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# The changes of digestive system inflammatory, oxidative stress, and histopathology factors following oral mesenchymal stem cells administration in rats with traumatic brain injury

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## Abstract

**Background and aims** Mucous mesenchymal stem cells can migrate to damaged areas, and their use is proposed as a new approach to treating diseases. The present study aimed to investigate the effect of oral mesenchymal stem cells (OMSCs) on inflammatory, oxidative stress, and histopathological indices in the tissues of the stomach, intestine, and colon after traumatic brain injury (TBI).

**Methods and materials** Adult male rats were randomly divided into four groups: Sham, TBI, Vehicle (Veh), and Stem cell (SC). Intravenous injection of OMSCs was performed at 1 and 24 h after injury. The inflammatory, oxidative stress, and histopathological indices of the tissues of the stomach, small intestine, and colon were evaluated 48 h after injury.

**Results** After TBI, IL-1β and IL-6 levels increased and IL-10 levels decreased in the tissues of the stomach, small intestine, and colon, but the administration of OMSCS prevented these changes to a large extent. Oxidative stress indices (MDA, PC, TAC, SOD, and CAT) showed an increase in oxidative stress after TBI, but oxidative stress was less severe in the OMSC group. The administration of OMSCs after TBI improved the histopathological outcome in the tissues of the stomach, small intestine, and colon.

**Conclusion** Administration of OMSCs in rats suffering from TBI can improve inflammatory, oxidative stress, and histopathological indices in the tissues of the stomach, small intestine, and colon, which shows the beneficial effect of using OMSCs in TBI.

**Keywords** Traumatic brain injury, Mesenchymal stem cell of the oral mucosa, Inflammation, Oxidative stress, Digestive system

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## Introduction

Traumatic brain injury (TBI) is one of the leading causes of disability and the third most common cause of death in the world [1, 2]. The main causes of TBI include road accidents, falls from heights, injuries during intense sports, and physical altercations [3]. Brain damage in TBI is divided into two: primary and secondary. Primary damage is caused by mechanical impact, and it follows a cascade of pathological and biochemical changes that lead to secondary damage and neuronal death [4]. Secondary damage is caused by the increase in reactive oxygen species (ROS), damage to the blood-brain barrier (BBB), activation of inflammation, membrane lipid peroxidation, neuronal destruction, and apoptosis [5–7]. TBI can lead to clinically significant adverse effects in other body organs, including gastrointestinal dysfunction [8, 9]. TBI can cause tissue damage and the accumulation of ROS species in the intestine [10]. The association between TBI and intestinal mucosal damage has also been studied. Research has shown that TBI leads to an increase in inflammation-related factors, including pro-inflammatory cytokines and ICAM-1, which can lead to structural changes in the intestine and dysfunction in its barrier, increased intestinal permeability, and translocation of intestinal bacteria and endotoxins [11–14]. This process may cause systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS).

Mesenchymal stem cells (MSC) are responsible for maintaining tissue homeostasis, and their use is one of the therapeutic approaches of interest in TBI research [15, 16]. The high potential for differentiation and regeneration, the ability to suppress inflammatory responses after brain damage, and the immune system regulation by MSCs are among the reasons for their use in TBI treatment research [17, 18]. Among the types of MSCs, oral mesenchymal stem cells (OMSCs) have been proposed for treating TBI due to their neuroprotective effects in vitro and in vivo [19, 20]. The results of research conducted on TBI model rats show a significant decrease in the level of inflammatory cytokines and an increase in neurological function in the group treated with mesenchymal stem cells [21]. Soltani et al. also showed that injecting OMSCs in animals with TBI reduces cerebral edema and intracranial pressure and improves neurological, motor, memory, and anxiety outcomes [22]. In addition, Kanazawa et al.'s study in 2011 showed that bone marrow-derived mesenchymal stem cells improve liver damage and regeneration [23]. Several studies have shown that MSCs can reverse the fibrotic process by inhibiting collagen deposition and TGF-β [24, 25].

Considering the systemic impact of TBI on other organs and the lack of effective and definitive treatments, continued therapeutic research in this field is essential. One promising approach involves using therapies with multifaceted targets, such as stem cells, to address the heterogeneous nature of TBI and prevent secondary damage [21]. OMSCs possess unique characteristics, including high differentiation potential, ease of access, rapid proliferation, and the ability to migrate to injured areas [26]. Given the pathophysiology of TBI, characterized by an acute inflammatory phase followed by a subacute phase, it is crucial to explore therapeutic interventions targeting both phases to effectively mitigate secondary injury [27–30]. In this study, we aimed to evaluate the effects of OMSCs—leveraging their well-documented neuroprotective properties, ease of administration, and demonstrated ability to migrate to injury sites [27, 30–33]—on inflammatory, oxidative stress, and histopathological outcomes in digestive system tissues (stomach, intestine, and colon) during the acute phase of TBI in male rats, recognizing the gastrointestinal damage often induced by TBI.

## Methods and materials

### Animals

Twenty-eight male Wistar rats weighing between 200 and 250 g were used in this study. This interventional-experimental study was approved by the Ethics Committee of Kerman University of Medical Sciences with the ethical code IR.KMU.AH.REC.1399.068.

