BENHA MEDICAL JOURNAL bmj

Print ISSN 1110-208X.

Online ISSN 2357-0016

Original article

The Role of Bone Marrow Mesenchymal Stem Cells and Glucosamine in the Treatment of Osteoarthritis Induced by Monosodium Iodoacetate in Adult Albino Rats

Marwa Abd Elbasset, Samia M. Manawy, Saadia A. Shalaby, Amal M. Elshazly, Hanan I. Elkerdasy

Anatomy and Embryology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to: Marwa Abd Elbasset, Anatomy and Embryology Department, Faculty of Medicine Benha University, Egypt.

Email: marwa.abdelbasset@fmed.bu.edu.eg

Received:
Accepted:

Abstract:

Background: Osteoarthritis plays a significant role in progressive degeneration of articular cartilage. **Aim:** This study was performed to assess the role of (BM-MSCS) or glucosamine alone and a combination of both in the treatment of knee osteoarthritis. **Methods:** Fifty adult male albino rats were divided into five groups; Group **I: Control group (10 rats)** were divided equally into subgroup a: rats were fed on a standard diet without any drug. subgroup b: rats were injected by 50 µL physiological saline. **Group II: Osteoarthritis group (10 rats)** were injected by (MIA) (3 mg/50 µL). **Group III: Glucosamine treated group (10 rats) after** induction of OA they were given glucosamine sulfate (40 mg/kg/day orally). **Group IV: BM-MSCS treated group (10 rats))** after induction of OA they were injected by BM-MSCS 10^7 cells. **Group V: Glucosamine & BM-MSCS treated group (10 rats)** after induction of OA they were given glucosamine sulfate and were injected by BM-MSCS. After sacrificing rats at the end of the experiment, articular cartilage samples were prepared for light and electron microscopic examinations. **Results:** Osteoarthritic rats showed histological changes which were decrease in thickness of cartilage, degenerated chondrocytes and fissuring of matrix. Slightly irregular surface and shrunken chondrocytes which appeared disorganized and few in glucosamine group was observed. Incomplete recovery in the MSCs group while restoration of the thickness, structure, and regular surface of the articular cartilage was detected in glucosamine and BM-MSCS treated groups. **Conclusion:** Treatment with glucosamine and MSCs showed improved histological architecture of the articular cartilage of osteoarthritic rats.

Keywords: Osteoarthritis, glucosamine, Mesenchymal stem cells.

Introduction

Osteoarthritis (OA) is a common joint disorder affecting millions of people in the developed countries. Approximately 13% of women and 10% of men older than 60 years have experienced symptomatic knee OA. The number of people suffering from knee OA may increase due to aging and increasing obesity rate of the general population [1].

The pathologic hallmarks of OA include progressive degeneration of articular cartilage and changes in subchondral bone architecture. The etiology of OA is not fully understood. At the cellular level, disease progression has been divided into a biosynthetic and degenerative phase. The biosynthetic phase begins with an extracellular matrix (ECM) damage that the chondrocytes fail to adequately repair. During the degenerative phase, ECM production is inhibited, and the chondrocytes release proteolytic enzymes which accelerate cartilage loss [2].

Glucosamine is a type of glycosaminoglycan (GAG), an amino saccharide, and the preferred substrate for the biosynthesis of proteoglycans, such as aggrecans, which maintain the cartilage integrity and function. Glucosamine reduces proteoglycan loss; delays cartilage generation and joint space narrowing and improves osteoarthritic pain in OA patients [1].

The MSC-based cell therapy is a relatively safe treatment that holds great potential for OA, evidenced by a positive effect on pain and knee function [3]. The MSCs act via alternate mechanisms to cell replacement, trophic mechanisms to promote tissue regeneration through modulation of the host environment and/or stimulation of endogenous progenitors [4].

Materials and methods

I-Materials:

This study is an experimental research approved by the Research Ethics Committee (MD 15-11-2021) of Faculty of Medicine, Benha University.

