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Original Article

Ginger Ethanolic Extract Mitigates Oxidative Stress and Apoptotic Signaling Following Serum Deprivation and Hydrogen Peroxide Exposure in Bone Marrow Mesenchymal Stem Cells

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ABSTRACT

Engraftment of bone marrow mesenchymal stem cells (BM-MSCs) exhibited promise for treating various disorders; however, oxidative stress-related lesions impair their survival and therapeutic potential during transplantation. Promoting their antioxidant capacity is therefore important. Here, we investigated whether ginger, as a medicinal plant, could attenuate oxidative stress-induced apoptosis in rat BM-MSCs in vitro and elucidated the underlying molecular pathways. rBM-MSCs were pretreated with serum-free media containing different concentrations of ginger ethanolic extract (3, 6, 12, 25 and 50 µg/mL), followed by hydrogen peroxide (H_2O_2) exposure (300 µM) for 3.5 h. Cell viability was assessed by a water-soluble tetrazolium salt assay. The antioxidant activity of the ethanolic extract of ginger (EEG) was also evaluated by total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) assays. The expression of proapoptotic and antiapoptotic proteins was examined by western blotting. All experiments were performed in triplicate using independent biological replicates. The results indicated that the ethanolic extract of dried ginger rhizome meaningfully elevated the viability of rBM-MSCs and protected them against H_2O_2-induced oxidative stress. Treatment with EEG (50 µg/mL) resulted in statistically significant reductions in lipid peroxidation, the Bax/Bcl-2 ratio, and cleaved caspase-3 levels (all $P < 0.001$), along with a statistically significant increase in TAC ($P < 0.01$). Our data support that EEG pretreatment may also protect rBM-MSCs from oxidative stress and boost their therapeutic efficacy by downregulating the proapoptotic/antiapoptotic protein ratio following transplantation.

Keywords: Antioxidant, Apoptosis, Ginger extract, H_2O_2, Mesenchymal stem cells

INTRODUCTION

Stem cell-based therapy is a recently developed strategy that has attracted the attention of numerous researchers and has the potential to address a variety of age-related and degenerative disorders [1]. Among the different types of stem/stromal cells, mesenchymal stem cells (MSCs) have the potential to be an effective treatment for a wide range of conditions [2]. This is due to their high self-renewal power, multilineage differentiation capacity, ease of isolation from different organs, great efficiency, and immunomodulatory potency [3, 4].

Although MSC-based therapy exhibits great therapeutic potential, several challenges, such as low survival rate and apoptosis and necrosis, have been reported [5-7]. Therefore, BM-MSC protection from apoptosis is essential for cell therapy to be successful. Oxidative stress is defined as a state in which the body's antioxidant network cannot adequately neutralize excessive reactive oxygen species (ROS), which are generated in injured tissues or organs as a result of the pathophysiology of numerous disorders [8-10]. In the damaged area, engrafted MSCs are negatively affected by this acute condition, leading to enhanced anoikis [11, 12].

Highly reactive oxygen-based intermediates encompass nitric oxide, superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2), which can oxidatively damage the structure and function of lipids, proteins, and DNA [6, 13]. Antioxidant supplements and medicinal plants with antioxidant properties have been demonstrated to minimize cellular dysfunction under oxidative stress [8, 14-17].

As a member of the Zingiberaceae family, ginger (*Zingiber officinale* Roscoe) has long served both nutritional and therapeutic intentions. It possesses a diverse range of well-documented bioactive constituents, notably phenolics and terpenoids. Among these, the phenolics, especially paradols, gingerols, and shogaols are chiefly responsible for its different bioactivities [18]. Recent research has revealed that ginger possesses biological properties, including anti-inflammatory [19], antibacterial [20], antioxidant [21, 22], and anticancer activities [23]. Numerous studies have demonstrated the effectiveness of ginger in preventing oxidative stress. In cell models, the fundamental mechanisms of antioxidant action have been studied [24, 25].

Therefore, in the ongoing research, we aimed to investigate the protective effects of dried Chinese ginger rhizome extract on oxidative stress and mitochondrial apoptosis caused by hydrogen peroxide and serum deprivation in BM-MSCs.