### Study groups

28 male Wistar rats were randomly divided into four groups: Sham: male rats that were subjected to all the necessary stages of causing a diffuse traumatic brain injury except the weight falling on the head. Traumatic brain injury (TBI): male rats that were anesthetized and had diffuse TBI. Vehicle (TBI + Veh): male rats that received the same volume of saline phosphate buffer solution as the stem cell recipient group (100 µl) intravenously, but without the stem cells, 1 and 24 h after TBI [34]. Stem cell (TBI + SC): male rats that received an effective number of $2 \times 10^6$ rat oral mucosal stem cells in 100 µl of phosphate saline buffer intravenously 1 and 24 h after TBI, to target the acute inflammatory and subacute phases of TBI pathophysiology [22, 27–30]. The inflammatory, oxidative stress, and histopathological indices of the stomach, small intestine, and colon tissues were assessed 48 h after injury to capture the peak of pathological changes and evaluate the early therapeutic effects of OMSCs [29, 30, 35].

### Brain injury preparation and induction method

Anesthesia was induced in all animals by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) to ensure animal welfare during the procedure [30]. In all groups except for the Sham group, TBI was induced using

the Marmarou method, a well-established closed-skull model for replicating diffuse and moderate brain injuries. This method involves dropping a 300-gram weight from a height of 2 m, which reliably causes moderate TBI, as validated in previous studies including our own [5, 22, 30, 36, 37]. Following TBI induction, the animals were monitored to confirm respiration and then returned to their respective cages.

#### Stem cell preparation method

The oral mucosa tissue of anesthetized mice was prepared and transported to the laboratory. After washing and disinfection, the pieces of mucosa were digested with collagenase. The collagenase enzyme activity was neutralized with L-DMEM solution. The cells were filtered and centrifuged to separate the vascular stem cells. Then, they were filtered with a 70 mm strainer and cultured in a DMEM medium with PBS and antibiotics. The cultures were kept in an incubator with a temperature of 36.5 °C and 5% $CO^2$, and the culture medium was changed every three days. The cultures were examined daily with a microscope, and the medium was changed if necessary. After the cells reached the limit, they were passaged with trypsin and prepared for intravenous injection [22, 38].

#### Tissue preparation

Tissue sampling was performed after the animals were sacrificed under deep and painless anesthesia. Euthanasia was conducted under deep anesthesia induced by intraperitoneal administration of ketamine (200 mg/kg) and xylazine (20 mg/kg). The depth of anesthesia was confirmed by the absence of a response to painful stimuli. To ensure complete circulatory arrest, the abdominal aorta was transected. This method adhered to the American Veterinary Medical Association (AVMA) guidelines to minimize pain and distress [39].

The stomach and intestine samples were homogenized in phosphate buffer (0.05 M, pH 7), with a fixed tissue weight of 100 mg per sample homogenized in 1 mL of buffer. The homogenates were then centrifuged at 7000 rpm at 4 °C for 2 min, and the supernatant was collected for subsequent analysis.

#### Inflammatory and anti-inflammatory interleukin measurement method

The level of inflammatory and anti-inflammatory activity was measured by measuring IL-1β, IL-10, and IL-6 factors in the tissues of the stomach or intestine using the ELISA method and special kits (KPG-IL-1β, KPG-IL-10, and KPG-IL-6, respectively, Karmania Pars Gene Company, Iran) based on the reaction between antigen and antibody, and finally, the absorbance was read at 450 nm. After entering the absorbance and concentration of the standard solutions into the Excel program, the standard curve was prepared. The concentration of the samples was determined based on absorption and using the standard curve line Eqs. [40–42].

#### Malondialdehyde measurement method

Malondialdehyde (MDA), the result of the peroxidation of membrane lipids, is considered an oxidant and was measured using the thiobarbituric acid method. In this method, after preparing the homogenized tissue samples, absorption was recorded at a wavelength of 532 nm. Then, a standard curve was created, and lipid peroxidation levels were measured in terms of nanomoles per milligram of protein using a KPG-MDA kit made by Karmania Pars Gene Company, Iran [43, 44].

#### Protein carbonyl (PC) content measurement method

Protein carbonyl content is an index of protein oxidation and indicates oxidant activity, which was measured using the 2, 4 dinitrophenol hydrazine (DNPH) method. In this method, using a special kit (KPG-PC, Karmania Pars Gene, Iran), the difference between the absorbance of homogenized tissue samples was determined at a wavelength of 366 nm, and the results were expressed as nanomoles per milligram of protein with an extinction coefficient of 22/Mm/ Cm [43, 45].

#### Total antioxidant capacity (TAC) activity measurement method

Total antioxidant activity was measured using the ferric reduction of antioxidant power (FRAP) method. The supernatant of homogenized and centrifuged samples was used to measure the antioxidant activity. Using the appropriate kit (KPG-TAC, Karmania Pars Gene, Iran), the absorbance of the samples was measured at a wavelength of 593 nm, and the results were expressed as nanomoles per milligram of protein [43, 46].

#### Catalase enzyme activity measurement method

Potassium phosphate buffer and hydrogen peroxide were added to a specific volume of tissue extract, and the absorbance was read at 240 nm for 1 min (every 15 s). Then, the decrease in absorbance was calculated per minute, and the level of enzyme activity was calculated per unit for the amount of tissue protein. One unit of catalase enzyme is the amount of enzyme that decomposes one millimole of hydrogen peroxide per minute (KPG-CAT, Karmania Pars Gene, Iran) [47, 48].