1. Animals

This study was carried out on 50 adult albino rats, weighing between 200 and 250 mg, obtained from laboratory animal house unit of Kasr Al-Ainy Faculty of Medicine, Cairo University and housed in plastic cages at 20 ± 2°C and 14 h:10 h. light: dark, rats were fed a standard diet and water, at the laboratory animal house unit of Kasr Al-Ainy, Faculty of Medicine, Cairo University. The experimental protocol was approved by the Research Ethical Committee (MD 15-11-2021) of the Faculty of Medicine, Benha University. After an accommodation period of 1 week, the experiment was done from 5 July 2023 to 5 August 2023.

2. Chemicals

Monosodium Iodoacetate: It was obtained from El Naser Pharmaceutical Chemical Company. It was acquired as a powder then, dissolved as 3 mg in 50 µL of saline was injected into the left knee using a 27-gauge needle.

Glucosamine sulfate: It was obtained from EVA Pharma Company, Cairo, Egypt. It was acquired as an oral soluble powder presented as a sachet 1500 mg dissolved in 375 ml water (4 mg per 1ml) and given as 40 mg/kg/day orally.

3. Bone Marrow Mesenchymal Stem cells (BM-MSCs):

The BM-derived MSCs (which were processed and cultured for 14 days) were used in a single dose (1×10^7cells / ml) suspended in one ml phosphate buffer saline (PBS); were injected intravenously in the rat tail vein.

II- Methods:

Experimental design:

The fifty adult albino male rats were divided into five main groups:

Group I (normal control): Ten rats which were divided equally into two subgroups:

Subgroup a: 5 rats were fed on standard diet without any drug.

Subgroup b: 5 rats were injected by 50 µL physiological saline.

Group II (osteoarthritis group): Ten rats which were injected by MIA; 50 µL physiological saline containing 3 mg with a 27-gauge needle into the articular space of the left knee joint as a single dose [5].

Group III (glucosamine treated group): Ten rats were injected by MIA then given glucosamine sulfate in a dose of 40 mg/kg/day orally [6] diluted in saline solution (Na Cl) 0.9% for 4 weeks.

Group IV (BM-MSCS treated group): Ten rats were injected by MIA then injected by BM-MSCS at a dose of (1×10^{7} ^{cells}) were resuspended in 1 ml Phosphate Buffered Saline through the rat tail vein [7].

Group V (glucosamine and BM M-SCS treated group): Ten rats afte r induction of osteoarthritis were given both glucosamine and BM- MSCS in the same doses and duration as in group III & group IV.

At the end of the experiment, the rats were anesthetized by diethyl ether. Animals were sacrificed by cervical dislocation. The skin above left knee joint was removed, and the knee joint was exposed, then the knee joint was cut in sagittal plane [6]. The specimen contained tibia, femur with articular cartilages and menisci. The specimen was fixed in 10% formaldehyde for 48 hours then was decalcified. After processing for making paraffin blocks, 7-µm sections were cut and stained then examined.

Labeling of MSCs with PKH26

Mesenchymal stem cells were harvested during the 4th passage and were labeled with PKH26 which is a red fluorochrome. MSCs were labeled with PKH26 supplied by Sigma Company (Saint Louis, Missouri USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution. Cells were injected intravenously into rat tail vain. After 4 weeks, knee joint articular cartilages were examined with a

fluorescence microscope to detect and trace the cells [(8)].

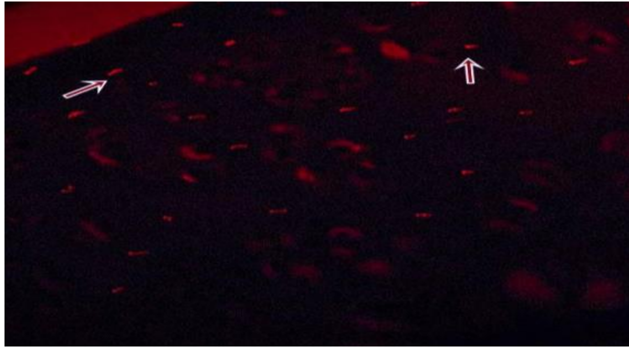

Fig. (1): A photomicrograph showing Scattered positive fluorescence PKH-26 labeled cells (red fluorescent) (↑) in the articular cartilage matrix. **(fluorescence microscope X 200).**

Histopathological study:

Knee joint articular cartilage samples have been prepared for a light microscopic study by fixation in 10 % formalin and were handled to make paraffin sections at 7 µm thickness. The sections were stained with:

• Hematoxylin and Eosin stain.