MATERIALS AND METHODS

Plant Collection and Ethanolic Extract Preparation

Dried Chinese ginger rhizomes (*Zingiber officinale* Roscoe) were obtained from a local market in Kerman, Iran, and were verified at the Herbal and Traditional Medicines Research Center (HTMRC), Kerman University of Medical Sciences. A voucher specimen (No. H107) was archived at the HTMRC Herbarium for future reference. The dried rhizomes were powdered (40-mesh) and thoroughly homogenized to minimize sampling bias and ensure representative sampling. Three independent portions of the homogenized powder were randomly collected and subjected to separate ethanolic extractions using the maceration method with 96% ethanol under identical experimental conditions. Each extraction represented one independent biological replicate and was processed separately throughout all subsequent experiments. Each extract was concentrated using a rotary evaporator, dried in an oven at 40°C, and stored at −20°C until use.

Preparation of Different Concentrations of the Ethanolic Extract of Ginger (EEG)

The dried extract was weighed and dissolved in dimethyl sulfoxide (DMSO) (<0.05% of the final solution). Different concentrations (3, 6, 12, 25, and 50 μg/mL) of EEG were prepared from the fresh stock solution.

Isolation and In Vitro Expansion of Primary Rat Bone Marrow Mesenchymal Stem Cells (rBM-MSCs)

Six adult male Wistar rats, weighing 200–250 g and aged 6–8 weeks, were sourced from the institutional animal house maintained at the Semnan University of Medical Sciences, Semnan, Iran. Animals were housed under standard laboratory conditions (22 ± 2°C, 12 h light/dark cycle, 55 ± 10% humidity) with free access to food and water and allowed to acclimatize for one week before the experiments. For bone marrow isolation, rats were first anesthetized with an intraperitoneal injection of ketamine (100 mg/kg)/xylazine (10 mg/kg). Then, hindlimbs were completely shaved and disinfected with 70% ethanol. After this step, rats received a lethal injection of ketamine (300 mg/kg)/xylazine (30 mg/kg), promptly followed by cervical dislocation. Then, femurs were cut off, cleaned, and then opened at both ends. Afterward, using a 23-gauge syringe, bone marrow was flushed with high-glucose DMEM containing 1% penicillin/streptomycin. The cell culture constituents were sourced from Gibco (Invitrogen, Carlsbad, CA, USA). Following centrifugation, cells were plated in high-glucose DMEM supplemented with 15% fetal bovine serum and 1% penicillin/streptomycin. Cultures were maintained at 37°C in a humidified incubator with 5% CO_2. The medium was refreshed 24 h post-initial seeding. The adherent cells at passage 3 were considered MSCs. Bone marrow cells from two rats were pooled to generate one independent primary rBM-MSC culture, resulting in three independent biological replicates used throughout the study. The study followed a completely randomized experimental design. Three independent ethanolic ginger extracts (biological replicates) and three independent primary rBM-MSC cultures (biological replicates) were generated separately. Each independent cell culture was exposed to every experimental treatment using each independent ginger extract under identical experimental conditions. Within each biological replicate, each treatment condition was evaluated in triplicate wells (technical replicates), and all experiments were independently repeated three times [26].

rBM-MSCs Characterization

Flow cytometric characterization was performed using a completely randomized experimental design to confirm the mesenchymal phenotype of the isolated rBM-MSCs. Cells at passage 3 were collected from three independent primary rBM-MSC cultures (biological replicates), each established independently under identical isolation and culture conditions. The harvested cells from culture flasks were washed with PBS, and centrifuged at 1500 rpm for 5 min. The cell pellet was resuspended in PBS to obtain a final volume of 1 mL. Subsequently, 100 μL of the cell suspension was transferred into individual flow cytometry tubes and incubated with 2 μL of fluorochrome-conjugated monoclonal antibodies against CD44 (FITC, 203906, BioLegend), CD45 (FITC, 202205, BioLegend), CD34 (PE, Sc-7324, Santa Cruz Biotechnology), and CD90 (PerCP/Cy5.5, 202515, BioLegend). Samples were incubated for 30 min at 4°C in the dark. Following incubation, cells were washed with 500 μL of PBS and centrifuged at 1500 rpm for 5 min. The final cell pellet was resuspended in 250 μL of PBS and subjected to flow cytometric analysis using a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The obtained data were analyzed using the FlowJo software [27].