#### Superoxide dismutase enzyme activity measurement method

EDTA, nitroblue tetrazolium (NBT), riboflavin, and methionine were added to the homogenized tissue, and after mixing, the mixture was maintained at 37 °C. The absorbance of the sample and control was read at

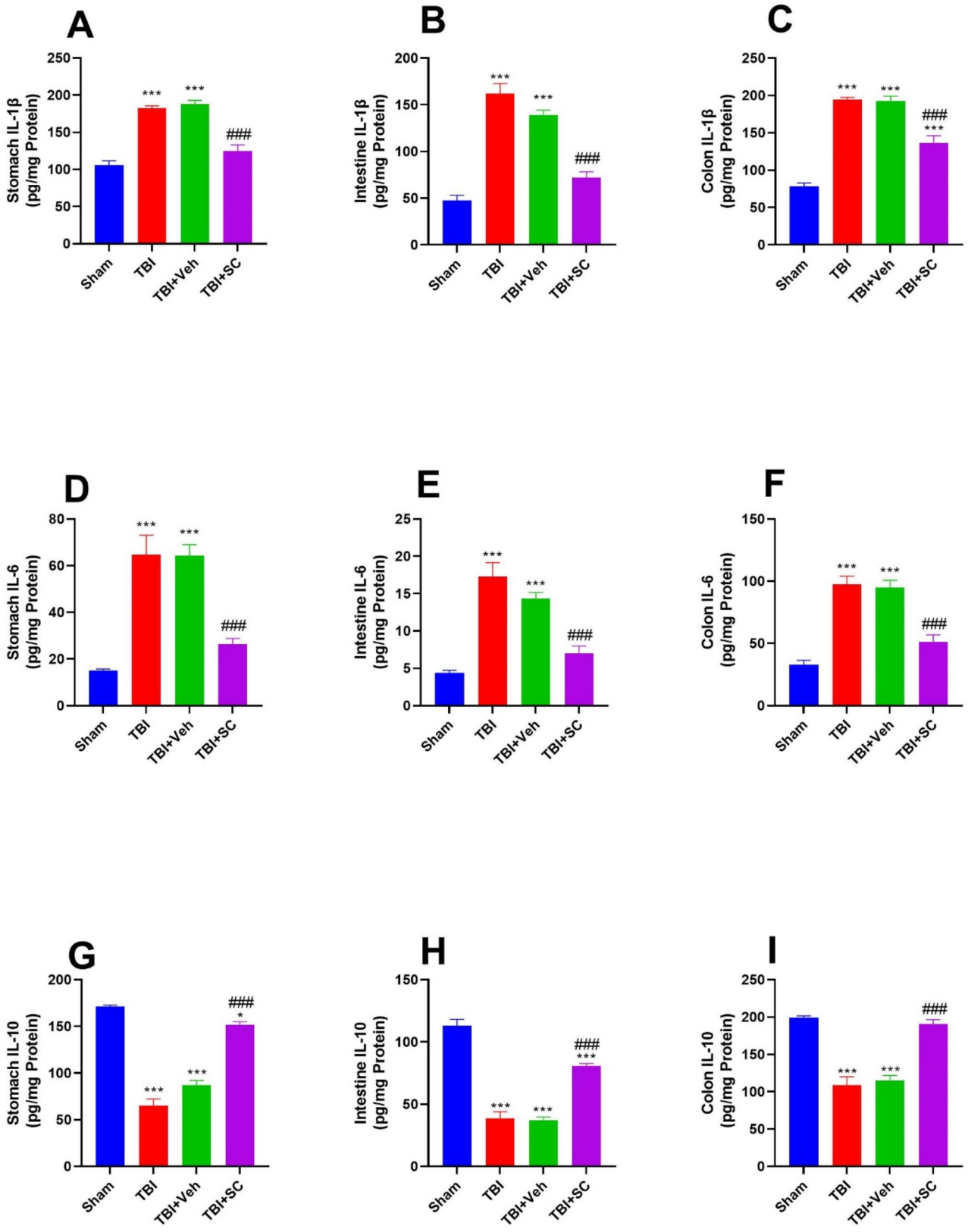


**Fig. 1** The level (mean ± SEM) of IL-1β, IL-6, and IL-10 in different study groups ($n = 7$ in each group). **A:** gastric IL-1β levels; **B:** IL-1β levels of the small intestine; **C:** colonic IL-1β levels; **D:** Gastric IL-6 levels; **E:** IL-6 levels of the small intestine; **F:** IL-6 levels of the colon; **G:** gastric IL-10 levels; **H:** IL-10 levels of the small intestine; **I:** colonic IL-10 levels. *** $P < 0.001$ compared to the Sham group. * $P < 0.05$ compared to the Sham group. ### $P < 0.001$ compared to the TBI and TBI + Veh groups. Veh: Vehicle; SC: Stem cell; TBI: Traumatic brain injury

