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Original Article

Ameliorative effects of undifferentiated and differentiated BM-MSCs in MIA-induced osteoarthritic Wistar rats: roles of NF-κB and MMPs signaling pathways

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Abstract: Objectives: Osteoarthritis (OA) is a degenerative joint condition that is persistent. OA affects millions of people throughout the world. Both people and society are heavily economically burdened by osteoarthritis. There is currently no medication that can structurally alter the OA processes or stop the disease from progressing. Stem cells have the potential to revolutionize medicine due to their capacity to differentiate into chondrocytes, capacity to heal tissues and organs including osteoarthritic joints, and immunomodulatory capabilities. Therefore, the goal of the current investigation was to determine how bone marrow-derived mesenchymal stem cells (BM-MSCs) and chondrogenic differentiated mesenchymal stem cells (CD-MSCs) affected the treatment of OA in rats with monosodium iodoacetate (MIA)-induced osteoarthritis. Methods: Male Wistar rats were injected three times with MIA (1 mg)/100 μL isotonic saline to induce osteoarthritis in the ankle joint of the right hind leg. Following the MIA injection, the osteoarthritic rats were given weekly treatments of 1 × 10^6 BM-MSCs and CD-MSCs into the tail vein for three weeks. Results: The obtained results showed that in osteoarthritic rats, BM-MSCs and CD-MSCs dramatically decreased ankle diameter measurements, decreased oxidized glutathione (GSSG) level, and boosted glutathione peroxidase (GPx) and glutathione reductase (GR) activities. Additionally, in rats with MIA-induced OA, BM-MSCs and CD-MSCs dramatically boosted interleukin-10 (IL-10) serum levels while considerably decreasing serum anticitrullinated protein antibodies (ACPA), tumour necrosis factor-α (TNF-α), and interleukin-17 (IL-17) levels as well as ankle transforming growth factor-β1 (TGF-β1) expression. Analysis of histology, immunohistochemistry, and western blots in osteoarthritic joints showed that cartilage breakdown and joint inflammation gradually decreased over time. Conclusions: It is possible to conclude from these results that BM-MSCs and CD-MSCs have anti-arthritic potential in MIA-induced OA, which may be mediated *via* inhibitory effects on oxidative stress, MMPs and inflammation through suppressing the NF-κB pathway. In osteoarthritis, using CD-MSCs as a treatment is more beneficial therapeutically than using BM-MSCs.

Keywords: Osteoarthritis, undifferentiated BM-MSCs, chondrogenic BM-MSCs, NF-κB, MMPs

Introduction

Osteoarthritis (OA) is a condition that affects both young and old people's musculoskeletal systems according to Liu *et al*. [1]. The normal function of the synovial joints is impacted by osteoarthritis [2]. Both people and society are heavily burdened financially by osteoarthritis [3]. It is the most prevalent type of arthritis and one of the main factors contributing to the

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impairment of 303 million people worldwide [2]. The physical and financial burden of OA is enormous due to an ageing population and the fact that it is one of the primary causes of disability in the elderly [1, 4].

Age, obesity, joint trauma, genetics, gender, crystal deposition disorders-like gout, and metabolic/endocrine diseases like diabetes are the most important risk factors for the onset of OA [5]. The hallmarks of OA progression are inflammation, chondrocyte apoptosis, and cartilage extracellular matrix (ECM) loss, which cause articular cartilage to degrade [6].

Currently, it is unclear how to pinpoint the exact cause of OA in particular, and the probability that OA will progress depends on a variety of factors. It can be suggested that dysbiosis of the gut microbiota, which results in obesity, insulin resistance, and systemic inflammation, predisposes people to the development of OA [7]. The majority of therapy attempts, however, have only been successful in symptom reduction (pain relief) [8].

Despite significant research, there is currently no cure for OA or effective way to stop it from progressing [9]. The monosodium iodoacetate (MIA)-induced model of OA is an excellent experimental model that, in contrast to surgical models, is easy to construct and results in OA alterations that are the same as those observed in human beings [10]. As inflammatory cytokines are raised and anti-inflammatory cytokines are decreased, it mimics the inflammation process [11].

Mesenchymal stem cell (MSC) therapies have attracted attention recently because they help to protect, regenerate, and restore damaged and degenerating arthritic joints [12-14].

For the development of cell-based therapeutic strategies for tissue repair and regenerative medicine, bone marrow-derived mesenchymal stem cells (BM-MSCs) hold significant promise. In several investigations, both in animal models and even in clinical research, the impact of mesenchymal stem/stromal cell-based therapy on cartilage repair has been demonstrated [15, 16]. One of the first types of stem cells to be used in the clinic are BM-MSCs because they are simple to isolate, have the capacity to differentiate into multiple lineages, and can multiply extensively *in vitro* [17]. Adult MSCs can differentiate into cells of ectodermal (neurons), endodermal, and mesodermal origins including osteoblasts, chondrocytes, and adipocytes [18]. An intra-articular injection of chondrocytes improves the condition of the damaged cartilage, stops further cartilage degradation, and prompts cartilage healing [19]. Because they share traits with the host tissue, differentiated MSCs are advantageous for replacing damaged tissue. According to several research reports, transplanting chondrogenic differentiated MSCs (CD-MSCs) has beneficial results in treating OA [20]. In order to cure OA, it may be necessary to differentiate MSCs into certain chondrogenic cells through their manipulation for transplant into patients. The modification of extracellular niches, which is typically mediated by “cocktails” made up of growth factors, signalling molecules, and/or genetic manipulations, is what causes stem cells to differentiate. This process is regulated by intrinsic and extrinsic regulators as well [21].

The aim of this work is to assess the *in vivo* effects of undifferentiated BM-MSCs and CD-MSCs in alleviating oxidative stress, joint degradation, and inflammation caused by monosodium iodoacetate (MIA)-induced ankle OA in the rat model.

Materials and methods

Experimental animals

In the current study, 40 male Wistar rats were employed. They were 6-7 weeks old and weighed between 110 and 120 g. The Helwan Farm, Holding Company for Biological Products and Vaccines (VACSERA), Egypt, provided the animals for this study. Prior to the start of the investigation, animals were kept under observation for about 7 days to get monitor for any diseases. The animals were kept in polypropylene cages with stainless steel ventilated covers in the Animal House of the Department of Zoology, Faculty of Science, Beni-Suef University, Egypt, at a standard temperature (20-25°C) and regular daily lighting cycle (10-12 h/day), as well as with a balanced standard diet and water readily available at all times. The experimental study using Wistar rats was approved by the ethics committee for the care and use of animals, Faculty of Science, Beni-Suef University, Egypt (Ethical approval number: 020-118).

Induction of OA

The ankle joint of the right hind leg was injected three times with 100 µL of physiological saline containing 1 mg of MIA (Sigma-Aldrich, St. Louis, MO) to cause OA [22].

Isolation and culture of BM-MSCs

The technique for isolating and cultivating BM-MSCs is based on ideas from Pittenger *et al*. [23], Aggarwal and Pittenger [24], Chaudhary and Rath [25], as well as our earlier works [12, 26].

Isolation of BM-MSCs: The techniques of Pittenger *et al*. [23] and Aggarwal and Pittenger [24] were used to isolate stem cells. Ten mL of finely prepared marrow was carefully placed over 1 mL of Ficoll-Hyopaque solution (1.077 g/cm^3; Sigma, St. Louis, Missouri, USA) in sterile centrifuge tubes. The mixture was centrifuged for 20 minutes at 22°C at a speed of 1500-1800 rpm. The supernatant was carefully aspirated and discarded after centrifugation to avoid disrupting the cell suspension-Ficoll interface. A sterile pipette was used to transfer the concentrated marrow aspirate into another sterile tube. A final suspension was created by adding phosphate buffered saline to the cell suspension in a 15-mL conical tube and thoroughly washing the mixture by centrifuging for 10 minutes at 1500-1800 rpm and 4°C. The supernatant was decanted and the process was repeated several times.

Culture of BM-MSCs: The cell pellet was quickly washed in phosphate buffer saline (PBS) after the supernatant had been decanted, and it was then suspended in full DMEM (DMEM supplemented with 15% FBS, 0.36% sodium hydrogen carbonate, and 1% penicillin-streptomycin solution). The number of living and dead cells was counted using a hemocytometer at a 100× magnification after the cells were stained with trypan blue solution (0.2%) to measure the viability of the cells (the number of viable cells relative to the total number of cells). In sterile T-25 cm^2 Greiner cell culture flasks with canted necks, 25 × 10^6 cells were seeded at a density of 1 × 10^6 cells/cm^2 area, and they were then placed in a 37°C, 5% CO_2-humidifed incubator (Biobase, Model: BJPX-C50; South Gongye Road, Jinan, Shandong Province, China). Each incubation took place for 4 days, during which dead, floating, and non-adherent cells were removed until the cells had reached 70 to 80% confluence. Following this, adherent cells were treated with two sterile PBS washes (pre-warmed at 37°C) and trypsinized for two to three minutes every seven to ten days of culture using 1 to 2 mL of trypsin (0.25%)/EDTA (1 mM) (pre-warmed at 37°C). Using an inverted biological microscope, cells were checked to ensure that adhering cells had detached (Novel, Model: NIB-100; Jiangsu, China). The addition of 3-5 mL of full DMEM stopped the action of trypsin. Cells were then gathered and centrifuged for 5 minutes at 3000 rpm. After decanting the produced supernatant, DMEM was added, and centrifugation was used to wash the cell pellet. The cell pellet was resuspended in full media and divided into 1:2 and 1:3 ratios for the first, second, and subsequent passes. Osteoarthritic rats were quickly injected with 1 × 10^6 cells/rat in an incomplete DMEM at a viability level of greater than 95% in the lateral tail vein.

Chondrogenic differentiation of BM-MSCs

We followed existing techniques to carry out *in vitro* chondrogenesis [27]. Trypsinization, washing, and the transfer of 7.5 × 10^5 cells into fresh T75 flasks were performed on expanded MSC. Treatment with STEMPROTM Chondrogenesis Differentiation medium (Thermo Fisher Scientific, MA, USA) resulted in the induction of chondrogenic differentiation [28]. Three times a week, differentiation medium was changed throughout the incubation of monolayers at 37°C and 5% CO_2. To confirm chondrocyte morphology after 7 days, the monolayer was examined under a microscope. The transition of a fibroblastic, spindle-shaped MSC to a rounded, polygonal morphology resembling chondrocyte monolayers was deemed to be > 90% differentiating. Chondro-MSCs were not cultivated beyond the first passage in order to prevent dedifferentiation. After being visually verified, chondro-MSCs were trypsinized, washed, and counted.

Experimental design

Wistar rats were divided into four groups with ten animals, as shown below: 1) Normal Group: Each normal rat received injections of equivalent volume of isotonic sterile saline in which the treatments were dissolved into the tail vein

at days 7, 14, and 21. 2) Osteoarthritic control group (OA control) received injections of 1 mg MIA over the course of three days in the ankle joint of the right hind leg. In addition, the rats in this group received injections of equivalent volume of isotonic sterile saline in which the treatments were dissolved into the tail vein at days 7, 14, and 21 following MIA injection. 3) Osteoarthritic treated with BM-MSCs Group (OA+BM-MSCs); rats in this osteoarthritic group received MIA injections into the ankle joint of the right hind leg over the course of three days, as well as BM-MSC injections (1×10^6 cells/rat) into the tail vein 7, 14, and 21 days later [29]. 4) Osteoarthritic treated with CD-MSCs Group (OA+CD-MSCs); rats in this osteoarthritis group received injections of MIA in the ankle joint of the right hind leg on three consecutive days, as well as CD-MSCs (1×10^6 cells/rat) into the tail vein at the 7, 14, and 21 days following the injection of MIA [29].

Blood and organ sampling

We collected blood samples from the jugular vein at the end of the trial under diethyl ether anaesthesia. Anticoagulant-free blood was collected into tubes, allowed to clot, and then centrifuged for 15 minutes at 3000 rpm. In a hurry, the clear supernatant sera were gathered, divided into three portions for each individual animal, and kept at -20°C until required. Postmortem, the hind leg ankles were removed and kept at -70°C for protein analysis in Western blots.

