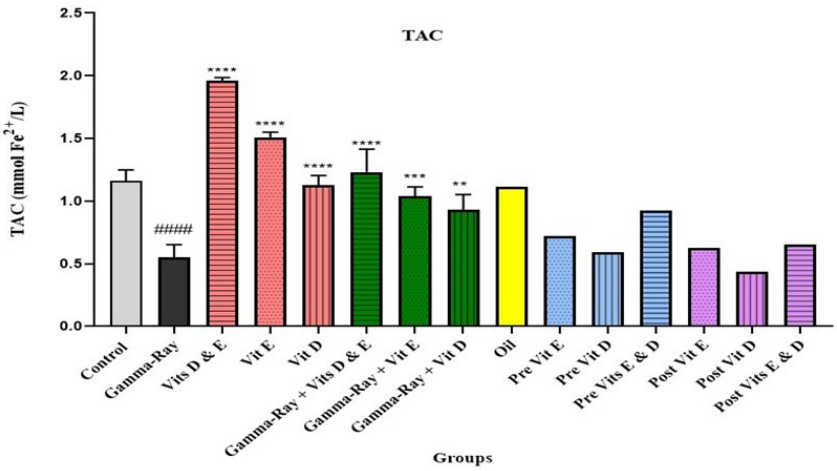


Figure 4, TAC was significantly lower in the gamma-irradiated group than in the control group ($p < 0.001$). TAC values were significantly higher in the non-irradiated groups treated with vitamin D, vitamin E, or their combination than in the irradiated group ($p < 0.001$). Among the irradiated groups, combined vitamin D and E treatment was associated with the most pronounced improvement in TAC ($p < 0.001$), followed by vitamin E treatment ($p < 0.005$) and vitamin D treatment ($p < 0.01$). However, no significant improvements in TAC levels were observed in the pre-treatment or post-treatment groups compared to the gamma-irradiated group.



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Figure 4. Total antioxidant capacity (TAC) in the bone marrow of the experimental rats. The effects of vitamin D, vitamin E, and their combination on TAC following gamma irradiation are shown. Data are expressed as mean ± SEM (n = 3). $^{\#\#\#\#}p < 0.001$ indicates a significant difference between the gamma-irradiated and control groups. $^{**}p < 0.01$, $^{***}p < 0.005$, and $^{**}p < 0.001$ indicate significant differences between the vitamin-treated groups and the gamma-irradiated group.

Evaluation of catalase (CAT) activity in bone marrow tissue

As illustrated in

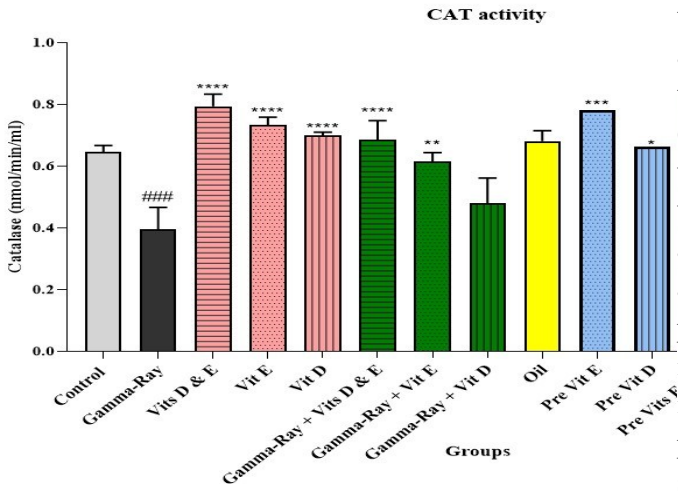


Figure 5, CAT activity was significantly lower in the gamma-irradiated group than in the control group ($p < 0.001$). Administration of vitamin D, vitamin E, or their combination in non-irradiated animals was associated with significantly higher CAT activity than that observed in the gamma-irradiated group (all $p < 0.0001$). Under irradiated conditions, vitamin E and the combined administration of vitamins D and E significantly increased CAT activity compared with irradiation alone ($p < 0.01$ and $p < 0.0001$, respectively). Notably, vitamin D pretreatment prior to irradiation also significantly increased CAT activity compared with the gamma-irradiated group ($p < 0.05$. In contrast, vitamin D post-treatment did not produce a statistically significant change in CAT activity.