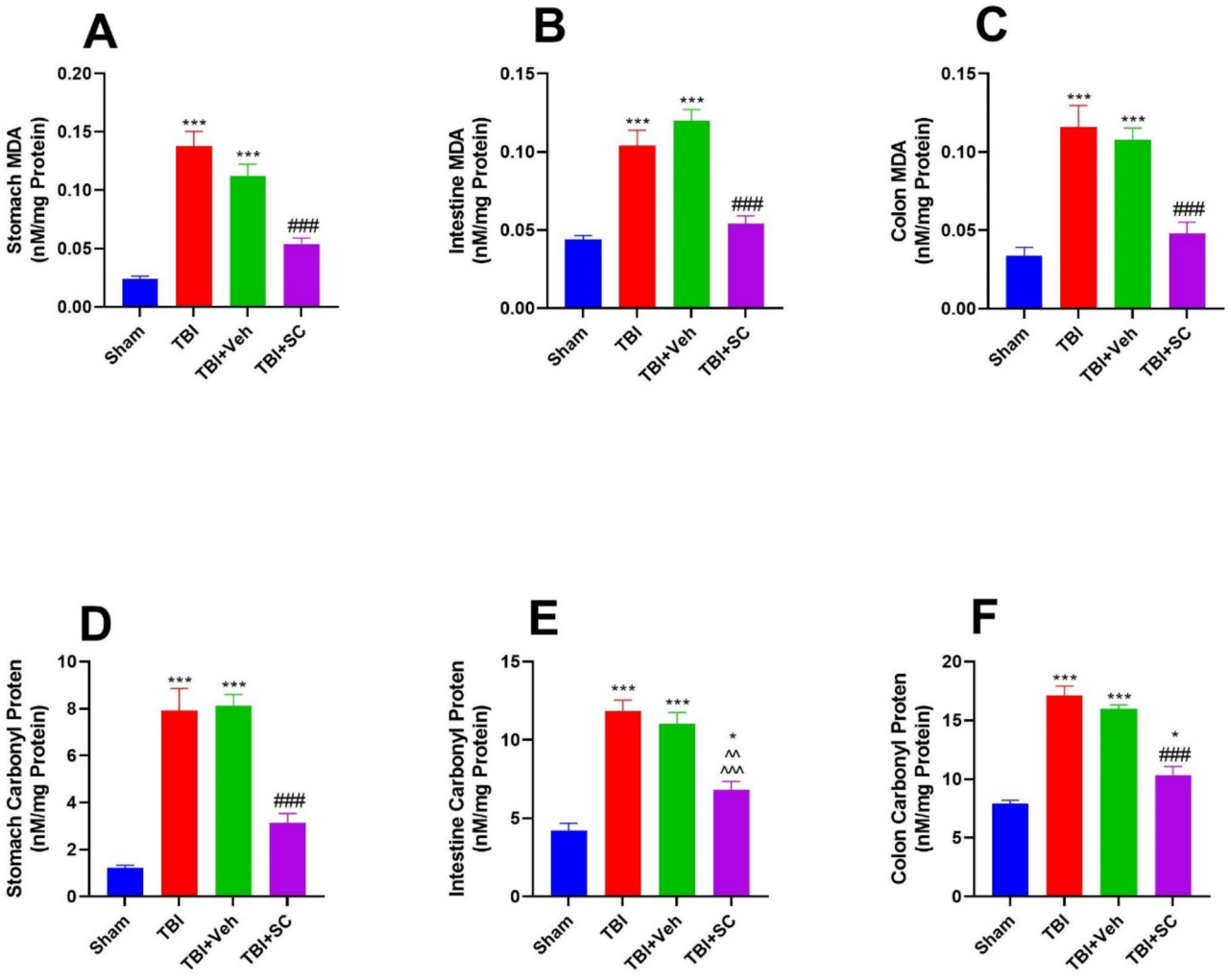


**Fig. 2** MDA and PC levels (Mean ± SEM) in different study groups ($n = 7$ in each group). **A:** MDA levels of the stomach tissue; **B:** MDA levels of the small intestine tissue; **C:** MDA levels of the colon tissue; **D:** PC levels of the stomach tissue; **E:** PC levels of the small intestine tissue; **F:** PC levels of the colon tissue. *** $P < 0.001$ compared to the Sham group. * $P < 0.05$ compared to the Sham group. ### $P < 0.001$ compared to the TBI and TBI + Veh groups. ^^^ $P < 0.001$ compared to the TBI group. ^^ $P < 0.01$ compared to the TBI + Veh group. Veh: vehicle; SC: stem cell; TBI: traumatic brain injury

a wavelength of 560 nm. The level of enzyme activity was calculated as units per milligram of sample protein (KPG-SOD, Karmania Pars gene, Iran) [48, 49].

### Evaluation of histopathological outcome

In order to measure the histopathological changes of the digestive system, 4-micrometer sections were prepared from the stomach, small intestine, and large intestine. For each organ, at least 3 cross-sections were selected from different regions with a distance of at least 100 µm from each other to ensure representative sampling. Sections were stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed by a pathologist who was blinded to the experimental groups and by light microscope (Olympus, CX33, Tokyo, Japan). Histopathological scoring in small and large intestine was done according to predefined criteria: Villous distortion, lamina propria edema, inflammation, vascular congestion in villous tips, epithelial damage, goblet cell loss, mucosal atrophy and or hyperplasia [50–52]. For the stomach, in the rats subjected to TBI, the following changes: Inflammation, congestion, ulcer and erosion was also assessed [53]. Each category was scored based on the severity and extent of injury on a scale of 0 to 4 (in 0.5-point increments), where 0 indicated no injury and 4 indicated severe injury. The sum of the category scores was considered as the total histopathology score.

### Assay replication

All biochemical and enzymatic assays, including the measurement of inflammatory cytokines, oxidative stress markers, and antioxidant enzyme activity, were performed in triplicate for each sample to ensure reproducibility and reliability. The average values of these