Ankle measurement

We noticed changes in the anteroposterior diameters (cm) of the osteoarthritic and normal ankles. A micrometer was used to measure the diameter of the ankles [30]. On days 0, 7, 14, and 21 following OA induction, measurements were taken. Additionally, a camera took pictures of the right leg.

Detection of serum cytokine levels

The level of tumour necrosis factor-α (TNF-α) in serum was determined. By using an ELISA kit (Catalog Number: 438204) bought from Bio-Legend (San Diego, California) in accordance with the instructions of the manufacturer. The interleukin-10 (IL-10) level in serum was determined by using an ELISA kit (Catalog Number: PR1000) acquired from PharmPak (R&D Systems, Minneapolis, MN, USA) and following the instructions of the manufacturer. The levels of interleukin-17 (IL-17) and Anti-citrullinated protein antibodies (ACPA) in serum were measured using ELISA (enzyme-linked immunosorbent assay) kits (Catalog Number: ER0035 for IL-17 and ER1453 for ACPA, respectively) bought from Wuhan Fine Biotech Company (China) in accordance with the manufacturer's recommendations.

Estimation of serum oxidative stress and anti-oxidant defense biomarkers

Utilizing specialized ELISA kits purchased from BioVision (CA, USA) in accordance with the manufacturer's instructions, we were able to identify the activities of oxidised glutathione (GSSG), glutathione peroxidase (GPx), and glutathione reductase (GR) in serum. The ELISA plate reader was used to measure each ELISA kit (Stat Fax 2200, Awareness Technologies, Florida, USA).

Western blot analysis

Using Western blot analysis, the ankle joint tissue was found to contain the following proteins: matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS), tumour necrosis factor receptor (TNFR), nuclear factor-κB (NF-κB) p50, NF-κB p65, and IκB (nuclear factor of kappa light polypeptide). Identical amounts of protein (30 g) were extracted and run on 10% sodium dodecyl sulfate-polyacrylamide gels (SDSPAGE). Additionally, proteins were moved to polyvinylidene fluoride (PVDF) membranes, and blocking was done overnight using 5% skim milk in TBS with Tween 20. The primary TNFR, NF-κB p50, NF-κB p65, IκB, MMP-3, MMP-9, and iNOS antibodies were applied to the membranes at 4°C for 40 minutes. Following TBST washing, exposure to the appropriate secondary antibodies, and development with a kit that produces enhanced chemiluminescence, membranes were used (BioRad, Oregon, USA). The produced blots were finally scanned, and ImageJ software (NIH, USA) was used to measure the band intensity.

Histopathological and immunohistochemical examination

The right ankle joint of the hind leg was removed and saline-washed following sacrifice (21 days

after MIA injection). In 10% neutral buffered formalin, ankle joints were fixed for 48 hours. The fixed tissues were delivered to the pathology section of the National Cancer Institute at Cairo University, Egypt, for processing, blocking in paraffin wax, sectioning, and staining with hematoxylin and eosin (H&E) for ankle joint. Using paraffin blocks containing 10% formic acid and replacing them twice weekly for two weeks, the right hind leg ankle joint was decalcified. Using a surgical blade, the end point of decalcification was physically evaluated. Following thorough decalcification, the samples underwent phosphate buffer solution (PBS) washing, ethanol dilution, and paraffin wax embedding. Finally, to assess the histopathological alterations and severity of arthritis, 5 µm thick cross sections of these blocks were stained with hematoxylin-eosin (H&E) [31] and viewed using a light microscope. A pathologist performed an unbiased histopathological investigation of bone, cartilage, and synovial inflammation. For immunohistochemical investigation, the ankle joint samples that were embedded in paraffin were cut into sections that were 5 µm thick. These sections were then placed on slides with a positive charge (Thermo Fisher Scientific, Pittsburgh, PA, USA) and subjected to immunostaining. In overall, after deparaffinization, rehydration, antigen retrieval, and sealing, the sections were incubated in a 3% H_2O_2 solution for 15 minutes. Subsequently after blocking, the tissue sections were incubated with transforming growth factor-β1 (TGF-β1) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:200, and kept at a temperature of 4°C overnight. Following the washing step with phosphate-buffered saline, the sections treated with the peroxidase-labeled secondary antibody (diluted 1:200) were left to incubate for a duration of 30 minutes. The reaction with the 3,3-diaminobenzidine (DAB) substrate allowed for the visualization of the bound antibody complex, and hematoxylin was used to counterstain the slides (ABclonal Inc., China). The immunohistochemically stained sections were subsequently examined at low power (×100) and high power (×400) using light microscopy. A brown color indicated a positive reaction. We used ImageJ (1.51d), a free software program, to assess the integrated positive reaction intensities to measure the intensity of the TGF-β1 positive reaction.

Data statistical analysis

The data were presented as mean ± standard error of mean (SEM). The statistical investigation was carried out using the Statistical Package of Social Sciences (SPSS) program version 22 (SPSS, Cary, NC, USA), which included a one-way analysis of variance (ANOVA) and a Duncan's multiple range test [32]. A difference between groups was considered as significant when it reached $P < 0.05$.

Results

Effect on right ankle anteroposterior diameter

With the exception of time zero, osteoarthritic rats displayed a marked increase in the right ankle anteroposterior diameter as compared to healthy control rats at each checkpoint (**Figure 1**). On the other hand, at all check timepoints following MIA injection, the right ankle anteroposterior diameter was noticeably reduced in the osteoarthritic rats treated with BM-MSCs and CD-MSCs. The use of BM-MSCs and CD-MSCs in the first, second, and third weeks of treatment of the arthritic rats led to a significant decrease in the elevated values of right ankle anteroposterior diameter as compared to the osteoarthritic control rats (**Figure 1**).

Effect on ACPA level

The serum ACPA level significantly increased (+267.83%) when MIA-induced osteoarthritic rats were compared to healthy control rats ($P < 0.05$). The treatment of MIA-induced osteoarthritic rats with BM-MSCs caused a substantial ($P < 0.05$) decrease in the elevated ACPA; the measured percentage change was -50.35% in comparison to the osteoarthritic control. The treatment of MIA-induced osteoarthritic rats with CD-MSCs caused a substantial ($P < 0.05$) decrease in the elevated ACPA; the measured percentage change was -53.43% in comparison to the osteoarthritic control. CD-MSC treatment is the most effective in reduction of the elevated ACPA level in arthritic rats (**Figure 2**).

Effect on serum TNF-α (Th1 cytokine) level

Rats with MIA-induced osteoarthritis had significantly ($P < 0.05$) higher serum levels of TNF-α (Th1 Cytokine), with a percentage change of +697.17% in comparison to healthy control

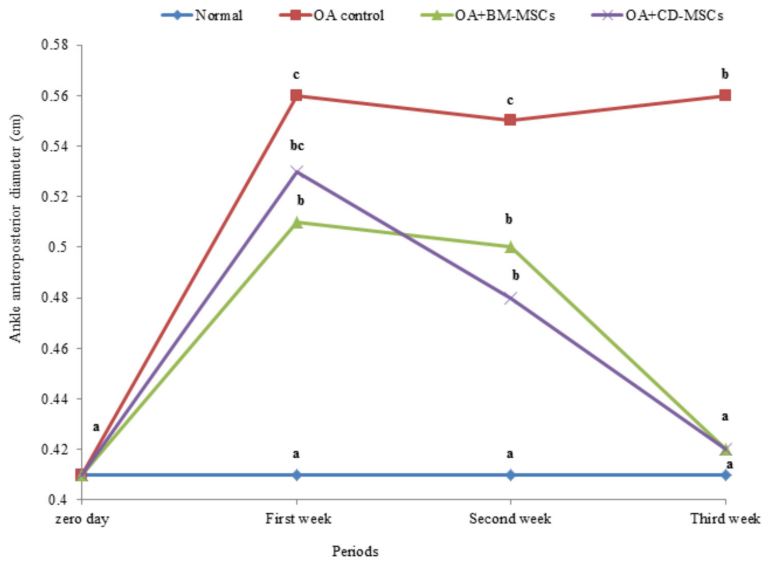

Figure 1. Ankle anteroposterior diameter in normal control, osteoarthritic, and osteoarthritic-treated groups. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs.

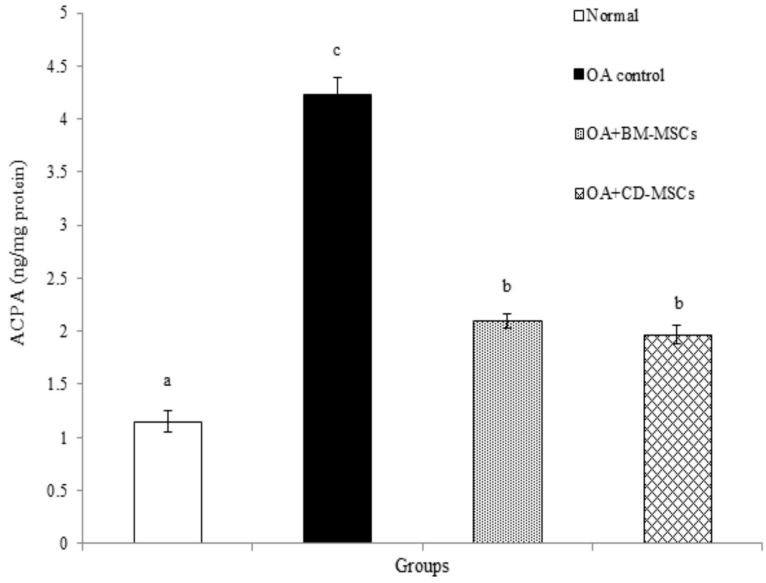

Figure 2. Effect of treatment with BM-MSCs and CD-MSCs on serum ACPA level in in MIA-induced osteoarthritic rats. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; ACPA: Anticitrullinated protein antibodies.

rats. A significant ($P < 0.05$) reduction in the increased TNF-α was observed in the MIA-induced osteoarthritic rats treated with BM-MSCs and CD-MSCs; the documented percentage changes were -73.28% and -74.05%, respectively, in contrast to the osteoarthritic control. Significant ($P < 0.05$) reductions in the increased TNF-α level were seen in rats treated with CD-MSCs for osteoarthritis (**Figure 3**).

Effect on serum IL-17 (Th17 cytokine), and IL-10 levels

When compared to normal control rats, MIA-induced osteoarthritic rats had significantly ($P < 0.05$) higher blood IL-17 levels, with a percentage change of +291.36%. In contrast to IL-17, MIA-induced osteoarthritic rats had significantly lower serum levels of IL-10 (-73.9%) ($P < 0.05$). When MIA-induced osteoarthritic rats were treated with BM-MSCs and CD-MSCs, the elevated IL-17 level was significantly ($P < 0.05$) reduced; the reported percentage changes were, respectively, -56.04% and -60.36% in comparison to the osteoarthritic control.

Contrary to IL-17, the lowered IL-10 level was significantly improved ($P < 0.05$) in osteoarthritic rats treated with BM-MSCs and CD-MSCs; the assessed percentage changes were +206.2% and +228.6%, respectively. The most effective method for treating IL-10 and IL-17 levels in osteoarthritic rats was to provide CD-MSCs (**Figure 3**).