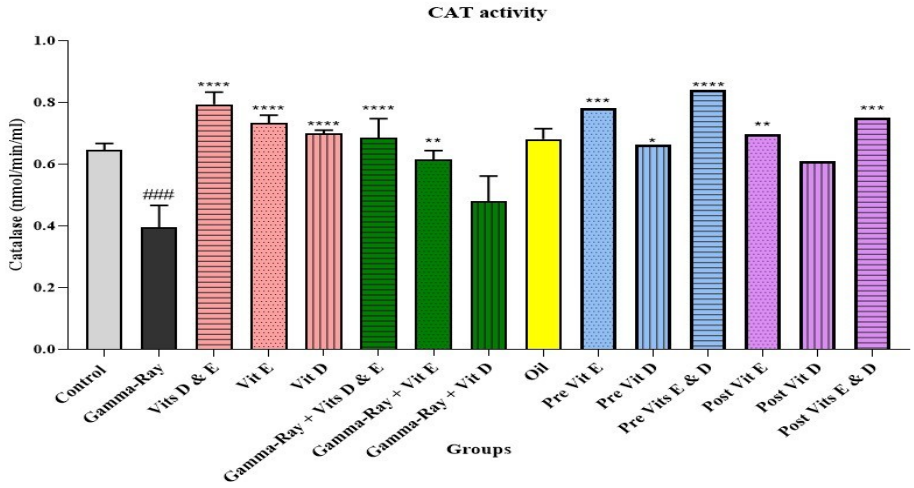


Figure 5. Catalase (CAT) activity in the bone marrow of rats across the experimental groups. The effects of vitamin D, vitamin E, and their combined administration under irradiated and non-irradiated conditions are shown. Data are presented as mean ± SEM (n = 3). $^{\#\#\#\#}p < 0.0001$ indicates a significant difference between the gamma-irradiated and control groups; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{**}p < 0.0001$ indicate significant differences between the vitamin-treated groups and the gamma-irradiated group.

Evaluation of glutathione peroxidase (GSH) levels in bone marrow tissue

As shown in

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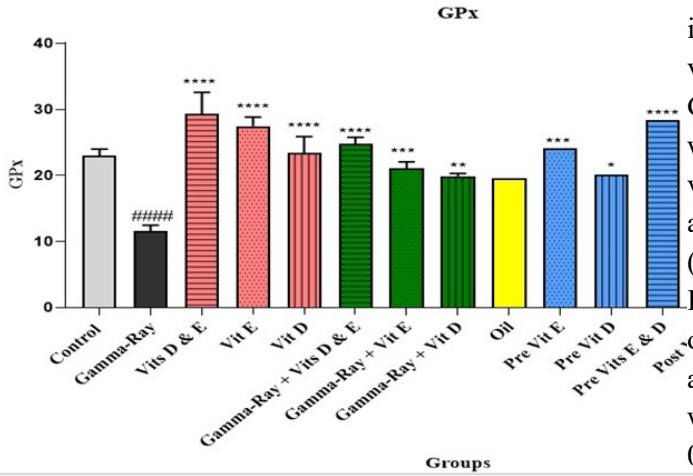


Figure 6, gamma irradiation significantly reduced glutathione peroxidase (GPx) activity compared with the control group ($p < 0.001$). In the non-irradiated groups, treatment with vitamin D, vitamin E, or their combination was associated with significantly higher GPx activity than that observed in the gamma-irradiated group. Among the irradiated groups, treatment with the combination of vitamins D and E resulted in the highest observed GPx activity and a significant increase compared with irradiation alone ($p < 0.001$). Vitamin E and vitamin D alone also significantly increased GPx activity compared with the gamma-irradiated group ($p < 0.005$ and $p < 0.01$, respectively).