• Masson's Trichrome.

•Immunohistochemical stain: immunohistochemical avidin-biotin peroxidase technique [(8)] for detection of a proinflammatory cytokine; inducible nitric oxide synthase (iNOS).

Slide visualization and image photographing were performed in the Anatomy Department, Faculty of Medicine, Benha University, Egypt. For such purpose, Nikon Eclipse 80i upright microscope (Nikon Corporation, Japan) with a fitted digital camera, Toup CamTM Xcam full HD camera (ToupTek Europe, Ultramacro Ltd., UK) was used.

• Transmission electron microscopic (TEM) examination:

Ultrastructure examination of articular cartilage samples fixed in 2.5% glutaraldehyde, was done according to [(9)]; the ultrathin slices were studied in the faculty of medicine, at Tanta University, Egypt, using a TEM JEoL JEM-100 S.

Morphometric study

The cartilage thickness, the percentage of number of iNOS positive chondrocytes in immunohistochemically stained sections and area percentage of collagen fibers in Masson's trichrome stained sections were measured using Leica Qwin 500 image analyzer computer system (Leica Microsystems Ltd, Cambridge, UK).

Statistical analysis:

The results were analyzed using SPSS version 19 (SPSS Inc., Chicago, Illinois, USA). Post HOC LSD test was used to contrast differences between the groups. A value of $P \leq 0.05$ was accepted as statistically significant [(8)].

Results

Histopathological results

Hematoxylin and Eosin results:

Group I (control group): The hyaline articular cartilage covering both femoral condyles and tibial plateau had a smooth surface and consisted of chondrocytes surrounded by extracellular matrix (Fig.2a).

Group II (osteoarthritis group): The articular cartilage showed irregular surface and fibrillation confined to the superficial zone (Fig.2b).

Group III (glucosamine treated group): The articular cartilage shows irregular degenerated surface and shrunken chondrocytes which appear disorganized and few (Fig.2c).

Group IV (BM-MSCS treated group): The stained sections of this group revealed restored cartilage thickness and zonal layers arrangement, but the tidemark was still faint (Fig.2d).

Group V (glucosamine and BM-MSCS tre ated group): The stained sections of this gr oup revealed repair of the articular cartilag e. This was evidenced by restoration of th e thickness, structure, and regular surface of the articular cartilage that became as in the c ontrol group (Fig.2e).

Masson's trichrome results:

The Masson's trichrome stain showed tha t in group I and group V, the matrix of articu lar cartilage was well stained with green col or reflecting normal content of collagen fibe rs. However, group II had extensive red colo ration reflecting marked reduction of collag en fibers in the matrix. Group III and group IV showed increase in the Masson's trichrom e–stained areas for collagen with a reduction in the red coloration compared to that of gro up II (Fig.3).

Immunohistochemical results:

Immune histochemical stained sections f or iNOS showed negative reaction in group I and group V. Group II showed positive re action. Whereas, few iNOS-positive chondr ocytes were seen in group III and fewer iN OS-positive chondrocytes were seen in grou p IV as compared to the untreated group (F ig.4).

Transmission electron microscopic result:

Group l (Control group): The articular cart ilage showed spherical chondrocyte with a r ounded, eccentrically located nucleus with fi nely dispersed nuclear chromatin, abundant r ough endoplasmic reticulum, clusters of mit ochondria, lipid and glycogen vacuoles (Fig. 5a, 6a).

Group II (osteoarthritis group): The artic ular cartilage showed shrunken chondrocyte s with dark vacuolated nuclei, irregular cyto plasmic membrane. The cytoplasm containe d different sizes of cavitations, damaged mit ochondria, degenerated rough endoplasmic r eticulum and granules. The cell membrane had multiple cytoplasmic processes (Fig.5b, 6b).

Group III (glucosamine treated group): T he articular cartilage showed elongated chon drocyte with elongated dark nucleus. Moreo ver, in most cells we observed a significant d

ecrease in the cytoplasmic organelles and str uctures, particularly in the extension of the r ough endoplasmic reticulum (Fig.5c,6c).