Water-Soluble Tetrazolium Salt (WST-1) Assay

This step was designed to determine the protective concentration of EEG on rBM-MSCs against H_2O_2-induced oxidative stress. For this purpose, rBM-MSCs were cultured in 96-well plates at a seeding density of 1×10^4 cells per well. After 24 h, the seeded rBM-MSCs were

randomly divided into seven groups (three wells were considered for each group): control group (which received only serum-free media), H_2O_2 group (which received serum-free media for 24 h and then cells were exposed to 300 µM H_2O_2 for 3.5 h), and treatment groups (which received serum-free media containing different concentrations of EEG (3, 6, 12, 25, and 50 µg/mL) for 24 h and then were washed with phosphate-buffered saline (PBS) twice, and exposed to 300 µM H_2O_2 for 3.5 h. After treatment, cell viability was assessed using the WST-1 assay [28, 29]. Briefly, 10 µL of WST-1 reagent was dispensed into each well followed by a 2-hour incubation at 37°C. After gentle shaking, optical density was determined at a wavelength of 450 nm using a microplate spectrophotometer. All experiments also included three independent biological replicates.

Thiobarbituric Acid Reactive Substances (TBARS) Assay

MDA levels were used as an index of lipid peroxidation, as previously described [30, 31]. To evaluate MDA levels, rBM-MSCs (1.2×10^5 cells/well) were seeded in 12-well plates. After 24 h, the rBM-MSCs were randomly divided into four groups: control group (which received only serum-free medium), H_2O_2 group (which received serum-free media for 24 h and then were exposed to 300 µM H_2O_2 for 3.5 h), and two treatment groups were exposed to serum-free medium supplemented with EEG at concentrations of 25 and 50 µg/mL (which were selected based on their superior protective effects observed in the WST assay) for 24 h and then were washed with phosphate-buffered saline (PBS) twice, and exposed to 300 µM H_2O_2 for 3.5 h. After the treatment period, cells were detached with trypsin/EDTA and washed twice with ice-cold PBS, followed by sonication on ice for lysis. After cell lysis, the protein concentration of each sample was estimated using the bicinchoninic acid (BCA) protein assay. Then, 100 µL of cell lysate was mixed with 200 µL of a working solution containing thiobarbituric acid and trichloroacetic acid in HCl. Following incubation at 95°C for 60 min, the samples were allowed to cool at room temperature. After a 5-min centrifugation at 10000×g, the optical density was determined at 532 nm. MDA concentrations were determined using a standard curve generated with 1,1,3,3-tetramethoxypropane (TMP). MDA levels were also normalized to the total protein content of each sample. The normalized values were then expressed as fold change relative to the control group [32]. Each experimental condition was assessed in triplicate wells, and the entire experiment was independently repeated three times.

Total Antioxidant Capacity (TAC) Determination

Ferric reducing antioxidant power (FRAP), an indicator of total antioxidant capacity (TAC), was used to determine the ability of BM-MSCs to convert the ferric 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ-Fe^{+3}) complex to its ferrous (TPTZ-Fe^{+2}) form [33]. The experimental design was identical to that described for MDA measurement. After cell lysis, the 10 µL of cell lysate (10^6 cells per sample) was added to 140 µL of a fresh working mixture (acetate buffer (pH 4.6), TPTZ mixture (10 mM in 40 mM hydrochloric acid), and ferric chloride solution ($FeCl_3.6H_2O$) at a ratio of 10:1:1). Following incubation at 37 °C for 10 minutes, the optical density of the mixture was determined at a wavelength of 593 nm using a microplate spectrophotometer (a BioTek ELX800). TAC was determined using a standard curve generated with $FeSO_4 \cdot 7H_2O$. TAC levels were normalized to the total protein content determined by the BCA assay. The normalized values were then expressed as a fold change relative to the control group. Each experimental condition was assessed in triplicate wells, and the entire experiment was independently repeated three times.