Effect on serum oxidative stress and antioxidant defense system

Effect on serum GSSG content: The serum GSSG level significantly increased ($P < 0.05$) in the MIA-induced osteoarthritic rats; the reported percentage change was +421.88% in comparison to the healthy controls. In rats treated with

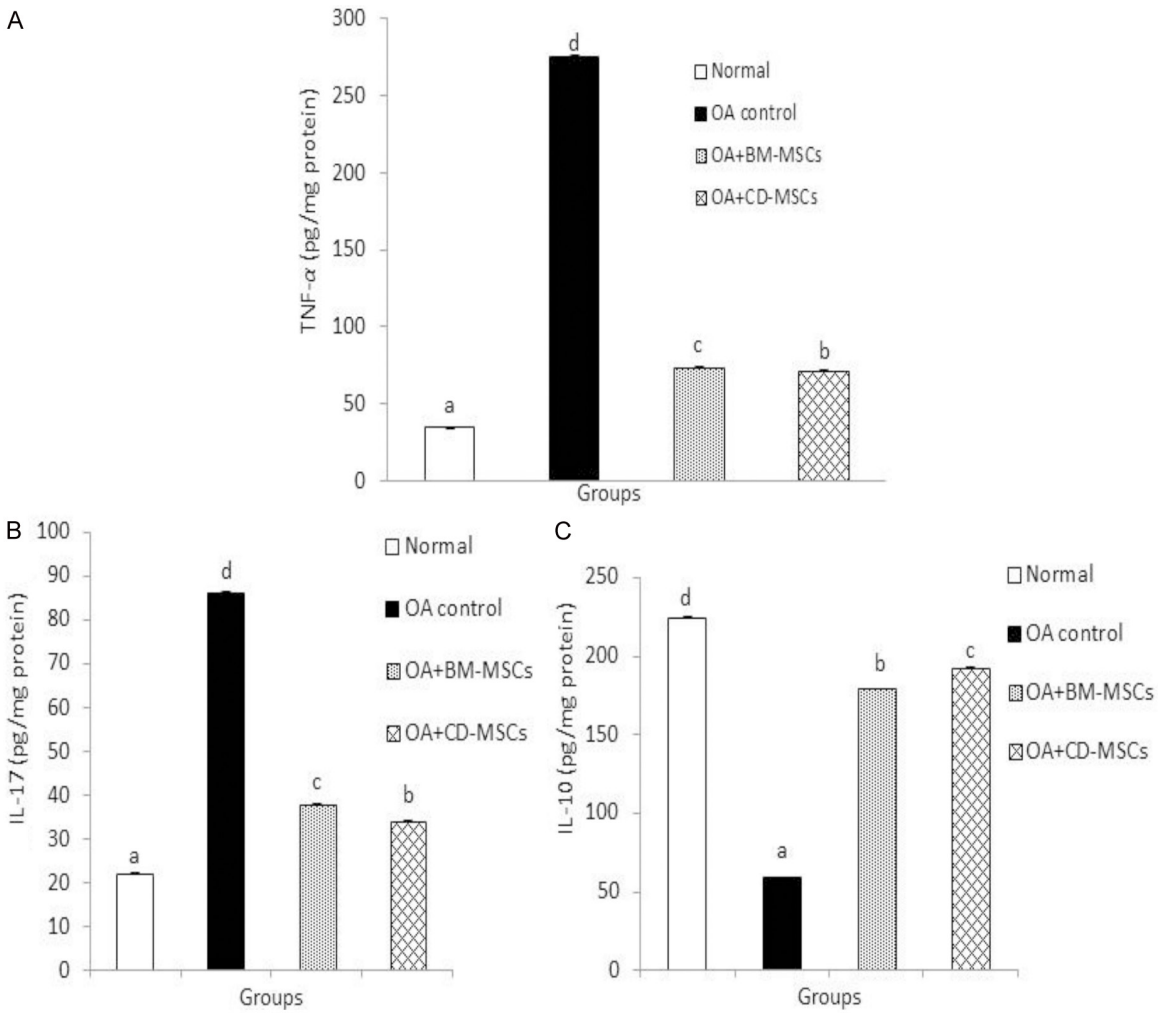

Figure 3. Effect of treatment with BM-MSCs and CD-MSCs on serum TNF-α, IL-17, and IL-10 levels in MIA-induced osteoarthritic rats. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; TNF-α: tumour necrosis factor-α; IL-17: interleukin-17; IL-10: interleukin-10.

BM-MSCs and CD-MSCs for MIA-induced osteoarthritis, the raised GSSG level was significantly ($P < 0.05$) decreased; the reported percentage changes were -63.47% and -64.67%, respectively, in contrast to the osteoarthritic controls. The use of BM-MSCs and CD-MSCs has the same positive impact on serum GSSG content (**Figure 4**).

Effect on serum GPx and GR activities: MIA-induced osteoarthritic rats exhibited reduced blood GPx and GR activities when compared to the healthy control group, with percentage reductions of -82.34% and -78.31%, respectively. The decreased GPx and GR activities in the MIA-induced osteoarthritic rats were significantly improved ($P < 0.05$) by the administration of BM-MSCs and CD-MSCs, with percentage changes of +457.92% and +286.11% for BM-MSCs and +326.71% and +319.44 % for CD-MSCs, respectively. The BM-MSCs impact on boosting GPx activity was the strongest, with a percentage change of +457.92%. The most significant change in GR activity was caused by CD-MSCs, with a percentage change of +319.44% (**Figure 4**).

Effects on ankle tissue protein expression

Effect on TNFR and iNOS protein expression: In comparison to the normal control group, MIA-induced osteoarthritic rats showed a significant ($P < 0.05$) increase in the expression of the proteins TNFR (+272%) and iNOS (+223.81%) in

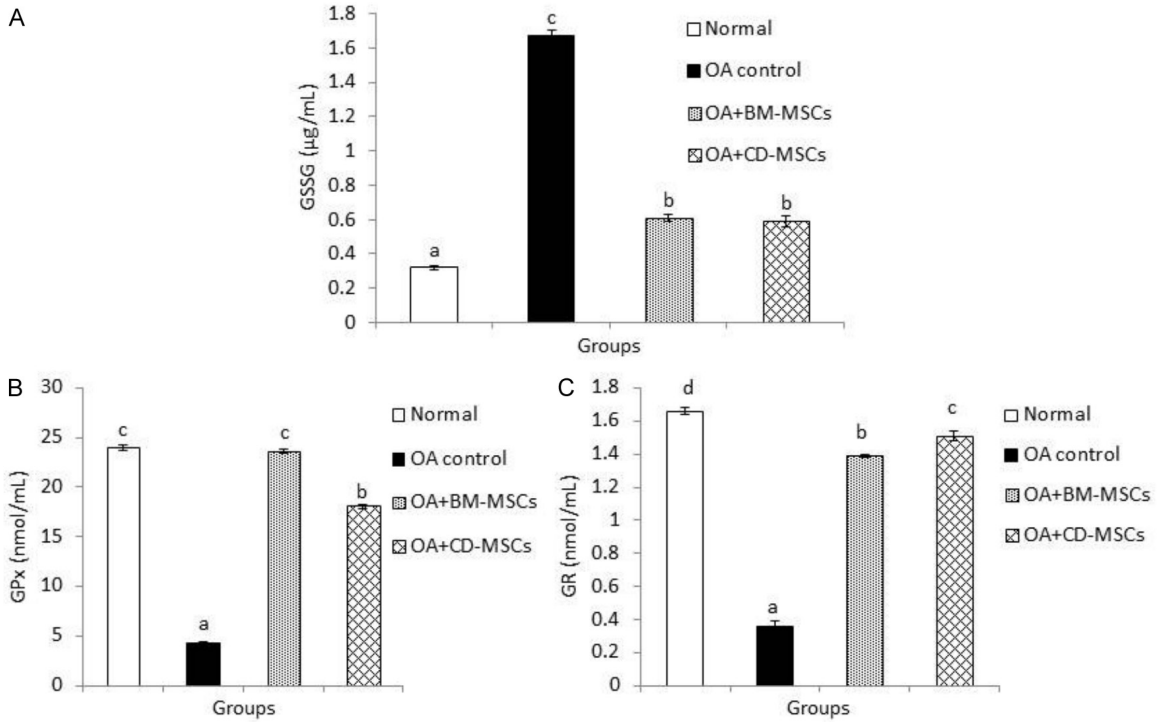

Figure 4. Effect of treatment with BM-MSCs and CD-MSCs on serum GSSG level and serum activities of GPx and GR in MIA-induced OA rats. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; GSSG: oxidised glutathione; GPx: glutathione peroxidase; GR: glutathione reductase.

the ankle tissue. The administration of BM-MSCs and CD-MSCs to MIA-induced osteoarthritic rats potentially reduced ($P < 0.05$) the changed protein expression of TNFR and iNOS; the corresponding percentage changes were -50.27% and -30.29% for BM-MSCs and -62.37% and -60.59% for CD-MSCs, respectively. The most effective treatment for reducing TNFR and iNOS protein expressions seemed to be CD-MSCs (**Figure 5**).

Effect on NF-κB P50, NF-κB P65, and IκB protein expression: MIA-induced osteoarthritic rats showed a significant ($P < 0.05$) increase in the expression of the proteins NF-κB P50 (+260.53%), NF-κB P65 (+309.09%), and IκB (+192.08%) in the ankle tissue in contrast to the normal control group. In MIA-induced osteoarthritic rats, the administration of BM-MSCs and CD-MSCs significantly decreased ($P < 0.05$) the changed protein expression of NF-κB P50, NF-κB P65, and IκB; the recorded percentage changes were -60.83%, -51.85%, and -36.95% as a consequence of BM-MSCs, and -64.48%, -63.21%, and -48.47% as a consequence of CD-MSCs. The most effective treatment for reducing NF-κB P65 and IκB protein expression seemed to be CD-MSCs. Furthermore, there was no discernible difference between BM-MSCs and CD-MSCs in how they affected NF-κB P50 protein expression (**Figure 6**).

Effect on MMP-3 and MMP-9 protein expression: In MIA-induced osteoarthritic rats, ankle tissue MMP-3 and MMP-9 protein expression significantly ($P < 0.05$) increased, with percentage changes of +298.9% and +314.42%, respectively, compared to the normal control group. The administration of BM-MSCs and CD-MSCs to MIA-induced osteoarthritic rats potentially reduced ($P < 0.05$) the changed protein expression of MMP-3 and MMP-9; the corresponding percentage changes were -57.95% and -64.97% for BM-MSCs and -69.54% and -66.59% for CD-MSCs, respectively. The most effective treatment for reducing MMP-3 protein expression seemed to be CD-MSCs. Moreover,

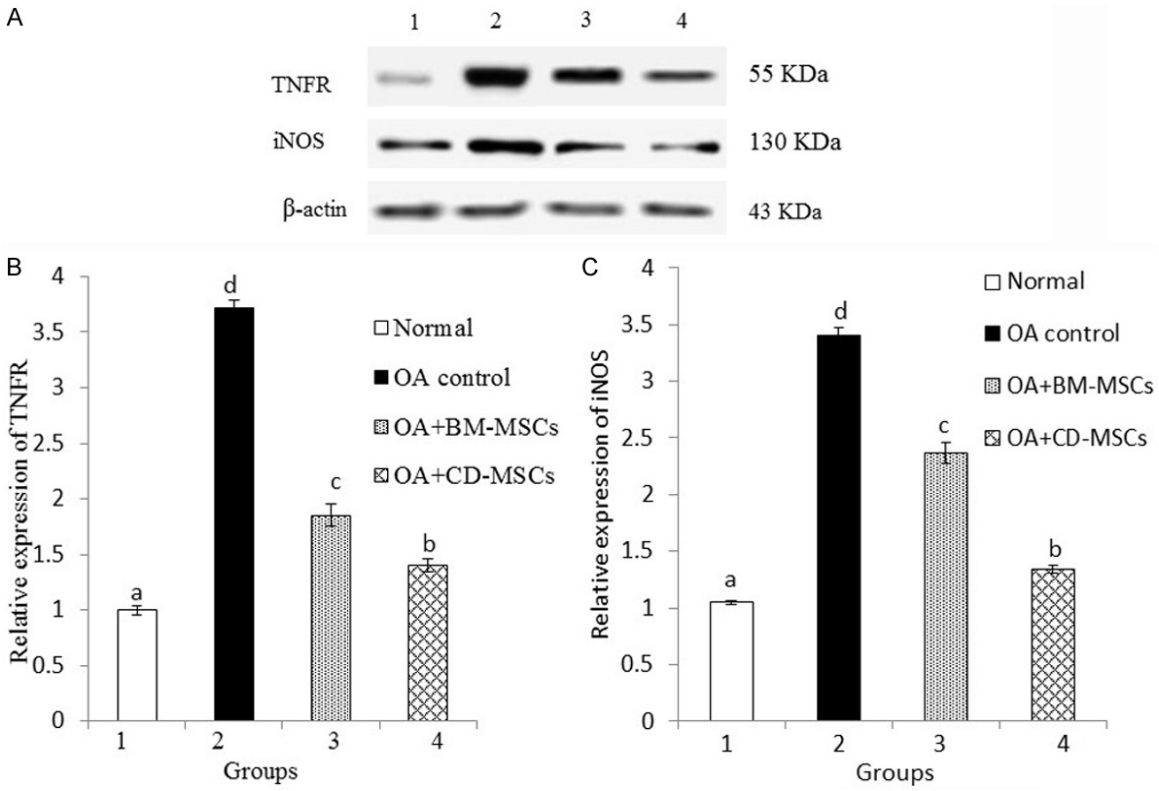

Figure 5. Effect of BM-MSCs and CD-MSCs on ankle tissue TNFR and iNOS protein expressions in MIA-induced osteoarthritic rats. A: Immunoblots of TNFR and iNOS protein expressions; B: Statistical analysis result of TNFR protein expression; C: Statistical analysis result of iNOS protein expression. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; TNFR: tumour necrosis factor receptor; iNOS: inducible nitric oxide synthase.

there was no significant difference between the effects of BM-MSCs and CD-MSCs on MMP-9 activity (**Figure 7**).