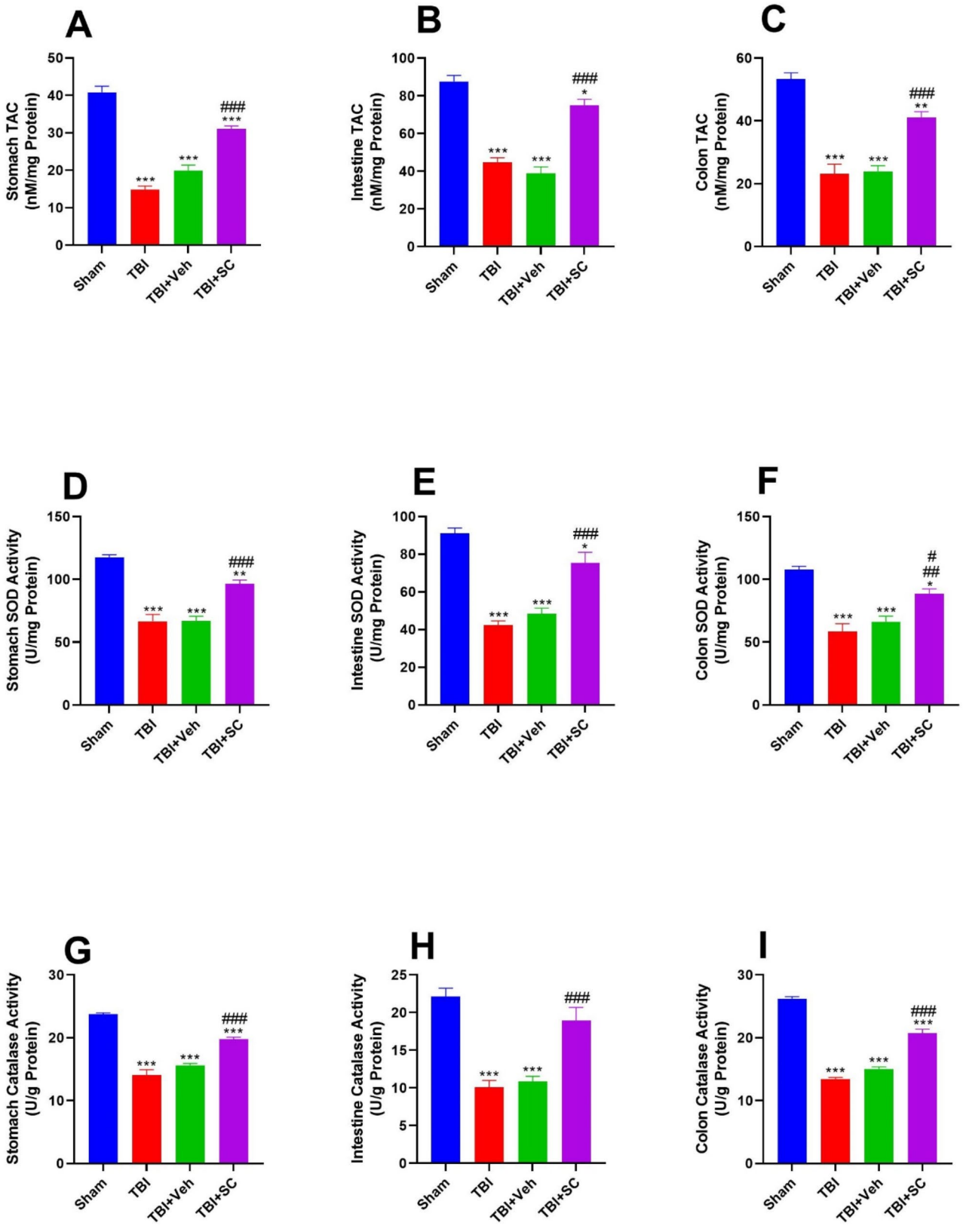


**Fig. 3** (See legend on next page.)

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**Fig. 3** TAC, SOD, and CAT levels (mean ± SEM) in different study groups ($n = 7$ in each group). **A:** TAC levels of the stomach tissue; **B:** TAC levels of the small intestine tissue; **C:** TAC levels of the colon tissue; **D:** SOD levels of the stomach tissue; **E:** SOD levels of the small intestine tissue; **F:** SOD levels of the colon tissue; **G:** CAT levels of the stomach tissue; **H:** CAT levels of the small intestine tissue; **I:** CAT levels of the colon tissue. *** $P < 0.001$ compared to the Sham group. ** $P < 0.01$ compared to the Sham group. * $P < 0.05$ compared to the Sham group. ### $P < 0.001$ compared to the TBI and TBI + Veh groups. ## $P < 0.01$ compared to the TBI group. # $P < 0.05$ compared to the TBI + Veh group. Veh: vehicle; SC: stem cell; TBI: traumatic brain injury

triplicates were calculated and used in the statistical analysis.

### Statistical analysis

The results were expressed as mean ± SEM. The distribution normality of the data was checked using the Shapiro-Wilk test. ANOVA test was used to compare the average data between different groups with normal distribution. In case of significance, Tukey's post hoc test was used to check the difference between the average data in different groups in normal distribution. In case of non-normal distribution, the Kruskal-Wallis test was used. $P < 0.05$ was considered as the minimum significance level. Statistical analysis of data was done using SPSS software version 22 [54].

## Results

### The OMSCs administration effect on IL-1β, IL-6, and IL-10 levels

The levels of IL-1β and IL-6 in the tissues of the stomach, small intestine, and colon in different study groups are shown in Fig. 1. In the TBI and TBI + Veh groups, the levels of IL-1β and IL-6 in the tissues of the stomach (F [3, 16] = 48.04 for IL-1β and F [3, 16] = 27.17 for IL-6), small intestine (F [3, 16] = 55.38 for IL-1β and F [3, 16] = 29.72 for IL-6), and colon (F [3, 16] = 77.63 for IL-1β and F [3, 16] = 34.54 for IL-6) increased after TBI compared to the Sham group. There was no difference in the increase rate between the TBI and TBI + Veh groups. However, the level of IL-1β and IL-6 in the OMSCs group in the stomach, small intestine, and colon was lower after TBI compared to the TBI and TBI + Veh groups (Figs. 1-A to 1-F). In addition, a decrease in the level of IL-10 was observed in the tissues of the stomach (F [3, 16] = 114.4), small intestine (F [3, 16] = 80.42), and colon (F [3, 16] = 45.20) in the TBI and TBI + Veh groups compared to the Sham group. However, the administration of OMSCs prevented this decrease to some extent, and the level of this index was higher in the SC group compared to the TBI and TBI + Veh groups (Figs.1-G to 1-I).