Western Blotting

The western blot procedure was applied as earlier reported. The experimental design was identical to that described for MDA measurement. Precisely, treated and untreated rBM-MSCs were first detached using trypsin, followed by two washes with cold PBS. They were then lysed using RIPA lysis buffer (Sigma-Aldrich, USA) containing a protease inhibitor cocktails. Whole-cell lysates were centrifuged at 12000×g for 10 min at 4°C. The resulting supernatant was gathered, and the protein content was estimated using the BCA protein assay kit. Following loading (50 µg) and separating equal volumes of protein using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), the separated proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes at 120 V for 60 min. To prevent non-specific interactions, the membranes were treated with 2% nonfat dry milk for one hour at room temperature. Afterward, the membranes were incubated overnight at 4°C with appropriately diluted primary antibodies. The primary antibodies (Santa Cruz Biotechnology Inc.; Dallas, TX, USA) included mouse monoclonal anti-Bax (sc-7480, 1:1000), mouse monoclonal anti-Bcl2 (sc-7382, 1:1000), mouse monoclonal anti-caspase 3 (sc-56053, 1:1000), and mouse monoclonal anti-β-actin (sc-47778, 1:1000). After washing the membranes three times with Tris-buffered saline containing 0.1% Tween 20 (TBST) for five min, the membranes were applied to mouse IgGκ BP-HRP (sc-516102, 1:1000) for one hour. Protein patterns were visualized using an enhanced chemiluminescence (ECL) detection reagent (Amersham Biosciences; Buckinghamshire, UK). Finally, the images were quantified with ImageJ software [34].

Statistical Analysis

Data represent the mean ± standard deviation (SD) from at least three independent biological replicates (n = 3). Statistical analyses were performed using GraphPad Prism version 10.5.0. Datasets were assessed for potential outliers using the ROUT test (Q = 1%) and for homogeneity of variances using the Brown–Forsythe test prior to statistical analysis. Multiple-group comparisons were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

RESULTS

rBM-MSC Characterization

Flow cytometric analysis of rBM-MSCs revealed positive staining for mesenchymal stem cell markers such as CD90 and CD44 (Fig. 1a-b) but negative staining for hematopoietic markers such as CD34 and CD45 (Fig. 1c-d).

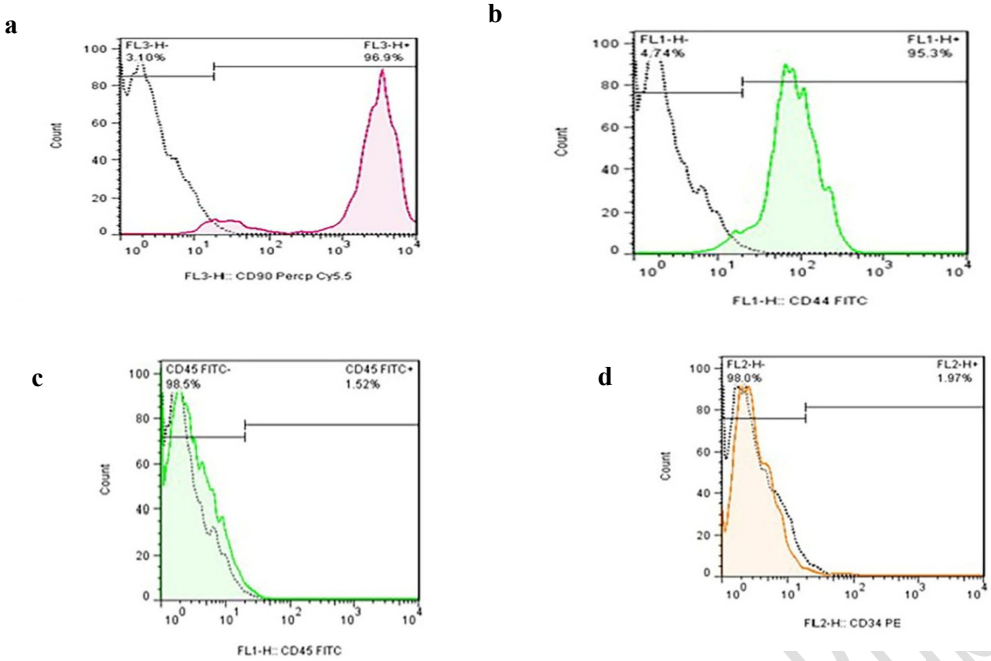

Fig. 1 The phenotypic characterizations of rBM-MSCs. The positive surface markers (a-b) of rBM-MSCs were CD90 and CD44. The negative surface markers (c-d) of rBM-MSCs were CD45 and CD34. All experiments included at least three independent biological replicates.