Histological and immunohistochemical changes of joint

The histological findings in ankle joint sections from the four experimental groups are depicted in **Figure 8**. Normal control rats (NC) had normal bone, cartilage, and fibrous joint capsule histological structure and no inflammation when examined in hematoxylin and eosin stained sections of the ankle joints (**Figure 8A** and **8B**). However, stained sections of osteoarthritic control rats revealed synovial hyperplasia with infiltration of numerous inflammatory cells (lymphocytes, macrophages, and occasionally plasma cells), substantial pannus development, and severe cartilage and bone degradation as the major histopathological alterations (**Figure 8C** and **8D**). Contrarily, moderate (++) osteoarthritis pathology was present in osteoarthritic rats treated with BM-MSCs (**Figure 8E** and **8F**) and CD-MSCs (**Figure 8G** and **8H**).

Immunostaining of sections of the cartilage, synovial membrane of the ankle joint of OA rats with anti-TGF-β1 showed strong expression of brown color density (**Figure 9C**; ×100 and **9D**; ×400) as compared with the normal control (**Figure 9A**; ×100 and **9B**; ×400), while it showed weak expression of brown color density in cells obtained from OA rats treated with BM-MSCs (**Figure 9E**; ×100 and **9F**; ×400) and CD-MSCs (**Figure 9G**; ×100 and **9H**; ×400) in comparison with cells of OA rats (**Figure 9C**; ×100 and **9D**; ×400). The image analysis of the intensity of brown colour indicated a significant increase ($P < 0.05$) in OA rats in comparison with the normal control and a significant

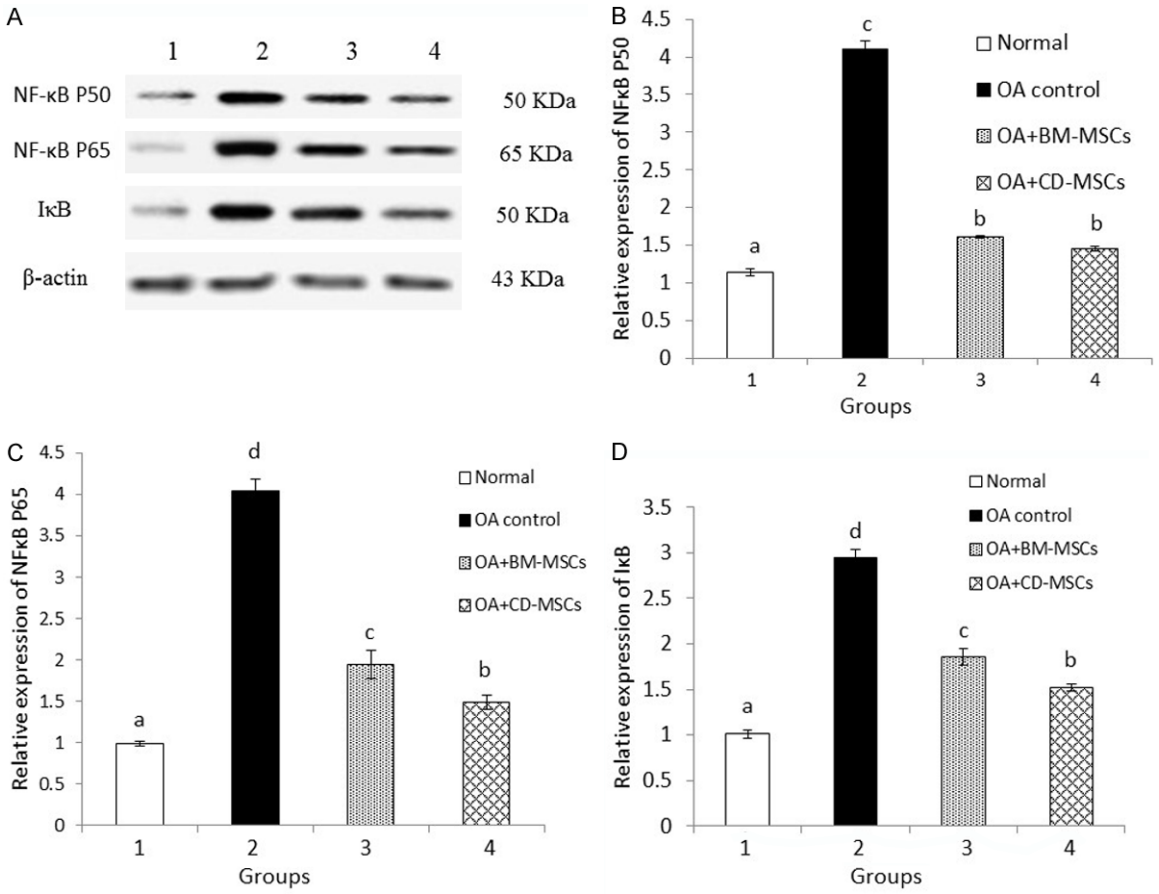

Figure 6. Effect of BM-MSCs and CD-MSCs on ankle tissue NF-κB P50, NF-κB P65, and IκB protein expressions in MIA-induced osteoarthritic rats. A: Immunoblots of NF-κB P50, NF-κB P65, and IκB protein expressions; B: Statistical analysis result of NF-κB P50 protein expression; C: Statistical analysis result of NF-κB P65 protein expression; D: Statistical analysis result of IκB protein expression. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; NF-κB P50: nuclear factor-κB p50; NF-κB P65: nuclear factor-κB p65; IκB: nuclear factor of kappa light polypeptide.

decrease ($P < 0.05$) in OA rats treated with BM-MSCs and CD-MSCs compared to OA controls (**Figure 10**).

Discussion

In the current investigation, MIA was injected intra-articularily to create an osteoarthritis model [30]. A common experimental model for preclinical studies is OA brought on by MIA. This model is frequently used to evaluate curative agents since testing is quick, it's easy to use, and it's similar to both animal and human OA [33]. The inhibitory effect of MIA on glyceraldehydes-3 phosphate dehydrogenase activity in chondrocytes disrupts glycolysis, decreases the production of proteoglycans, and ultimately results in cell death [34].

Here, the anterioposterior diameter of the right hind ankle was measured once a week for three weeks as a measure of joint swelling, allowing for the monitoring of both the progression of the disease and the effects of the tested medications. By the third week of the experiment, the arthritic control rats had anteroposterior diameters that were significantly larger than those of the normal rats at all points of observation. These results concur with those of Jimbo *et al*. [22], Cottom and Maker [35], Jimbo *et al*. [30], and Ahmed *et al.* [13]. However, the elevated values in the arthritic control rats were effectively lessened in the BM-MSCs and CD-MSCs-treated rats, and they were close to normal ranges. This research supports the findings of Ahmed *et al.* [13]. In our opinion, a significant decline of the right ankle anteroposte-

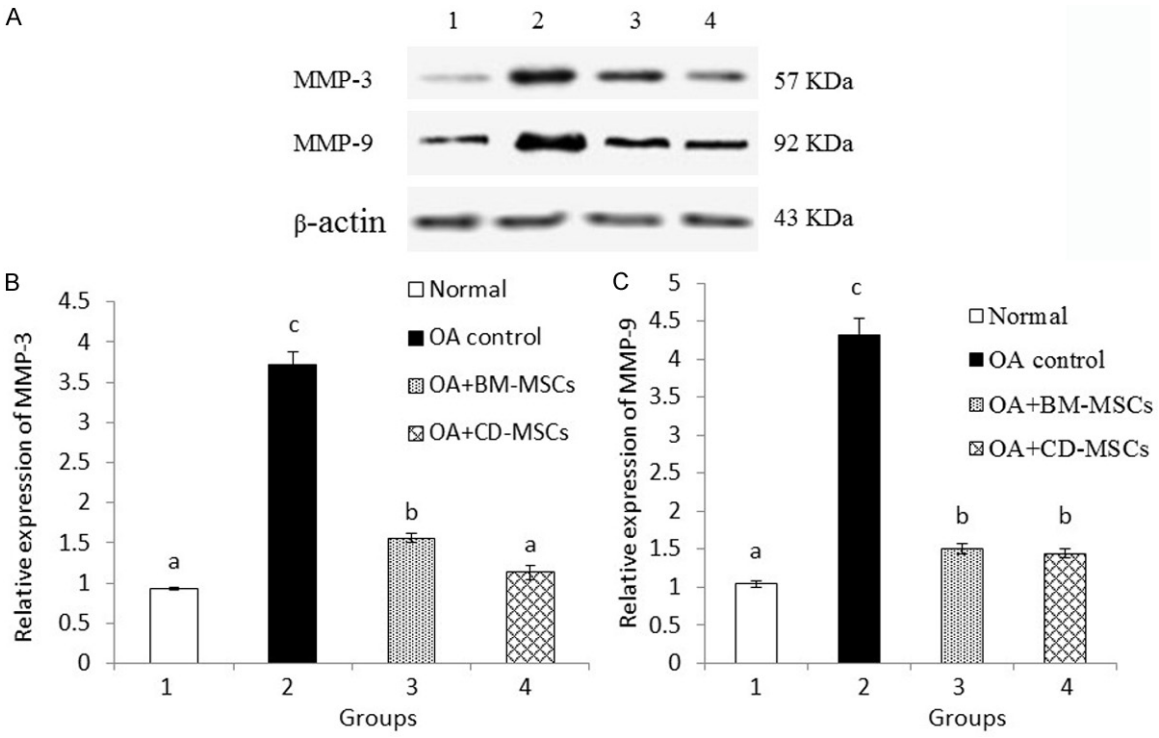

Figure 7. Effect of BM-MSCs and CD-MSCs on ankle tissue MMP-3 and MMP-9 protein expressions in MIA-induced osteoarthritic rats. A: Immunoblots of MMP-3 and MMP-9 protein expressions; B: Statistical analysis result of MMP-3 protein expression; C: Statistical analysis result of MMP-9 protein expression. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; MMP-3: matrix metalloproteinase-3; MMP-9: matrix metalloproteinase-9.

rior diameter of the OA treated rats in contrast to the arthritic rats is attributed to the BM-MSCs and CD-MSCs anti-inflammatory effects, which was evidenced by the present study.

Edema, which results from fluid leaking from blood vessel endothelial cells into the inflamed synovium, is what causes joint swelling, which is common in many types of arthritis [36]. The raised values of the diameter after MIA administration were significantly reduced as a result of the intra-injection of BM-MSCs. Similar to this, BM-MSC therapy reduced knee swelling, according to a study by Kehoe *et al*. [37]. This effect was attributed to changes in the synovial endothelial cells' permeability to soluble MSCs-produced substances.