### The effect of OMSC administration on MDA and PC levels

After TBI, an increase in MDA levels in the tissues of the stomach (F [3, 16] = 37.63), small intestine (F [3, 16] = 31.06), and colon (F [3, 16] = 21.55) was observed in the TBI and TBI + Veh groups compared to the Sham group, and this amount was not different between the TBI and TBI + Veh groups. However, the level of this index for oxidative stress after TBI in the stomach, small intestine, and colon tissues was lower in the SC group compared to the TBI and TBI + Veh groups (Fig. 2-A to 2-C). In addition, PC levels increased after TBI in the stomach tissue (F [3, 16] = 37.23) in the TBI and TBI + Veh groups and in the small intestine (F [3, 16] = 33.07) and colon tissues (F [3, 16] = 57.61) in the TBI, TBI + Veh, and SC groups compared to the Sham group. PC levels in the stomach, small intestine, and colon tissues were not different between the TBI and TBI + Veh groups. However, PC levels in the tissue of the stomach, small intestine, and colon in the SC group, which received OMSCs after TBI, were lower compared to the TBI and TBI + Veh groups (Figs. 2-D to 2-F).

### The effect of OMSC administration on TAC, SOD, and CAT levels

TAC and SOD levels decreased in the tissues of the stomach (F [3, 16] = 83.82 for TAC and F [3, 16] = 44.68 for SOD), small intestine (F [3, 16] = 57.70 for TAC and F [3, 16] = 39.25 SOD), and colon (F [3, 16] = 40.12 for TAC and F [3, 16] = 24.70 SOD) after TBI in the TBI, TBI + Veh, and SC groups compared to the Sham group. TAC and SOD levels in the tissue of the stomach, small intestine, and colon were not different between the two groups of TBI and TBI + Veh. However, a smaller decrease in TAC and SOD levels in the tissues of the stomach, small intestine, and colon was observed after administration of OMSCs after TBI, compared to the TBI and TBI + Veh groups (Figs. 3-A to 3-F).

In addition, after TBI, a decrease in CAT levels in the tissues of the stomach (F [3, 16] = 76.28) and colon (F [3, 16] = 185.3) was observed in the TBI, TBI + Veh, and SC groups compared to the Sham group, and the level of this index in the small intestine (F [3, 16] = 26.21) was lower in the TBI and TBI + Veh groups than the Sham group. CAT levels in different tissues were not different between the TBI and TBI + Veh groups. However, the level of CAT in the stomach, small intestine, and colon were higher in the SC group compared to the TBI and TBI + Veh groups (Figs. 3-G to 3-I).

### The effect of OMSC administration on histopathological indices

Following TBI, acute and chronic inflammatory cell infiltration rates (F [3, 16] = 43.48 and F [3, 16] = 43.48 respectively), vascular congestion and capillary dilation (F [3, 16] = 43.48), and submucosal edema (F [3, 16] = 88.11)