In the pretreatment groups, combined administration of vitamins D and E, vitamin E alone, and vitamin D alone significantly increased GPx activity compared with the gamma-irradiated group ($p < 0.001$, $p < 0.005$, and $p < 0.05$, respectively). Similarly, post-treatment with vitamin E or the combination of vitamins D and E significantly increased GPx activity compared with irradiation alone ($p < 0.05$ and $p < 0.005$, respectively), whereas post-treatment with vitamin D alone did not produce a statistically significant change.

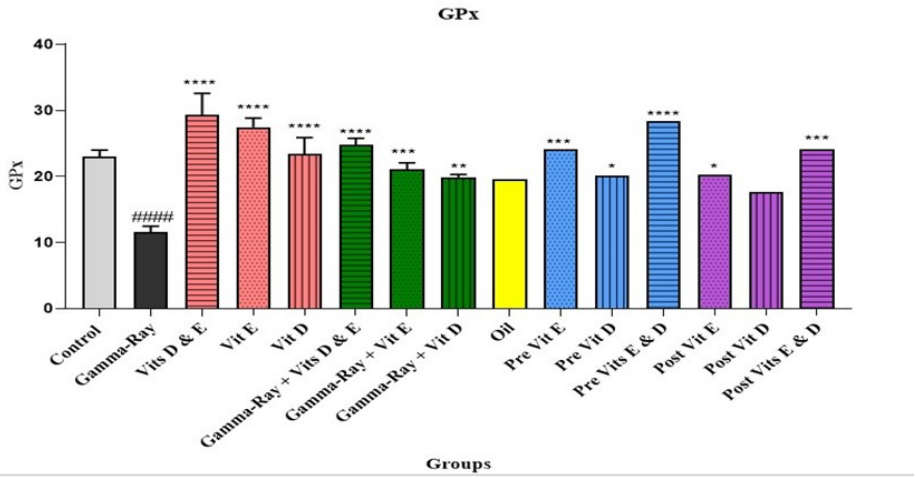


Figure 6. Glutathione peroxidase (GPx) activity in the bone marrow of rats across the experimental groups. The effects of vitamin D, vitamin E, and their combined administration under irradiated and non-irradiated conditions are shown. Data are presented as mean ± SEM (n = 3). $^{\#\#\#\#}p < 0.0001$ indicates a significant difference between the gamma-irradiated and control groups; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ indicate significant differences between the vitamin-treated groups and the gamma-irradiated group.

Discussion

The use of vitamins in the management of various human diseases has long been considered an important therapeutic strategy. Bone marrow tissue is particularly vulnerable to reactive oxygen species (ROS)-mediated damage, likely due to its high content of polyunsaturated fatty acids [19]. Therefore, antioxidant use to counter oxidative stress may help protect the bone marrow from radiation-induced injury. In the present study, the antioxidant effects of vitamins E and D on reducing oxidative stress in rat bone marrow were investigated.

Previous studies on bone marrow toxicity have demonstrated the crucial protective roles of antioxidants against such damage. In the current study, exposure to ionizing radiation (2 Gy) induced oxidative stress and caused harmful effects on the bone marrow, consistent with findings reported by Singh et al., Fotre et al., and Chen et al. [20-22]. Furthermore, our results indicated that gamma irradiation caused histopathological alterations and

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disrupted the antioxidant status in rat bone marrow. Additionally, radiation exposure altered the count and balance of blood cells.

The present findings indicated that gamma irradiation significantly reduced antioxidant capacity, as evidenced by reduced TAC and GPx levels and decreased CAT and SOD activities.