According to recent research, when compared to the healthy control rats, the osteoarthritic group had significantly higher serum levels of ACPA. These findings are in line with those of Ahmed *et al*. [13], who showed that sera from arthritic control rats had noticeably higher ACPA levels than those from the normal group. However, BM-MSCs or CD-MSCs treatment for OA rats resulted in a considerable drop in serum ACPA levels compared to the OA control group. These findings are consistent with Ahmed *et al*. [13]. These outcomes are in line with those reported by ElBatsh *et al*. [19], who claimed that microscopic analysis of joints injected with differentiated MSCs revealed reasonable cartilage surface. The surface area was smooth, showing no signs of inflammation, and had cartilage that was essentially identical in thickness to that of a normal ankle. These alterations take place as a result of differentiated MSCs that produce an abundance of extracellular matrix made up of molecules specific to cartilage, including aggrecan, type II collagen, and cartilage oligomeric matrix protein (PGs), which are essential components of cartilage tissue [38]. We believe that CD-MSCs have an anti-inflammatory impact since the rats supplemented with CD-MSCs showed a considerable reduction in the level of ACPA in their serum when compared to the arthritic rats (**Figure 11**).

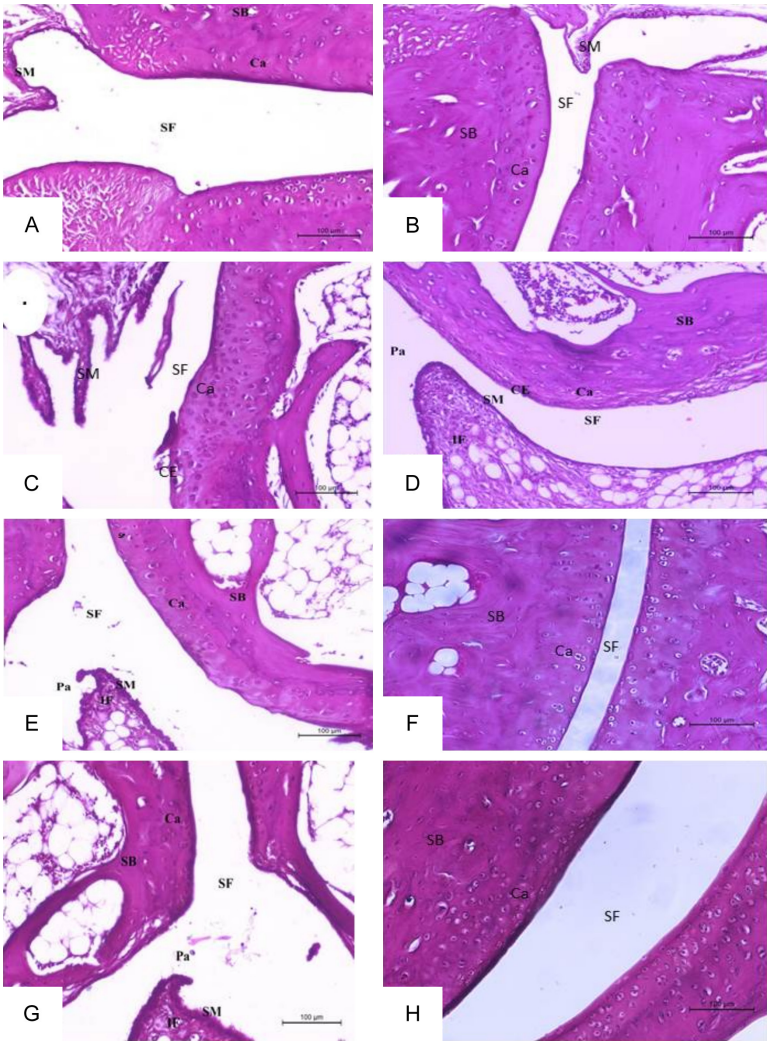

Figure 8. Photomicrographs of normal control rats (A, B), osteoarthritic rats (C, D), osteoarthritic rats plus BM-MSCs rats (E, F), and osteoarthritic rats plus CD-MSCs rats (G, H) show sections of the hind right leg ankle stained with H&E (×100). The photomicrographs of the hind ankle joints of the rats treated with BM-MSCs and CD-MSCs (C and D) showed moderate arthritic pathology. BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; Ca: cartilage; CE: cartilage erosion; SB: sponge bone; Pa: pannus; IF: inflammatory cells; SF: synovial fluid; SM: synovial membrane.

The pathophysiological mechanisms of arthritis are significantly influenced by TNF-α, a pro-inflammatory Th1 cytokine [39, 40]. Osteoclast differentiation and activation may be amplified by TNF-α and IL-17, which could lead to synovial hyperplasia, angiogenesis, cartilage degradation, and bone injury (**Figure 11**) [40]. The anti-inflammatory properties of Th2 cytokines, such as IL-4 and IL-10, on the other hand, lead to an improvement in arthritis and inflammation when their levels are raised [12, 13].

The osteoarthritic group in the current study displayed a pronounced increase in blood levels of TNF-α and IL-17 and a significant decrease in serum levels of IL-10, indicating the development of inflammation in the rat joints. The results of Li *et al*. [41] and Abo-Aziza *et al*. [42] are all in agreement with this research. These elevated levels of pro-inflammatory cytokines may reflect their pivotal function in the pathophysiology of arthritis progression in animal models [43]. The MIA injection increases pro-inflammatory cytokines while decreasing anti-inflammatory cytokines, which supports the growth of the inflammatory response. The creation and release of growth factors (GFs), such as transforming growth factor (TGF), may also be inhibited by the inflammatory environment and the elevated levels of TNF-α and IL-17. This could reduce chondrogenesis and the development of chondrocytes from mesenchymal stem cells (**Figure 11**) [44].

Contrarily, when compared to the elevated levels of the osteoarthritic control animals, OA rats treated with BM-MSCs and CD-MSCs showed a significant reduction in serum TNF-α and IL-17 levels while exhibiting a significant elevation in the reduced serum IL-10 level. These results support those of Ragab *et al*. [45]. Strong agreement exists between the results of the current investigation and those reported by Abo-Aziza *et al*. [42], who found a significant drop in serum TNF-α levels at weeks 2 and 4, respectively, of BM-MSC therapy. In contrast, the level of IL-10 didn't start to rise considerably until week 4 after transplantation. These findings support Li *et al*. [41], who reported that MSCs have been shown to inhibit the development of immature CD4+ T

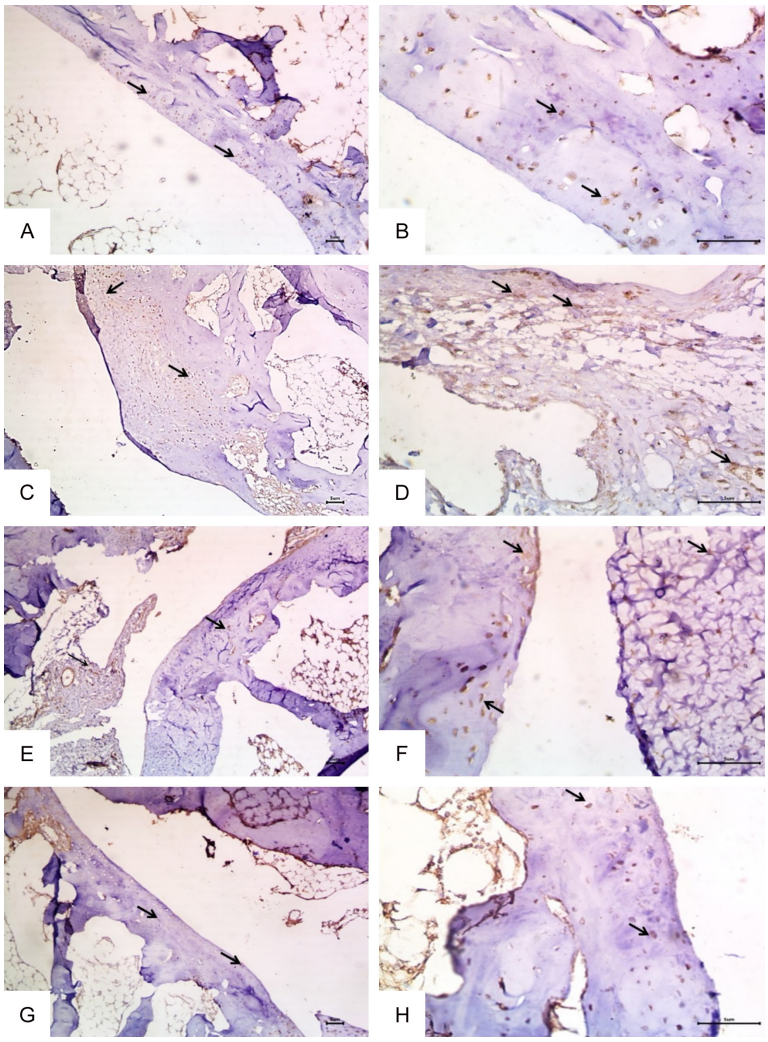

Figure 9. Photomicrographs of normal control rats (A, B), osteoarthritic rats (C, D), osteoarthritic rats plus BM-MSCs rats (E, F), and osteoarthritic rats plus CD-MSCs rats (G, H) show sections of the hind right leg ankle stained immunohistochemically. The photomicrographs of normal ankle (A; ×100 and B; ×400) exhibited weak expression of TGF-β1. The photomicrographs of the hind ankle joints of the rats treated with BM-MSCs (E; ×100 and F; ×400) and CD-MSCs (G; ×100 and H; ×400) showing the lower amount of TGF-β1 (arrow; brownish yellow color) in the cytoplasm and nuclei of cells than in arthritic rats (C; ×100 and D; ×400). BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs.

cells into Th17 cells *in vitro*. In addition, they might lessen the production of IL-17 and the activation of Th17 cells. These outcomes are also consistent with Chang *et al*. [46], who found that MSC-differentiated chondrocytes reduced many inflammatory markers like TNF-α (**Figure 11**).

According to the results of the current study, arthritic rats' ankle joints express considerably more TNFR than normal rats do. TNF-α stimulates the death receptors or TNFRs in addition to its necrotic actions, triggering the extrinsic cascade of apoptosis [47, 48].

As opposed to the osteoarthritic control group, the osteoarthritic rats treated with BM-MSCs or CD-MSCs showed a significantly lower level of TNFR expression in the tissues of the ankle joint.

When compared to the normal control rats in the current investigation, the serum GPx and GR activities in the MIA-induced osteoarthritic rats were dramatically reduced, while the serum GSSG level was noticeably raised. These findings also agree with those of Ahmed *et al*. [12] and Ragab *et al*. [45]. According to Lepetsos and Papavassiliou [49], ROS inhibits the production of ATP and mitochondrial oxidative phosphorylation in cultured chondrocytes, which ultimately reduces the production of collagen and proteoglycans and causes cartilage breakdown.

Contrarily, intravenous treatment of BM-MSCs and CD-MSCs to MIA-induced osteoarthritic rats resulted in a considerable decrease in the blood GSSG level while a marked increase in the GPx and GR activity. It has been shown that MSCs promote chondrogenesis by regenerating the ECM of articular cartilage [50]. Injections of BM-MSC reduced the loss of collagen type II in OA joints. Ahmed *et al*. [12] postulated that BM-MSCs would boost the antioxidant defense system at the expense of oxidative stress in tissues, preventing the subsequent inflammatory process (**Figure 11**). This outcome is consistent with studies by Chang *et al*. [51] who discovered that MSC therapy raised the GPx expression in diseases such Friedreich's ataxia, severe acute pancreatitis, and small intestinal

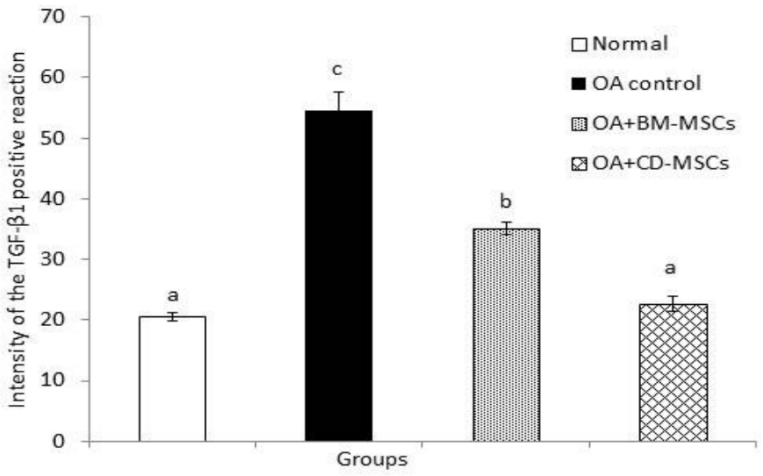

Figure 10. Effect of treatment with BM-MSCs and CD-MSCs on intensity of the TGF-β1 positive reaction in MIA-induced osteoarthritic rats. The means, each of which has a unique symbol (letter), differ significantly from one another at $P < 0.05$ for each period. OA: osteoarthritis; BM-MSCs: bone marrow-derived mesenchymal stem cells; CD-MSCs: chondrogenic differentiated MSCs; TGF-β1: transforming growth factor-beta 1.

ischemia/reperfusion (I/R) injury. The significantly greater GPx and GR activity in the treated rats' serum when compared to the arthritic rats supports our hypothesis that CD-MSCs have an anti-oxidative effect (**Figure 11**).