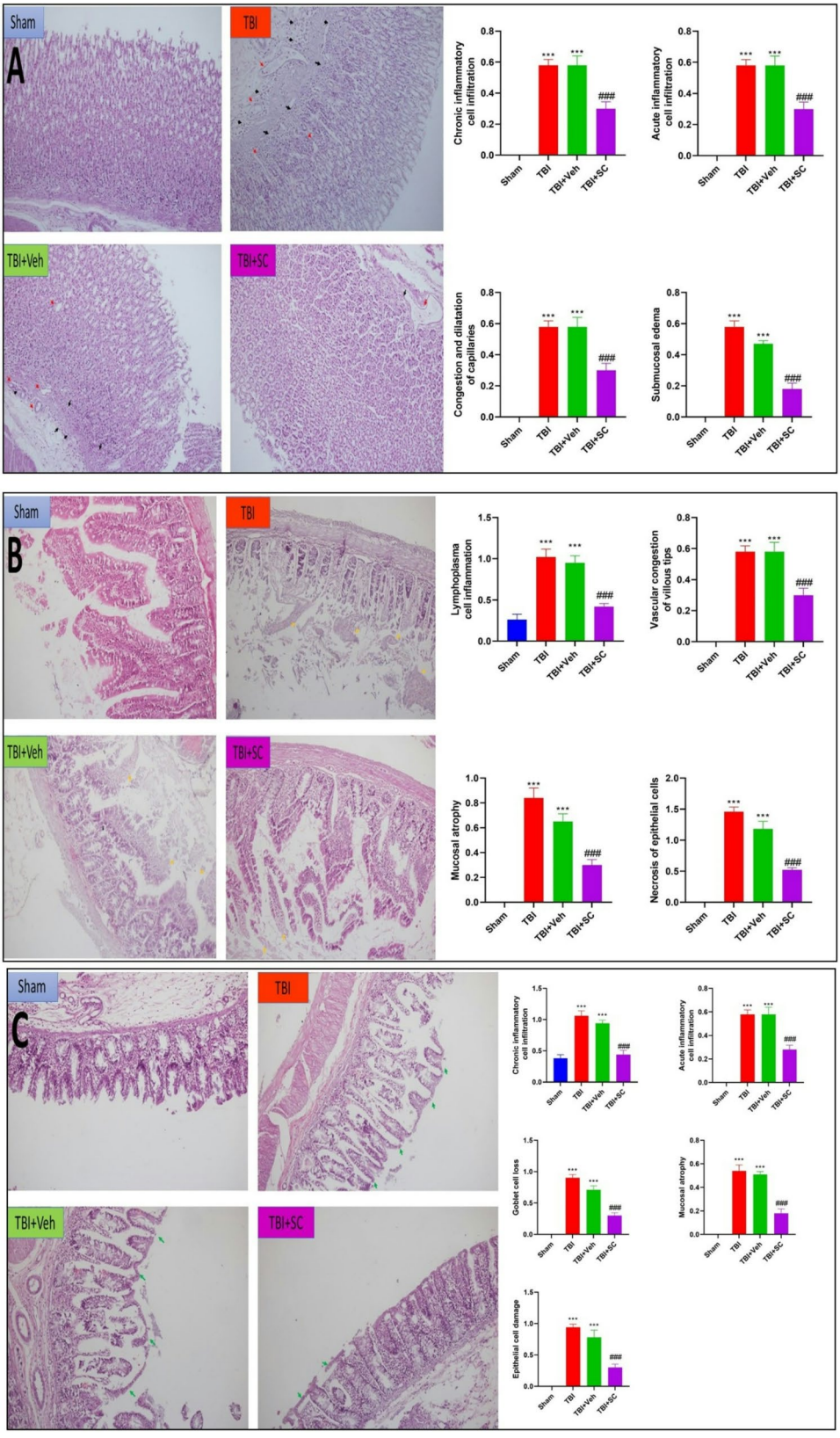


**Fig. 4** (See legend on next page.)

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**Fig. 4** Histopathological changes of the stomach, small intestine, and colon tissues following traumatic brain injury in the study groups ($n = 7$ in each group). Results based on hematoxylin and eosin staining with ×100 magnification. (**A**) Histopathological changes and H&E-stained figures of the stomach tissue are shown as follows: infiltration of acute and chronic inflammatory cells (black arrow), vascular congestion, and capillary dilation (red arrow); (**B**) Histopathological changes and H&E-stained figures of the small intestine tissue are shown as follows: lymphoplasma cells infiltration in lamina propria along with necrosis of epithelial cells on top of the villi (oranges arrows); (**C**) Histopathological changes and H&E-stained figures of the colon tissue are shown as follows: colonic mucosa with atrophy and moderate chronic and mild acute inflammatory cells infiltration in lamina propria (that extends to muscularis mucosa and sub mucosa) along with epithelial cells surface damage (Extension of the subepithelial space with moderate lifting of epithelial layer) (green arrows). *** $P < 0.001$ compared to the Sham group. ### $P < 0.001$ compared to the TBI and TBI + Veh groups. Veh: vehicle; SC: stem cell; TBI: traumatic brain injury

of the stomach tissue increased compared to the Sham group. This increase was not different between the TBI and TBI + Veh groups. Following the administration of OMSCs, this increase was lower than in the TBI and TBI + Veh groups (Fig. 4-A). In the small intestine tissue, in the TBI and TBI + Veh groups, the level of lymphoblast cell inflammation ($F [3, 16] = 25.11$), necrosis ($F [3, 16] = 77.26$), vascular congestion of villous ridges ($F [3, 16] = 43.48$), and mucosal atrophy ($F [3, 16] = 44.13$) increased compared to the Sham group. This increase was not different between the TBI and TBI + Veh groups. Following the administration of OMSCs, there was less change compared to the TBI and TBI + Veh groups (Fig. 4-B). In the colon tissues of the TBI and TBI + Veh groups, acute and chronic inflammatory cell infiltration ($F [3, 16] = 48.12$ and $F [3, 16] = 27.68$ respectively), goblet cell loss ($F [3, 16] = 72.10$), mucosal atrophy ($F [3, 16] = 59.67$), and epithelial cell damage ($F [3, 16] = 39.43$) increased compared to the Sham group. This increase was not different between the TBI and TBI + Veh groups. Following the administration of OMSCs, there was less change compared to the TBI and TBI + Veh groups (Fig. 4-C).

## Discussion

The effect of administration of OMSCs after TBI on the level of inflammation, oxidative stress, and gastrointestinal tissue damage was investigated in this study. Male rats received OMSCs intravenously at 1 and 24 h post-TBI to target acute and subacute phases, with assessments conducted 48 h post-injury to evaluate early therapeutic effects on inflammation, oxidative stress, and histopathology of gastrointestinal tissues. The results showed that after TBI, the level of inflammatory factors IL-1β and IL-6 increased in the tissues of the stomach, small intestine, and colon, but this increase was significantly lower in the group receiving OMSCs. In addition, the level of the anti-inflammatory factor IL-10 decreased, but in the intervention group with OMSCs, this decrease was significantly lower. In the investigation of oxidative stress, the results of the study showed that the level of oxidant factors MDA and PC increased after TBI in the tissues of the stomach, small intestine, and colon. However, in the intervention group with OMSCs, there was less change. In addition, the level of antioxidant factors TAC, SOD, and CAT decreased in the mentioned tissues after TBI, but in the intervention group with OMSCs, there was less change. The histological studies also showed that the administration of OMSCs after TBI improved the histopathological outcome in the tissues of the stomach, small intestine, and colon.