It is well established that exposure to ionizing radiation, whether resulting from accidental events or radiotherapy, disrupts the normal structure and function of biological systems. The extent of cellular injury depends on several factors, including tissue sensitivity and radiation dose [23]. Ionizing radiation induces oxidative stress by generating ROS, including hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{-\bullet}$), and hydrogen peroxide (H_2O_2), thereby disrupting the balance between pro-oxidant and antioxidant systems within cells [24, 25]. These reactive species can interact with vital cellular components, including lipids, DNA, and proteins, leading to cellular damage [26].

Exposure to ionizing radiation also causes a marked reduction in hematological parameters [27]. In the present study, irradiation induced significant disturbances in hematological indices, as evidenced by decreases in Hb concentration, RBC count, WBC count, platelet count, hematocrit, MCV, and MCH. These findings are consistent with previous reports [28]. Radiation may induce apoptosis and impair the growth, proliferation, viability, and migratory capacity of stem cells [29].

According to the findings reported by Omaima et al., the reduction in RBC count may be attributed to structural alterations in membrane proteins and intracellular peptides, together with increased intracellular viscosity, which may reduce erythrocyte deformability and accelerate their removal from the circulation. In addition, radiation-induced renal injury may impair erythropoietin production, thereby contributing to a reduction in RBC count [30, 31].

At the tissue level, our histopathological findings strongly correlated with these hematological and biochemical disruptions. Exposure to gamma radiation severely disrupted the bone marrow architecture, leading to marrow aplasia, destruction of the stromal network (manifesting as a honeycomb appearance), and increased bone marrow adiposity. However, interventions with vitamins D and E effectively preserved the residual hematopoietic pockets and minimized adipocyte replacement. This structural preservation confirms that mitigating ROS directly protects the highly radiosensitive hematopoietic niches

This may lead to depletion of factors required for erythroblast differentiation, impaired release of reticulocytes from the bone marrow, and loss of circulating cells due to hemorrhage. The observed decrease in Hb concentration may also be related to the destruction of erythrocyte precursors in the bone marrow, resulting in impaired iron utilization. Moreover, ionizing radiation may delay iron incorporation and reduce hemoglobin association with the erythrocyte membrane [32].

The present study further showed that vitamin D supplementation alone improved antioxidant defense parameters in the vitamin D-treated groups. In the groups receiving vitamin D in combination with irradiation, antioxidant defenses were also improved compared with those in the irradiated group, suggesting that vitamin D may attenuate radiation-induced oxidative imbalance. This effect was reflected by increased TAC and GPx levels and enhanced SOD and CAT activities. Moreover, while both pre-treatment and post-treatment were associated with improved antioxidant defense parameters, pre-treatment regimens were notably more effective. This suggests that the prophylactic presence of vitamin D is crucial for priming cellular defense mechanisms before the radiation-induced ROS cascade initiates, exerting protective effects on the antioxidant system in rat bone marrow. Vitamin D is a fat-soluble vitamin that occurs primarily in two forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) [32, 33]. Oxidative stress plays a central role in cellular injury under pathophysiological conditions because excessive ROS production can overwhelm antioxidant defenses and promote cellular damage [34]. Under physiological conditions, antioxidant defense mechanisms help maintain cellular redox homeostasis by regulating oxidant levels [35]. These defenses include enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, as well as nonenzymatic antioxidants, including albumin and GSH. Nutritional factors,

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including vitamins and minerals, may also modulate redox homeostasis [34]. Accordingly, vitamin D has been proposed to exert indirect antioxidant effects. Wiseman reported over two decades ago that vitamin D reduced iron-dependent lipid peroxidation in liposomal membranes. Structural similarities among vitamin D, cholesterol, and ergosterol have been proposed to contribute to the membrane-stabilizing properties of vitamin D [36]. Furthermore, vitamin D binds to the nuclear vitamin D receptor (VDR), thereby regulating the expression of various target genes [36]. Through VDR-dependent pathways, vitamin D may attenuate oxidative stress by enhancing intracellular GSH levels and modulating antioxidant enzymes, including SOD and GPx, while potentially reducing ROS generation [37]. Recent studies have reported that vitamin D supplementation may reduce oxidative stress biomarkers [38, 39]. Moreover, a meta-analysis of neurodegenerative diseases suggested that a vitamin D level of approximately 75 nmol/L may be associated with better bone health and neurological outcomes, possibly through attenuation of oxidative stress and improved mitochondrial metabolism [40].