The secretome, a collection of paracrine molecules released by MSCs, contains a wide range of proteins with a variety of biological properties, such as immunological modulation, anti-apoptotic effects, and antioxidative properties. The ability of MSCs and their secretome to scavenge free radicals, activate the antioxidant defense system, and change cellular bioenergetics is what is thought to be responsible for their antioxidative actions [52]. Additionally, ROS generation can be inhibited and oxidative stress decreased by MSC immunosuppressive abilities. In recent studies, BM-MSCs improved antioxidant activity and reduced oxidative stress in rats with severe acute pancreatitis by causing nuclear translocation of Nrf-2, a newly discovered regulator of cellular resistance to oxidants, via the PI3K/AKT signalling pathway [53].

The NF-κB pathway plays a critical role in cartilage breakdown, MMP modulation, and inflammation associated with chondrocytes [54]. Many inflammatory signalling pathways, such as nuclear factor-kappa B (NF-κB), have been connected to the regulation of OA [55] as a result of the rising inflammatory milieu that injured cartilage is subjected to [56]. An NF-κB classical-canonical pathway can be activated in chondrocytes and synoviocytes of articular joints by mechanical stress or cytokines (IL-1β and TNF-α). The process begins with the activation of IB kinase (IKK), which causes the proteasome to phosphorylate and degrade IB. Next, NF-κB p65 and NF-κB p50 protein are liberated and moved from the cytoplasm to the nucleus [57]. A variety of inflammation-related substances, such as inducible nitric oxide synthase (iNOS), matrix metalloproteinase proteins, IL-6, IL-1β, and TNF-α, are then produced by activated chondrocytes and synoviocytes, and these cytokines further activate the signalling cascade [58] (**Figure 11**). According to our findings, NF-κB p50 and NF-κB p65 were strongly inhibited by BM-MSCs and CD-MSCs in OA rats. These results are in line with those of Wang *et al*. [59] and Mancuso *et al*. [60]. These findings support a study by Murakami *et al*. [61] who found MSCs reduced the expression of NF-κB p65 in activated macrophages and chondrocytes, which in turn reduced the release of inflammatory cytokines. In a study published in 2018, Wang *et al*. [62] proposed that MSC injection could reduce excessive TNF-α, an NF-κB activator, and prevent the phosphorylation of the NF-κB p65 subunit in spinal cord injury.

Our data from the current study showed that the IκB protein level was much lower in osteoarthritic rats than in normal rats. These findings concur with those of Murakami *et al.* [61]. The IκB protein level increased noticeably in the osteoarthritic rats treated with BM-MSCs and CD-MSCs in contrast to the untreated osteoarthritic rats. These findings support the findings of Murakami *et al*. [61], who found that MSCs increased the expression of IκBα in activated macrophages and chondrocytes, which in turn

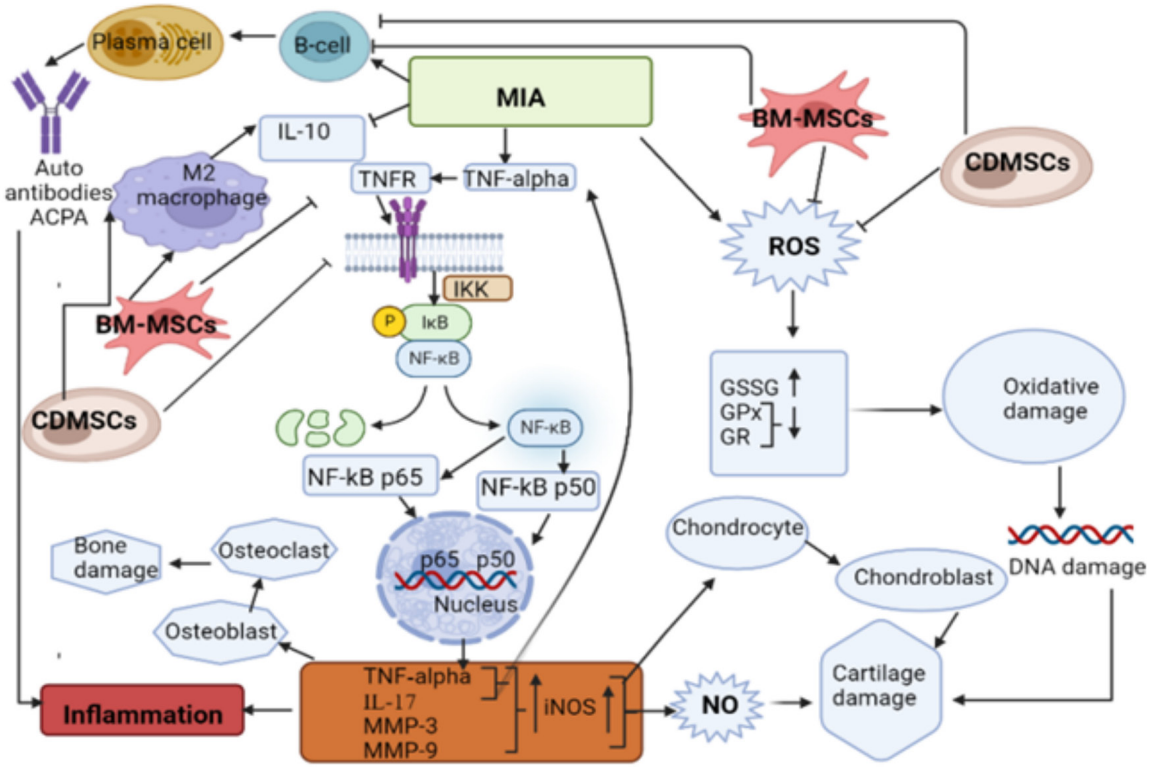

Figure 11. Schematic figure illustrating how BM-MSCs and CD-MSCs work in rats with osteoarthritis. MIA: monoiodoacetate; BM-MSCs: Bone marrow-derived mesenchymal stem cells; CD-MSCs: Chondrogenic differentiated mesenchymal stem cells; TNF-α: tumour necrosis factor-α; IL-10: interleukin-10; IL-17: interleukin-17; ROS: Reactive oxygen species; GSSG: reduced glutathione; GPx: Glutathione peroxidase; GR: Glutathione reductase; MMP-3: matrix metalloproteinase-3; MMP-9: matrix metalloproteinase-9; iNOS: inducible nitric oxide synthase; TNFR: Tumour necrosis factor receptor; NF-κB: nuclear factor-κB; IκB: nuclear factor of kappa light polypeptide; ACPA: Anticitrullinated protein antibodies; NO: nitric oxide.

reduced the production of the inflammatory cytokine IL-1β.

MMP-3, MMP-9, and MMP-13 are members of the family of inflammatory mediators known as matrix metalloproteinases (MMPs), which promote cartilage injury and ECM breakdown [63]. Our findings showed that the levels of the proteins MMP-3 and MMP-9 were much higher in the ankle joint tissues of osteoarthritic rats than in normal rats. These findings concur with Li *et al*. [64], who reported that MMP-9 was shown to be considerably elevated in OA, as well as with WANG and CAI [65] and Ahmed *et al*. [13]. Additionally, some investigations have shown that the presence of TNF-α reduced the expression of type II collagen and aggrecan in chondrocytes, which in turn stimulated the release of MMP-3 [66]. According to Liacini *et al*. [67] and Vincenti and Brinckerhoff [68], NF-κB and MAP kinases are involved in the induction of MMP-1, MMP-3, and MMP-13 RNA and protein expression by TNF-α or IL-1β (**Figure 11**). ROS may also have more indirect effects on the degradation of the extracellular matrix (ECM), such as increasing the expression of genes encoding metalloproteinases (MMPs), while NO controls the activation of metal-dependent proteases in articular chondrocytes and cartilage [69]. As opposed to the osteoarthritic rats, those treated with BM-MSCs and CD-MSCs showed a significant reduction in the levels of the MMP-3 and MMP-9 proteins. Our findings concur with those of Ahmed *et al*. [13], who demonstrated that BM-MSCs were successful in reducing MMP-9 expression. These findings concur with Chang *et al*. [46] who revealed that the injection of stromal cells and chondrocytes in the KOA model resulted in lower expressions of inflammatory and catabolic markers.

NF-κB signalling has also been connected to the expression of OA mediators that promote

inflammation and damage, such as the iNOS gene [70]. Nitric oxide (NO) is thought to be produced by an enzyme known as iNOS. By altering ECM homeostasis and cytokine expression, excessive NO generation by iNOS is thought to contribute to the pathogenesis of OA [52]. This leads to oxidative damage and chondrocyte death.

In our research, the injection of BM-MSCs and CD-MSCs dramatically decreased the expression of inducible iNOS in the osteoarthritic joints. According to Hamilton *et al*. [71], intra-articular injection of MSC may prevent the development of M1 macrophages by downregulating the amount of iNOS in macrophages.

Rats in the osteoarthritic control group showed significant histopathological changes three weeks after MIA administration, including severe cartilage and bone damage along with synovial hyperplasia, widespread pannus development, and infiltration of many inflammatory cells (lymphocytes, macrophages, and plasma cells) in H&E stained ankle joint tissue. These results corroborate those of Moqbel *et al*. [72], who reported erosion, loss of cartilage matrix, and synovium hyperplasia in the cartilage of the OA group. In earlier investigations, same behaviours were also demonstrated [46, 73].

The damaging effects of an inflammatory synovial pannus were hypothesized to be the cause of cartilage damage in the osteoarthritic group. The regions of contact with pannus tissue, which breaks down cartilage by enhancing proteolytic activity, are where cartilage erosion typically begins. Additionally, synovial fibroblasts have the potential to enter the articular cartilage of the affected joint or release damaging enzymes into the synovial fluid [74]. When mononuclear cells infiltrate the subintima of the synovium, they release substances like TNF-α, IL-1, and IL-6 that bind to the receptors on chondrocytes and cause the release of MMPs that can break down every component of the extracellular matrix as well as inhibit the production of type II collagen, which accelerates cartilage breakdown [75]. However, the previously stated histological lesions of arthritis were only moderately present in the rats from both treated osteoarthritic groups (BM-MSCs and CD-MSCs).

Other studies [19, 76] also noted the striking improvement in cartilage and synovium structure after MSCs treatment. This effect may result from the differentiation of stem cells into tissue cells, restoring lost morphology and function, or from the secretion of a variety of bioactive growth factors, including FGF, EGF, PDGF, and VEGF, which promote the growth of endothelial progenitor cells and create a repair environment with anti-apoptotic effects [77]. In our judgement, the treated rats' joint tissue was better in the CD-MSC-supplemented rats than it was in the arthritic rats.

TGF-β1 is a component of the transforming growth factor-β (TGF-β) superfamily of cytokines contributing to various cellular responses, such as apoptosis, proliferation, differentiation, and extracellular matrix production [78]. TGF-β1 is essential for the induction of arthritis related fibrosis [79].