Protective Effects of EEG on rBM-MSCs Against H_2O_2

Cell viability data met the assumptions of homogeneity of variance (Brown–Forsythe, $P = 0.8619$). As shown in Fig. 2, H_2O_2 (300 µM) significantly reduced rBM-MSC viability from 100 ± 8.50% in the control group to 68.00 ± 9.84% ($P < 0.001$). Pretreatment with EEG increased cell viability in a concentration-dependent manner, with the greatest protection observed at 50 µg/mL (91.67 ± 3.78%), which was not significantly different from the control group. Although the 25 (81.00 ± 2.64%) and 12 (79.33 ± 4.50%) µg/mL concentrations partially improved cell viability, they remained significantly lower than the control group ($P < 0.05$ and $P < 0.01$, respectively) and did not differ significantly from the H_2O_2 group. Lower concentrations (6 and 3 µg/mL) also failed to improve cell viability and did not differ from the H_2O_2-treated group (68.67 ± 8.32% and 67.00 ± 6.08%, respectively). Furthermore, the 50 µg/mL concentration showed significantly higher viability than the 6 and 3 µg/mL groups ($P < 0.05$ and $P < 0.01$, respectively). These findings indicate that EEG attenuated H_2O_2-induced cytotoxicity in rBM-MSCs, with a statistically significant cytoprotective effect observed only at 50 µg/mL.

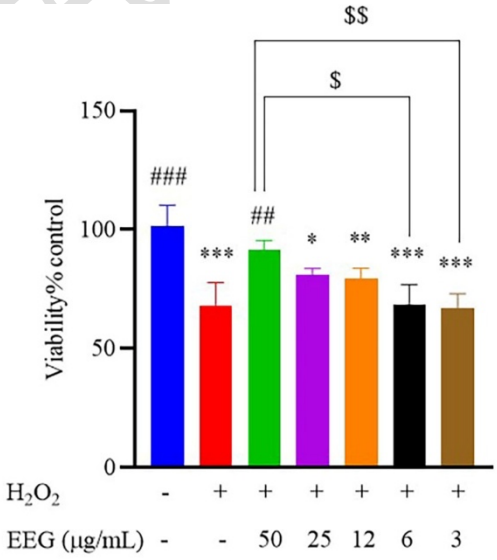

Fig. 2 The effect of the ethanolic extract of ginger (EEG) on rBM-MSC viability. Cells were pretreated with various concentrations (3-50 µg/mL) of EEG for 24 h, followed by exposure to H_2O_2 (300 µM) for 3.5 h. Values are presented as mean ± SD. Significance: ##$P < 0.01$ and ###$P < 0.001$ versus the H_2O_2-treated group; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus the control group. $$P < 0.05$ and $$$P < 0.01$ versus the EEG (50 µg/mL) group. All experiments included at least three independent biological replicates.

EEG Preconditioning Modulated TBARS Levels in rBM-MSCs Exposed to H_2O_2

TBARS analysis showed a significant overall difference among the experimental groups (one-way ANOVA, $P = 0.0002$). As shown in Fig. 3, MDA levels significantly increased following H_2O_2 exposure compared with the control group (1.70 ± 0.13 vs. 1.00 ± 0.12, $P < 0.001$). Pretreatment with EEG attenuated H_2O_2-induced lipid peroxidation, reducing MDA levels to 1.30 ± 0.08 and 1.13 ± 0.04 at concentrations of 25 and 50 μg/mL, respectively. Tukey's post hoc test revealed significant reductions in MDA levels in both EEG-treated groups compared with the H_2O_2 group ($P < 0.01$ and $P < 0.001$, respectively). While the EEG 25 μg/mL group remained significantly different from the control group ($P < 0.05$), no significant difference was observed between the EEG 50 μg/mL and the control group. No significant difference was also detected between the two EEG-treated groups, indicating that EEG pretreatment effectively protected rBM-MSCs against H_2O_2-induced oxidative damage.