The results further demonstrated that vitamin E supplementation alone improved antioxidant defense parameters in the vitamin E-treated groups. In addition, antioxidant defenses were improved in the groups receiving vitamin E in combination with irradiation compared with the irradiated group, suggesting that vitamin E may enhance antioxidant status during radiation exposure. This effect was reflected by increased TAC and GSH levels and enhanced SOD and CAT activities.

Vitamin E is a major lipid-soluble, chain-breaking antioxidant that scavenges free radicals in vivo. It exerts its antioxidant effects primarily within biological membranes and lipoproteins, where it can act in cooperation with water-soluble antioxidants such as vitamin C. Vitamins E and C may act synergistically to inhibit lipid and LDL oxidation [41, 42]. During the scavenging of lipid peroxyl radicals, vitamin E is converted into the α-tocopheroxyl radical. This radical can be reduced by vitamin C and other reducing agents, thereby contributing to the regeneration of active vitamin E. Alternatively, further oxidation of vitamin E may lead to the formation of oxidation products, including α-tocopheryl quinone, which has been proposed as an indicator of vitamin E oxidation and antioxidant activity [43]. Elevated levels of α-tocopheryl quinone have also been detected in human atherosclerotic plaques [44]. Vitamin E deficiency has been associated with several pathological conditions, including neurological disorders, ataxia, and infertility. The amelioration of some of these conditions by synthetic radical-scavenging antioxidants further supports the role of vitamin E as a chain-breaking antioxidant [45]. In this context, vitamins C and E may limit the propagation of lipid peroxidation through complementary chain-breaking antioxidant mechanisms [46].

Notably, the results demonstrated that the combined administration of vitamins D and E provided a highly pronounced radioprotective effects.

Overall, the present findings indicate that gamma irradiation induces oxidative stress, impairs antioxidant enzyme activities, and produces hematological and histopathological alterations in rat bone marrow. In contrast, vitamin D and vitamin E, administered either alone or according to pre-treatment and post-treatment regimens, attenuated these adverse effects and improved antioxidant defense status in the tissue. These findings suggest that vitamins D and E may be protective agents against ionizing radiation-induced bone marrow injury with combined prophylactic administration showing particularly promising effects.

Conclusion

The present findings demonstrated that exposure to 2 Gy of gamma irradiation induced substantial bone marrow toxicity, accompanied by significant biochemical and histopathological alterations. In contrast, rats treated with vitamin E or vitamin D, either alone or in combination, showed improved antioxidant defenses, as indicated by increased TAC levels and enhanced activities of superoxide dismutase, glutathione peroxidase, and catalase, together with partial or marked restoration of the altered bone marrow morphology. Notably, combined administration of vitamin E and vitamin D provided greater protection against radiation-induced bone marrow injury than either vitamin

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alone. These findings suggest that the combination of vitamins E and D may have potential as an adjunctive protective agent for reducing radiation-induced bone marrow toxicity. However, further studies are required to determine its optimal dosing, underlying molecular mechanisms, and safety in the context of radiotherapy.

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Authors' contributions
Mozhgan Jafari conducted the animal experiments, performed the statistical analyses, and drafted the original manuscript. Leila Mousavi and Leila Alvandi contributed to the histological evaluations and critically revised the manuscript. Abolfazl Ghafouri Khosroshahi supervised the study and contributed to its conception, design, and coordination, as well as to the preparation and final revision of the manuscript. Mahin Bashiri-Nahanjeh contributed to the writing and editing of the manuscript.

Conflict of Interest
The authors declare that they have no conflicts of interests.

Ethical Issues
All experimental procedures involving animals were conducted in accordance with the relevant institutional and national guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (Ethics approval code: [IR.UMSHA.REC.1402.169]).

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