Inflammation plays a vital role in secondary injury after TBI. It leads to the activation of central nervous system cells, such as microglia and astrocytes, which increase the secretion of inflammatory cytokines [55, 56]. This process can cause systemic inflammation and damage to other tissues [13]. After TBI, inflammatory factors in the tissues of the stomach, small intestine, and colon increased, and anti-inflammatory factors, such as IL-10, decreased. These changes were less in the intervention group with OMSCs. Using stem cells has been considered a therapeutic strategy for neurological diseases such as TBI. MSCs help treat TBI due to their high ability to suppress inflammation and differentiate and regulate the immune system [15, 16, 18]. MSCs can suppress inflammatory responses after brain injury and act as anti-inflammatory agents [57, 58]. Studies have shown that using OMSCs after TBI can reduce the changes of inflammatory factors such as IL-1β and IL-6 [59]. Administration of OMSCs has been shown to significantly increase IL-10 expression in the injured tissue [60]. It can prevent the proliferation of T cells and microglial cells and reduce the secretion of inflammatory cytokines. By releasing anti-inflammatory cytokines and immunological regulatory factors, they can increase the survival rate of damaged cells [61, 62]. TBI is associated with central and peripheral inflammatory responses, and controlling inflammation in TBI patients can have protective effects [63, 64]. Therefore, the use of OMSCs following TBI can have beneficial effects on the tissues of the stomach, small intestine, and colon by modulating inflammatory and anti-inflammatory factors. While the use of ketamine and xylazine as anesthetics during the TBI induction procedure ensured animal welfare, their potential neuroprotective properties might have influenced the inflammatory and oxidative stress responses observed in this study [65, 66]. Future studies could explore alternative anesthetic protocols to minimize potential confounding effects. Also, it should be noted that the use of an animal model may not fully replicate human responses, as species-specific differences

could impact the translational potential of the findings [67–69]. This limitation highlights the need for further validation in clinical settings or alternative preclinical models.

Oxidative stress is associated with producing oxygen free radicals, superoxide, hydrogen peroxide, nitric oxide, and proxy nitrite in response to TBI [70]. Excessive production of ROS causes peroxidation of cellular and vascular structures, DNA fragmentation, and inhibition of the mitochondrial electron transport chain [71, 72], and these processes can stimulate apoptosis and necrosis [4]. Antioxidant defense systems play an essential role in protection against oxidative damage [73–75]. This study showed that although TBI increases the levels of oxidant factors in the stomach, small intestine, and colon tissues, these changes were less in the intervention group with OMSCs. In addition, the reduction of antioxidant factors was lower in the intervention group with OMSCs. In this regard, the results of Radavi-Asgar et al.'s study showed that oxidant factors increase after brain damage and antioxidant factors decrease in the heart and kidney, changes moderated by using OMSCs [59]. In other studies, an increase in MDA and a decrease in SOD and CAT after brain injury have been reported [76, 77]. Through secreting immunoregulatory particles such as exosomes, MSCs reduce oxidative stress and promote extracellular matrix regeneration by inhibiting apoptosis and stimulating angiogenesis [78, 79]. MSC exosomes have antioxidant activity and can reduce inflammatory damage caused by oxidative stress [78, 80]. Therefore, the use of OMSCs after TBI can have beneficial effects on the tissues of the stomach, small intestine, and colon by modulating oxidative stress factors. However, the precise molecular and cellular mechanisms underlying these effects remain unclear and require further investigation. Additionally, parameters such as microbiome alterations, gut permeability, and changes in the gut-brain axis were not explored in this study. Addressing these aspects in future research would provide a more comprehensive understanding of the therapeutic potential of OMSCs.

The histopathological results showed that using OMSCs after TBI improved the stomach, small intestine, and colon tissues. In addition, the infiltration of inflammatory cells, vascular congestion, capillary dilation, and tissue edema decreased in the intervention group, and there was less damage to epithelial cells. TBI can lead to several physiological complications, including digestive system dysfunction, and increase intestinal permeability, bacterial translocation, and multi-system organ failure [8, 51, 81]. Studies have shown that TBI causes darkening of the intestinal villi and increases its permeability [50, 51]. Intestinal epithelial cells act as a physical barrier between the intestinal lumen and the underlying vessels and lymph nodes. This is mainly due to the structural integrity of cellular tight junctions, and loss of this integrity is associated with increased intestinal permeability and bacterial translocation [82, 83]. TBI increases pro-inflammatory cytokines such as TNF-α and IL-6, leading to intestinal permeability and epithelial cell damage [12, 84]. MSCs reduce oxidative stress and inflammatory response through the secretion of exosomes and vesicles by inhibiting apoptosis and stimulating angiogenesis [77–80]. These types of cells may help improve gastrointestinal function after TBI by modulating tight junction proteins and reducing intestinal permeability [85, 86]. However, this focus on the acute phase did not allow for the assessment of long-term outcomes, which are critical for understanding sustained therapeutic benefits and potential side effects of OMSCs. Future longitudinal studies are necessary to address this gap.

## Conclusion

The results of this study showed that the administration of OMSCs after TBI reduces inflammation and oxidative stress and improves the damage to the tissues of the stomach, small intestine, and colon. These results suggest that OMSCs can be an effective therapeutic method to reduce gastrointestinal complications after TBI.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12868-025-00936-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Conception or design of the work: Zahra Soltani, Saeed Karamoozian, Mohammad Khaksari. Data collection: Masoud Eslami, Nazanin Sabet, Farzaneh Rostamzadeh. Data analysis and interpretation: Zahra Soltani, Elham Jafari. Data interpretation and drafting the article: Zahra Soltani, Alireza Raji Amirhasani, Zakieh Keshavarzi. Critical revision of the article: Zahra Soltani, Masoud Eslami, Alireza Raji Amirhasani, Mohammad Khaksari, Zakieh Keshavarzi, Farzaneh Rostamzadeh, Nazanin Sabet, Elham Jafari, Saeed Karamoozian. All authors reviewed the manuscript.

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**Data availability**
All the data are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
This interventional-experimental study was approved by the Ethics Committee of Kerman University of Medical Sciences with the ethical code IR.KMU.AH.REC.1399.068. The animals used in our study were purchased from

the Animal Research Center of Kerman University of Medical Sciences. No animals were privately owned, and the research was conducted in accordance with the ethical standards.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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