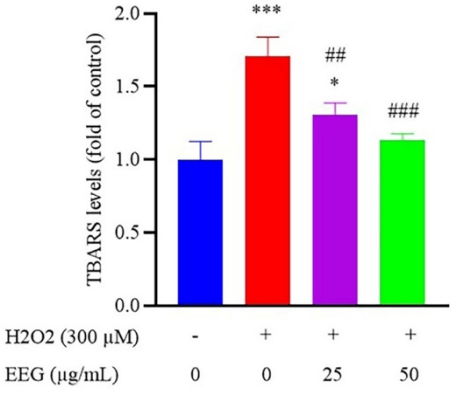

Fig. 3 Changes in TBARS content in rBM-MSCs treated with EEG 24 h before exposure to H_2O_2 (3.5 h, 300 μM). Values are presented as mean ± SD. Significance: ##$P < 0.01$ and ###$P < 0.001$ versus the H_2O_2-treated group; *$P < 0.05$ and ***$P < 0.001$ versus the control group. All experiments included at least three independent biological replicates.

EEG Preconditioning Enhanced TAC Levels in rBM-MSCs Exposed to H_2O_2

TAC analysis revealed a significant overall difference among the experimental groups (one-way ANOVA, $P = 0.0026$). According to Fig. 4, H_2O_2 exposure significantly reduced TAC levels compared with the control group (0.54 ± 0.08 vs. 1.00 ± 0.10, $P < 0.01$). Pretreatment with EEG increased TAC levels to 0.85 ± 0.13 and 0.93 ± 0.07 at concentrations of 25 and 50 μg/mL, respectively. Tukey's post hoc analysis demonstrated that both EEG-treated groups exhibited significantly higher TAC levels than the H_2O_2 group ($P < 0.05$ and $P < 0.01$, respectively). No significant differences were observed between the EEG-treated groups and the control group, or between the two EEG concentrations, indicating that EEG pretreatment effectively restored the antioxidant capacity of rBM-MSCs following H_2O_2-induced oxidative stress.

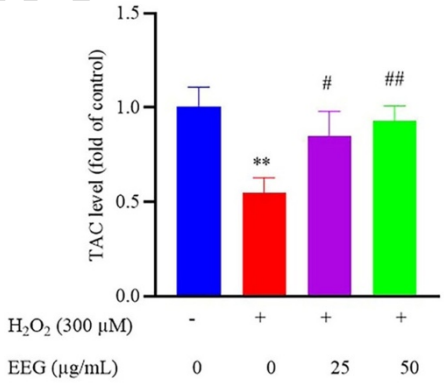

Fig. 4 Changes in TAC levels in rBM-MSCs treated with EEG 24 h before exposure to H_2O_2 (3.5 h, 300 μM). Values are presented as mean ± SD. Significance: #$P < 0.05$ and ##$P < 0.01$ versus the H_2O_2-treated group; **$P < 0.01$ versus the control group. All experiments included at least three independent biological replicates.

EEG Decreased H_2O_2-Induced Apoptosis of rBM-MSCs

One-way ANOVA demonstrated a significant difference among the experimental groups in the Bax/Bcl-2 ratio ($P < 0.0001$). As illustrated in Fig. 5a, H_2O_2 exposure markedly increased the Bax/Bcl-2 ratio compared with the control group (5.15 ± 0.29 vs. 1.00 ± 0.11, $P < 0.001$). Pretreatment with EEG attenuated this increase in a concentration-dependent manner, reducing the ratio to 3.24 ± 0.21 and 1.91 ± 0.27 at 25 and 50 μg/mL, respectively. Although both EEG-treated groups remained significantly different from the control group ($P < 0.001$, $P < 0.01$), they showed significantly lower Bax/Bcl-2 ratios than the H_2O_2 group ($P < 0.001$). Moreover, the 50 μg/mL concentration exerted a

significantly greater protective effect than 25 µg/mL (1.91 ± 0.27 vs. 3.24 ± 0.21, $P < 0.001$). In addition, one-way ANOVA revealed a significant difference among the experimental groups regarding caspase-3 protein expression ($P < 0.0001$). As shown in Fig. 5b, caspase-3 levels were markedly elevated in the H_2O_2-treated group compared with the control group (4.83 ± 0.36 vs. 1.00 ± 0.09, $P < 0.001$). Pretreatment with EEG significantly attenuated H_2O_2-induced caspase-3 overexpression, reducing its level to 3.77 ± 0.31 ($P < 0.01$) and 2.32 ± 0.21 ($P < 0.001$) at concentrations of 25 and 50 µg/mL, respectively. Tukey's post hoc analysis demonstrated significant differences between both EEG-treated groups and the H_2O_2 group ($P < 0.001$), indicating a concentration-dependent protective effect of EEG against H_2O_2-induced apoptosis.