Even though TGF-β signaling has a principal role in cartilage development and in maintaining articular chondrocyte homeostasis in synovial joints, in the present study, TGF-β is potentially involved in joint degeneration. The present immunohistochemical stained sections indicated that TGF-β potentially increased in the arthritic rats as compared to normal control. Similarly, a study by Dranitsina et al. [80] revealed that MIA-OA causes an increase in the expression of TGF-β1 genes in rat cartilage cells. Our results, in contrast to Halfaya et al. [81], indicated a significant rise in the level of TGF-β in OA rat joints compared with that of the control group [82]. Van der Kraan [83] postulated that an elevation of the TGF-β level could activate inflammation that may be involved in OA pathogenesis by altering cellular differentiation and causing joint deterioration. Additionally, studies have reported that TGF-β signaling mediated by Smad2/3 may be involved in OA progression by inducing the recruitment of MSCs and osteoprogenitors to the subchondral bone, ending with aberrant bone remodeling that initiates and worsens osteoarthritis. Nevertheless, the activation and catabolic role of TGF-β in OA requires further investigation [84].

Additionally, our outcomes are consistent with Wei et al. [85] and Ahmed et al. [13] who revealed that BM-MSCs successfully lowered the expression level of TGF-β1 as compared to arthritic rats. On the contrary, a compensatory anti-inflammatory response is observed in synovial membranes [13].

Our results suggest that BM-MSC and CD-MSC therapy regulates and decreases OA-induced inflammation, delays cartilage degeneration, and improves cartilage regeneration through paracrine activity. This is based on biochemical, molecular, histological, and immunohistochemical outcomes [86]. Despite the fact that the study's objectives were met, there were some possible drawbacks, including the fact that the relevant mechanism underlying the effects of BM-MSCs and CD-MSCs on OA has not yet been further confirmed because of an insufficiency of data and inability to predict the pathway and safety in clinical studies.

Overall, the underlying mechanisms of BM-MSCs and CD-MSCs as a treatment for cartilage degeneration in an MIA-induced OA rat model were illustrated and summarized in **Figure 11**.

Conclusion

The findings of the current study demonstrated that over a three-week period, intravenous injections of BM-MSCs and CD-MSCs dramatically improved the biochemical, molecular, and histological outcomes of rats with OA caused by MIA. However, the process of cartilage regeneration likely takes long time to develop. The impact of BM-MSCs and CD-MSCs on the development of OA should therefore be investigated over a long period of time.

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Disclosure of conflict of interest

None.

Abbreviations

ECM, extracellular matrix; OA, Osteoarthritis; MIA, monoiodoacetate; MSC, Mesenchymal stem cell; BM-MSCs, Bone marrow-derived mesenchymal stem cells; CD-MSCs, Chondrogenic differentiated mesenchymal stem cells; TNF-α, tumour necrosis factor-α; IL-10, interleukin-10; IL-17, interleukin-17; ELISA, enzyme-linked immunosorbent assay; ROS, Reactive oxygen species; GSSG, reduced glutathione; GPx, Glutathione peroxidase; GR, Glutathione reductase; MMP-3, matrix metalloproteinase-3; MMP-9, matrix metalloproteinase-9; iNOS, inducible nitric oxide synthase; TNFR, Tumour necrosis factor receptor; NF-κB, nuclear factor-κB; IκB, nuclear factor of kappa light polypeptide; SPSS, Statistical Package of Social Sciences; ACPA, Anticitrullinated protein antibodies.

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References

[1] Liu W, Wang H, Su C, Kuang S, Xiong Y, Li Y and Gao S. The evaluation of the efficacy and safety of oral colchicine in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trails. Biomed Res Int 2022; 2022: 2381828.

[2] Kloppenburg M and Berenbaum F. Osteoarthritis year in review 2019: epidemiology and therapy. Osteoarthritis Cartilage 2020; 28: 242-8.

[3] Vincent TL. Of mice and men: converging on a common molecular understanding of osteoarthritis. Lancet Rheumatol 2020; 2: e633-e645.

[4] AlKuwaity KW, Mohammad TN, Hussain MA, Alkhanani AJ and Ali AMB. Prevalence and determinant factors of osteoarthritis of the knee joint among elderly in Arar, KSA. Egypt J Hosp Med 2018; 72: 5173-5177.

[5] Mohammadinejad R, Ashrafizadeh M, Pardakhty A, Uzieliene I, Denkovskij J, Bernotiene E, Janssen L, Lorite GS, Saarakkala S and Mobasheri A. Nanotechnological strategies for osteoarthritis diagnosis, monitoring, clinical management, and regenerative medicine: recent advances and future opportunities. Curr Rheumatol Rep 2020; 22: 12.

[6] Mi B, Wang J, Liu Y, Liu J, Hu L, Panayi AC, Liu G and Zhou W. Icariin activates autophagy via down-regulation of the NF-κB signaling-mediated apoptosis in chondrocytes. Front Pharmacol 2018; 9: 605.

[7] Korotkyi O, Kyriachenko Y, Kobyliak N, Falalyeyeva T and Ostapchenko L. Crosstalk between gut microbiota and osteoarthritis: a critical view. J Funct Foods 2020; 68: 103904.

[8] Cooper C, Chapurlat R, Al-Daghri N, Herrero-Beaumont G, Bruyère O, Rannou F, Roth R, Uebelhart D and Reginster JY. Safety of oral non-selective non-steroidal anti-inflammatory drugs in osteoarthritis: what does the literature say? Drugs Aging 2019; 36 Suppl 1: 15-24.
[9] Wang Q, Lepus CM, Raghu H, Reber LL, Tsai MM, Wong HH, von Kaeppler E, Lingampalli N, Bloom MS, Hu N, Elliott EE, Oliviero F, Punzi L, Giori NJ, Goodman SB, Chu CR, Sokolove J, Fukuoka Y, Schwartz LB, Galli SJ and Robinson WH. IgE-mediated mast cell activation promotes inflammation and cartilage destruction in osteoarthritis. Elife 2019; 8: e39905.
[10] de Sousa Valente J. The pharmacology of pain associated with the monoiodoacetate model of osteoarthritis. Front Pharmacol 2019; 10: 974.
[11] Zahan OM, Serban O, Gherman C and Fodor D. The evaluation of oxidative stress in osteoarthritis. Med Pharm Rep 2020; 93: 12-22.
[12] Ahmed EA, Ahmed OM, Fahim HI, Mahdi EA, Ali TM, Elesawy BH and Ashour MB. Combinatory effects of bone marrow-derived mesenchymal stem cells and indomethacin on adjuvant-induced arthritis in Wistar rats: roles of IL-1β, IL-4, Nrf-2, and oxidative stress. Evid Based Complement Alternat Med 2021; 2021: 8899143.
[13] Ahmed EA, Ahmed OM, Fahim HI, Ali TM, Elesawy BH and Ashour MB. Potency of bone marrow-derived mesenchymal stem cells and indomethacin in complete Freund's adjuvant-induced arthritic rats: roles of TNF-α, IL-10, iNOS, MMP-9, and TGF-β1. Stem Cells Int 2021; 2021: 6665601.
[14] Ahmed RH, Galaly SR, Moustafa N, Ahmed RR, Ali TM, Elesawy BH, Ahmed OM and Abdul-Hamid M. Curcumin and mesenchymal stem cells ameliorate ankle, testis, and ovary deleterious histological changes in arthritic rats via suppression of oxidative stress and inflammation. Stem Cells Int 2021; 2021: 3516834.
[15] Hsu YK, Sheu SY, Wang CY, Chuang MH, Chung PC, Luo YS, Huang JJ, Ohashi F, Akiyoshi H and Kuo TF. The effect of adipose-derived mesenchymal stem cells and chondrocytes with platelet-rich fibrin releasates augmentation by intra-articular injection on acute osteochondral defects in a rabbit model. Knee 2018; 25: 1181-1191.
[16] Xia T, Yu F, Zhang K, Wu Z, Shi D, Teng H, Shen J, Yang X and Jiang Q. The effectiveness of allogeneic mesenchymal stem cells therapy for knee osteoarthritis in pigs. Ann Transl Med 2018; 6: 404.
[17] Mousa F, Abdel-Aziz KK, Abdel Gawad H, Mahmoud SS and Elgamel MS. Bone marrow-derived mesenchymal stem cells infusion ameliorates hyperglycemia, dyslipidemia, liver and kidney functions in diabetic rats. Int J Sci Res 2016; 5: 1624-1631.
[18] Macrin D, Joseph JP, Pillai AA and Devi A. Eminent sources of adult mesenchymal stem cells and their therapeutic imminence. Stem Cell Rev Rep 2017; 13: 741-756.
[19] ElBatsh MM, Eledel R, Noreldin RI, Mohamed AS and Omar TA. Effect of intra-articular injection of chondrocytes differentiated from mesenchymal stem cells in monosodium iodoacetate induced osteoarthritis in male rats. Bull Egypt Soc Physiol Sci 2020; 41: 140-154.
[20] Lai RC, Yeo RW and Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol 2015; 40: 82-88.
[21] Kristjánsson B and Honsawek S. Current perspectives in mesenchymal stem cell therapies for osteoarthritis. Stem Cells Int 2014; 2014: 194318.
[22] Jimbo S, Terashima Y, Takebayashi T, Teramoto A and Ogon I. A novel rat model of ankle osteoarthritis induced by the application of monoiodoacetate. Br J Med Med Res 2017; 6: 2.
[23] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S and Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-147.
[24] Aggarwal S and Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005; 105: 1815-1822.
[25] Chaudhary JK and Rath PC. A simple method for isolation, propagation, characterization, and differentiation of adult mouse bone marrow-derived multipotent mesenchymal stem cells. J Cell Sci Ther 2017; 8: 261.
[26] Ahmed OM, Hassan MA and Saleh AS. Combinatory effect of hesperetin and mesenchymal stem cells on the deteriorated lipid profile, heart and kidney functions and antioxidant activity in STZ-induced diabetic rats. Biocell 2020; 44: 27-39.
[27] Solchaga LA, Penick KJ and Welter JF. Chondrogenic differentiation of bone marrow-derived mesenchymal stem cells: tips and tricks. Methods Mol Biol 2011; 698: 253-78.
[28] Kalamegam G, Abbas M, Gari M, Alsehli H, Kadam R, Alkaff M, Chaudhary A, Al Qahtani M, Abuzenadah A, Kafienah W and Mobasheri A. Pelleted bone marrow derived mesenchymal stem cells are better protected from the deleterious effects of arthroscopic heat shock. Front Physiol 2016; 7: 180.
[29] van Buul GM, Siebelt M, Leijs MJ, Bos PK, Waarsing JH, Kops N, Weinans H, Verhaar JA,

Bernsen MR and van Osch GJ. Mesenchymal stem cells reduce pain but not degenerative changes in a mono-iodoacetate rat model of osteoarthritis. J Orthop Res 2014; 32: 1167-1174.
[30] Jimbo S, Terashima Y, Teramoto A, Takebayashi T, Ogon I, Watanabe K, Sato T, Ichise N, Tohse N and Yamashita T. Antinociceptive effects of hyaluronic acid on monoiodoacetate-induced ankle osteoarthritis in rats. J Pain Res 2019; 12: 191-200.
[31] Suvarna KS, Layton C and Bancroft JD. Bancroft's Theory and Practice of Histological Techniques E-Book. London, UK: Elsevier Health Sciences; 2017.
[32] Duncan BD. Multiple range test for correlated and heteroscedastic means. Biometrics 1957; 13: 359-64.
[33] South S, Crabtree K, Vijayagopal P, Averitt D and Juma S. Dose dependent effects of whole blueberry on cartilage health and pain in a monosodium iodoacetate (MIA) induced rat model of osteoarthritis. Curr Dev Nutr 2020; 4: 477.
[34] Beyreuther B, Callizot N and Stohr T. Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther 2007; 9: R14.
[35] Cottom JM and Maker JM. Cartilage allograft techniques and materials. Clin Podiatr Med Surg 2015; 32: 93-98.
[36] Middleton J, Americh L, Gayon R, Julien D, Aguilar L, Amalric F and Girard JP. Endothelial cell phenotypes in the rheumatoid synovium: activated, angiogenic, apoptotic and leaky. Arthritis Res Ther 2004; 6: 60-72.
[37] Kehoe O, Cartwright A, Askari A, El Haj AJ and Middleton J. Intra-articular injection of mesenchymal stem cells leads to reduced inflammation and cartilage damage in murine antigen-induced arthritis. J Transl Med 2014; 12: 157.
[38] Nam Y, Rim YA, Jung SM and Ju JH. Cord blood cell-derived iPSCs as a new candidate for chondrogenic differentiation and cartilage regeneration. Stem Cell Res Ther 2017; 8: 16.
[39] Zhang C. Flare-up of cytokines in rheumatoid arthritis and their role in triggering depression: shared common function and their possible applications in treatment (review). Biomed Rep 2021; 14: 16.
[40] Farrugia M and Baron B. The role of TNF-α in rheumatoid arthritis: a focus on regulatory T cells. J Clin Transl Res 2016; 2: 84-90.
[41] Li F, Li X, Liu G, Gao C and Li X. Bone marrow mesenchymal stem cells decrease the expression of RANKL in collagen-induced arthritis rats via reducing the levels of IL-22. J Immunol Res 2019; 2019: 8459281.
[42] Abo-Aziza FAM, Zaki AKA and Abo El-Maaty AM. Bone marrow-derived mesenchymal stem cell (BM-MSC): a tool of cell therapy in hydatid experimentally infected rats. Cell Regen 2019; 8: 58-71.
[43] Liu YL, Lin HM, Zou R, Wu JC, Han R, Raymond LN, Reid PF and Qin ZH. Suppression of complete Freund's adjuvant-induced adjuvant arthritis by cobratoxin. Acta Pharmacol Sin 2009; 30: 219-227.
[44] Kondo M, Yamaoka K and Tanaka Y. Acquiring chondrocyte phenotype from human mesenchymal stem cells under inflammatory conditions. Int J Mol Sci 2014; 15: 21270-21285.
[45] Ragab GH, Halfaya FM, Ahmed OM, Abou El-Kheir W, Mahdi EA, Ali TM, Almehmadi MM and Hagag U. Platelet-rich plasma ameliorates monosodium iodoacetate-induced ankle osteoarthritis in the rat model via suppression of inflammation and oxidative stress. Evid Based Complement Alternat Med 2021; 2021: 6692432.
[46] Chang YH, Wu KC and Ding DC. Induced pluripotent stem cell-differentiated chondrocytes repair cartilage defect in a rabbit osteoarthritis model. Stem Cells Int 2020; 2020: 8867349.
[47] Ahmed OM, Ali TM, Abdel Gaid MA and Elberry AA. Effects of enalapril and paricalcitol treatment on diabetic nephropathy and renal expressions of TNF-α, P53, Caspase-3 and Bcl-2 in STZ-induced diabetic rats. PLoS One 2019; 14: e0214349.
[48] Ahmed OM, Ebaid H, El-Nahass ES, Ragab M and Alhazza IM. Nephroprotective effect of *Pleurotus ostreatus* and *Agaricus bisporus* extracts and carvedilol on ethylene glycol-induced urolithiasis: roles of NF-κB, p53, bcl-2, bax and bak. Biomolecules 2020; 10: 1317.
[49] Lepetsos P and Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. Biochim Biophys Acta 2016; 1862: 576-591.
[50] Maheshwer B, Polce EM, Paul K, Williams BT, Wolfson TS, Yanke A, Verma NN, Cole BJ and Chahla J. Regenerative potential of mesenchymal stem cells for the treatment of knee osteoarthritis and chondral defects: a systematic review and meta-analysis. Arthroscopy 2021; 37: 362-378.
[51] Chang CL, Sung PH, Sun CK, Chen CH, Chiang HJ, Huang TH, Chen YL, Zhen YY, Chai HT, Chung SY, Tong MS, Chang HW, Chen HH and Yip HK. Protective effect of melatonin-supported adipose-derived mesenchymal stem cells against small bowel ischemia-reperfusion injury in rat. J Pineal Res 2015; 59: 206-220.
[52] Angeloni C, Gatti M, Prata C, Hrelia S and Maraldi T. Role of mesenchymal stem cells in counteracting oxidative stress-related neurodegeneration. Int J Mol Sci 2020; 21: 3299.
[53] Zhao D, Yu W, Xie W, Ma Z, Hu Z and Song Z. Bone marrow-derived mesenchymal stem cells

ameliorate severe acute pancreatitis by inhibiting oxidative stress in rats. Mol Cell Biochem 2022; 477: 2761-2771.
[54] Roman-Blas JA and Jimenez SA. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 2006; 14: 839-848.
[55] Huang X, Ni B, Xi Y, Chu X, Zhang R and You H. Protease-activated receptor 2 (PAR-2) antagonist AZ3451 as a novel therapeutic agent for osteoarthritis. Aging (Albany NY) 2019; 11: 12532-12545.
[56] Zha K, Sun Z, Yang Y, Chen M, Gao C, Fu L, Li H, Sui X, Guo Q and Liu S. Recent developed strategies for enhancing chondrogenic differentiation of MSC: impact on MSC-based therapy for cartilage regeneration. Stem Cells Int 2021; 2021: 8830834.
[57] Korotkyi O, Huet A, Dvorshchenko K, Kobyliak N, Falalyeyeva T and Ostapchenko L. Probiotic composition and chondroitin sulfate regulate TLR-2/4-mediated NF-κB inflammatory pathway and cartilage metabolism in experimental osteoarthritis. Probiotics Antimicrob Proteins 2021; 13: 1018-1032.
[58] Saito T and Tanaka S. Molecular mechanisms underlying osteoarthritis development: notch and NF-κB. Arthritis Res Ther 2017; 19: 94.
[59] Wang J, Liu Y, Ding H, Shi X and Ren H. Mesenchymal stem cell-secreted prostaglandin E2 ameliorates acute liver failure via attenuation of cell death and regulation of macrophage polarization. Stem Cell Res Ther 2021; 12: 15.
[60] Mancuso P, Raman S, Glynn A, Barry F and Murphy JM. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. Front Bioeng Biotechnol 2019; 7: 9.
[61] Murakami S, Lefebvre V and de Crombrugghe B. Potent inhibition of the master chondrogenic factor sox9 gene by interleukin-1 and tumor necrosis factor-alpha. J Biol Chem 2000; 275: 3687-3692.
[62] Wang L, Pei S, Han L, Guo B, Li Y, Duan R, Yao Y, Xue B, Chen X and Jia Y. Mesenchymal stem cell-derived exosomes reduce A1 astrocytes via downregulation of phosphorylated NFκB P65 subunit in spinal cord injury. Cell Physiol Biochem 2018; 50: 1535-1559.
[63] Szekanecz Z, Halloran MM, Volin MV, Woods JM, Strieter RM, Kenneth Haines G 3rd, Kunkel SL, Burdick MD and Koch AE. Temporal expression of inflammatory cytokines and chemokines in rat adjuvant-induced arthritis. Arthritis Rheum 2000; 43: 1266-1277.
[64] Li H, Li L, Min J, Yang H, Xu X, Yuan Y and Wang D. Levels of metalloproteinase (MMP3, MMP-9), NF-kappaB ligand (RANKL), and nitric oxide (NO) in peripheral blood of osteoarthritis (OA) patients. Clin Lab 2012; 58: 755-62.
[65] Wang XX and Cai L. Expression level of proteoglycan, collagen and type II collagen in osteoarthritis rat model is promoted and degradation of cartilage is prevented by glucosamine methyl ester. Eur Rev Med Pharmacol Sci 2018; 22: 3609-3616.
[66] Lefebvre V, Peeters-Joris C and Vaes G. Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim Biophys Acta 1990; 1052: 366-78.
[67] Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M and Zafarullah M. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res 2003; 288: 208-217.
[68] Vincenti MP and Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. Arthritis Res 2002; 4: 157-164.
[69] Murrell GA, Jang D and Williams RJ. Nitric oxide activates metalloprotease enzymes in articular cartilage. Biochem Biophys Res Commun 1995; 206: 15-21.
[70] Choi MC, Jo J, Park J, Kang HK and Park Y. NF-κB signaling pathways in osteoarthritic cartilage destruction. Cell 2019; 8: 734.
[71] Hamilton AM, Cheung WY, Gómez-Aristizábal A, Sharma A, Nakamura S, Chaboureau A, Bhatt S, Rabani R, Kapoor M, Foster PJ and Viswanathan S. Iron nanoparticle-labeled murine mesenchymal stromal cells in an osteoarthritic model persists and suggests anti-inflammatory mechanism of action. PLoS One 2019; 14: e0214107.
[72] Moqbel SAA, He Y, Xu L, Ma C, Ran J, Xu K and Wu L. Rat chondrocyte inflammation and osteoarthritis are ameliorated by madecassoside. Oxid Med Cell Longev 2020; 2020: 7540197.
[73] Park HJ, Lee CK, Song SH, Yun JH, Lee A and Park HJ. Highly bioavailable curcumin powder suppresses articular cartilage damage in rats with mono-iodoacetate (MIA)-induced osteoarthritis. Food Sci Biotechnol 2019; 29: 251-263.
[74] Pap T, Müller-Ladner U, Gay RE and Gay S. Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. Arthritis Res 2000; 2: 361-367.

[75] Tak PP and Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis. Arthritis Rheum 2000; 43: 2619-2633.
[76] Singh A, Goel SC, Gupta KK, Kumar M, Arun GR, Patil H, Kumaraswamy V and Jha S. The role of stem cells in osteoarthritis: an experimental study in rabbits. Bone Joint Res 2014; 3: 32-37.
[77] Augello A, Kurth TB and De Bari S. Mesenchymal stem cells: a perspective from *in vitro* cultures to *in vivo* migration and niches. Eur Cell Mater 2010; 20: 122-133.
[78] Wahl SM. Transforming growth factor-β: innately bipolar. Curr Opin Immunol 2007; 19: 55-62.
[79] Pohlers D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne RW and Wolf G. TGF-β and fibrosis in different organs - molecular pathway imprints. Biochim Biophys Acta 2009; 1792: 746-756.
[80] Dranitsina AS, Dvorshchenko KO, Korotkiy AG, Grebinyk DM and Ostapchenko LI. Expression of Ptgs2 and Tgfb1 genes in rat cartilage cells of the knee under conditions of osteoarthritis. Cytology and Genetics 2018; 52: 192-197.
[81] Halfaya FM, Ragab GH, Hagag U, Ahmed OM and Elkheir WA. Efficacy of hyaluronic acid in the treatment of MIA-induced ankle osteoarthritis in rats and its effect on antioxidant response element. J Vet Med Res 2020; 27: 119-127.
[82] Waly NE, Refaiy A and Aborehab NM. IL-10 and TGF-β: roles in chondroprotective effects of glucosamine in experimental osteoarthritis? Pathophysiology 2017; 24: 45-49.
[83] van der Kraan PM. Differential role of transforming growth factor-beta in an osteoarthritic or a healthy joint. J Bone Metab 2018; 25: 65-72.
[84] Hamdalla HM, Ahmed RR, Galaly SR, Ahmed OM, Naguib IA, Alghamdi BS and Abdul-Hamid M. Assessment of the efficacy of bone marrow-derived mesenchymal stem cells against a monoiodoacetate-induced osteoarthritis model in Wistar rats. Stem Cells Int 2022; 2022: 1900403.
[85] Wei Y, Xie Z, Bi J and Zhu Z. Anti-inflammatory effects of bone marrow mesenchymal stem cells on mice with Alzheimer's disease. Exp Ther Med 2018; 16: 5015-5020.
[86] Gupta PK, Chullikana A, Rengasamy M, Shetty N, Pandey V, Agarwal V, Wagh SY, Vellotare PK, Damodaran D, Viswanathan P, Thej C, Balasubramanian S and Majumdar AS. Efficacy and safety of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells (Stempeucel®): preclinical and clinical trial in osteoarthritis of the knee joint. Arthritis Res Ther 2016; 18: 301.