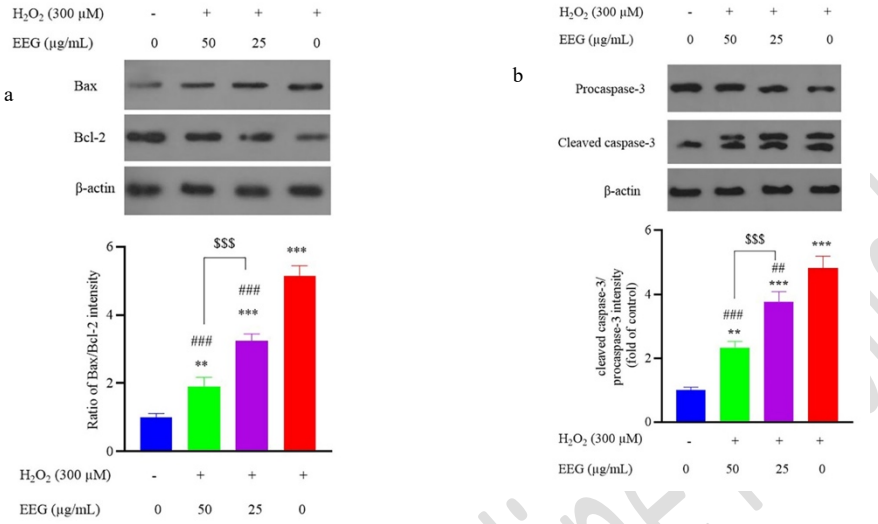

Fig. 5 The EEG attenuated H_2O_2-stimulated rBM-MSC apoptosis. Cells were pretreated with EEG (25 and 50 µg/mL) for 24 h, followed by H_2O_2 (300 µM) for 3.5 h. Values are presented as mean ± SD. Significance: ##$P < 0.01$ and ###$P < 0.001$ versus the H_2O_2-treated group; **$P < 0.01$, and ***$P < 0.001$ versus the control group. $$$$P < 0.001$ versus the EEG (50 µg/mL) group. All experiments included at least three independent biological replicates.

DISCUSSION

After implantation, most MSCs are destroyed [35, 36]. This may be related to excessive ROS and inflammation in the pathophysiological environment. Moreover, ROS can damage mitochondria and causes the release of cytochrome c and proapoptotic molecules, thereby inducing cell death. Therefore, developing an approach to preserve transplanted BM-MSCs is crucial to advancing stem cell-based therapy [37]. In the present research work, rBM-MSCs were treated with EEG for 24 h and then exposed to H_2O_2 (300 µM) for 3.5 h. The EEG was also observed to reduce the production of MDA, thus protecting cells against H_2O_2-stimulated cellular injury. Furthermore, EEG was found to elevate TAC and decrease rBM-MSC apoptosis through downregulation of the proapoptotic/antiapoptotic protein ratio. These protective effects may be linked to the antioxidant properties of ginger. Ginger's ability to diminish ROS may reduce lipid peroxidation as shown by the decreased levels of MDA. Ginger may also enhance cellular antioxidant defenses, as shown by increased TAC. Lowering oxidative stress may reduce the apoptosis and help the modulation of the proteins that are associated with apoptosis. Based on this rationale, we hypothesized that substances possessing antioxidant activity would be able to diminish oxidative stress at the ischemic site of injury and thereby promote the efficacy of stem cell therapy.

In the current research work, H_2O_2 (300 µM) decreased the viability of rBM-MSCs, which was consistent with previous studies [38, 39]. Furthermore, pretreating rBM-MSCs with ginger increased the viability of rBM-MSCs against H_2O_2-induced cytotoxicity. This result is consistent with previous studies showing that ginger extract was able to protect different cell types from H_2O_2-induced damage [22, 40].

H_2O_2 is one of the main indicators of intracellular ROS formation that can be converted into other free radicals, causing serious damage and eventually apoptosis [41]. Reactive oxygen species are potentially capable of prompting membrane lipid peroxidation. Membrane impairment can be triggered by lipid peroxidation and the generation of MDA [42, 43]. Consistent with the results of previous studies [39, 44], an increase in levels of oxidative stress products (MDA) was found in H_2O_2-induced rBM-MSCs. Our results showed that treatment with EEG (25 and 50 µg/mL) before exposure to H_2O_2 diminished MDA production and increased the level of TAC in rBM-MSCs. Our previous report indicated that ginger extract preconditioning meaningfully elevated the transcription of genes encoding catalase, superoxide dismutase, and glutathione peroxidase and reduced the lipid peroxidation caused by oxidative stress in cultured chondrocytes [22]. Ginger's strong antioxidant properties are attributed to its high total phenolic and flavonoid content [45], which have the ability to donate electrons to H_2O_2, neutralizing it to water [46].

Exposure to H_2O_2 exposes cells to elevated levels of ROS, which disrupt the equilibrium between oxidants and antioxidants, ultimately leading to necrosis and apoptosis [47]. In the early stages of apoptosis, the released cytochrome c activates caspase-9, which in turn triggers caspase-3 and initiates the cell death pathway. There is a direct relationship between a rise in the Bax/Bcl-2 ratio and upregulation of caspase-3 [48, 49]. In the current research work, H_2O_2 enhanced the levels of cleaved caspase-3 and the Bax/Bcl-2 ratio in rBM-MSCs. However, pretreatment with EEG meaningfully diminished cleaved/activated caspase-3 levels and the Bax/Bcl-2 ratio in rBM-MSCs, indicating that ginger

successfully mitigated oxidative injury by repressing H_2O_2-stimulated apoptosis. There are limited data showing that ginger attenuates oxidative stress and inhibits apoptotic signaling pathways in vitro or in vivo. Previous research conducted by Hao *et al*. showed that *Zingiber officinale* mitigated 6-hydroxydopamine-stimulated oxidative stress and apoptosis in PC12 cells by decreasing the Bax/Bcl-2 proportion and cleaved/activated caspase 3 levels [50]. Moreover, Yaghubi Beklar and colleagues demonstrated that administration of ginger extract at a dose of 100 mg/kg alleviated diazinon-stimulated hepatotoxicity by repressing oxidative stress and preventing caspase-3-dependent apoptotic signaling in rats [51].

CONCLUSION

The current research indicated that EEG exerted antiapoptotic and antioxidant effects in rBM-MSCs when exposed to H_2O_2. The viability of rBM-MSCs was enhanced after treatment with EEG, which was attributed to the antioxidant properties of ginger. Ginger ethanolic extract also led to a decrease in MDA levels, the Bax/Bcl-2 ratio, and cleaved/activated caspase 3 levels. Therefore, preconditioning with ginger may help improve the damaged microenvironment and enhance the therapeutic efficacy of MSCs for transplantation in the injured area. In light of these findings, future studies should investigate the in vivo protective actions of ginger-preconditioned BM-MSCs and clarify the mechanisms underlying their antioxidant and antiapoptotic activities.

Ethics Approval and Consent to Participate

All procedures related to rats were performed in agreement with the NIH Guide for the Care and Use of Laboratory Animals (Publication No.80−23, revised 1996) and were formally sanctioned by the Ethics Committee of Semnan University of Medical Sciences (IR.SEMUMS.REC.1400.218).

Consent for publications

Not Applicable

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interest

The authors have not declared any conflict of interests.

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Authors' contributions

Kobra Bahrampour Juybari conceptualized the project, supervised the work and contributed to writing, and editing of the manuscript's original draft. Arian Amirkhosravi administered the project and cooperated in the experiments. Mitra Mehrabani handled visualization and contributed to the investigation and methodology. Mehrnaz Mehrabani edited the manuscript and conducted the formal analysis. Fateme Naghibzade cooperated in the experiments. Mohammad Erfan Norouzmahani contributed to methodology. Sara Yaghoubi contributed to methodology. Maryamossadat Mirtajaddini Goki curated the data and contributed to the investigation. Mahla Amirzadeh contributed to the investigation. Sharareh Bancroft provided critical review and editing of the manuscript.

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