Group IV (BM-MSCS treated group): Th e articular cartilage showed no clear differen ces identified between control and treated ce lls except for being slightly circular with slig htly rounded nucleus and slight decrease in cytoplasmic organelles (Fig.5d, 6d).

Group V (glucosamine and BM-MSCS tre ated group): The articular cartilage show ed that the chondrocytes had restored no rmal shape and structure as noticed in the control group (Fig.5e, 6e).

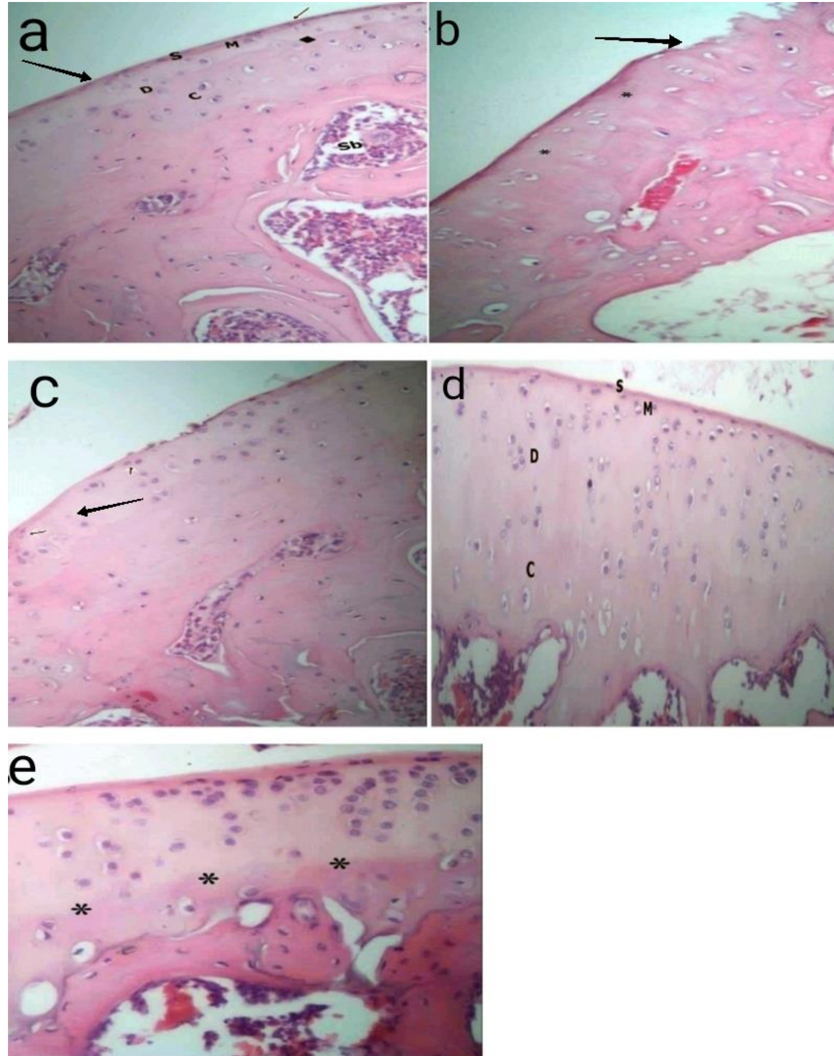

Fig. (2): Light photomicrograph of rat's knee joints of **a)** the control group (I) showing articular cartilage with smo oth non- fibrillated articular surface (↑). Different zones including superficial zone (s) midzone (m), deep zone (d), c alcified cartilage (c), and subchondral bone (Sb) is formed of bone marrow spaces and bone trabeculae. Tidemark is clearly defined between deep and calcified zones. **b)** OA group (II) showing articular cartilage with fibrillatio n (↑), Areas with low intensity of intercellular matrix and hypo- cellularity in mid zone (*) with absence of tid emark are noticed. **c)** Glucosamine treated group (III) showing few, disorganized and shrunken chondrocytes (↑) . **d)** BM-MSCS treated group (IV) showing restoration of normal zonal arrangement including superficial zone (s), midzone (m), deep zone (d), calcified cartilage. **e)** Glucosamine and BM-MSCS treated group (V) showin g well defined tidemark (*). **(H&E X 200)**.

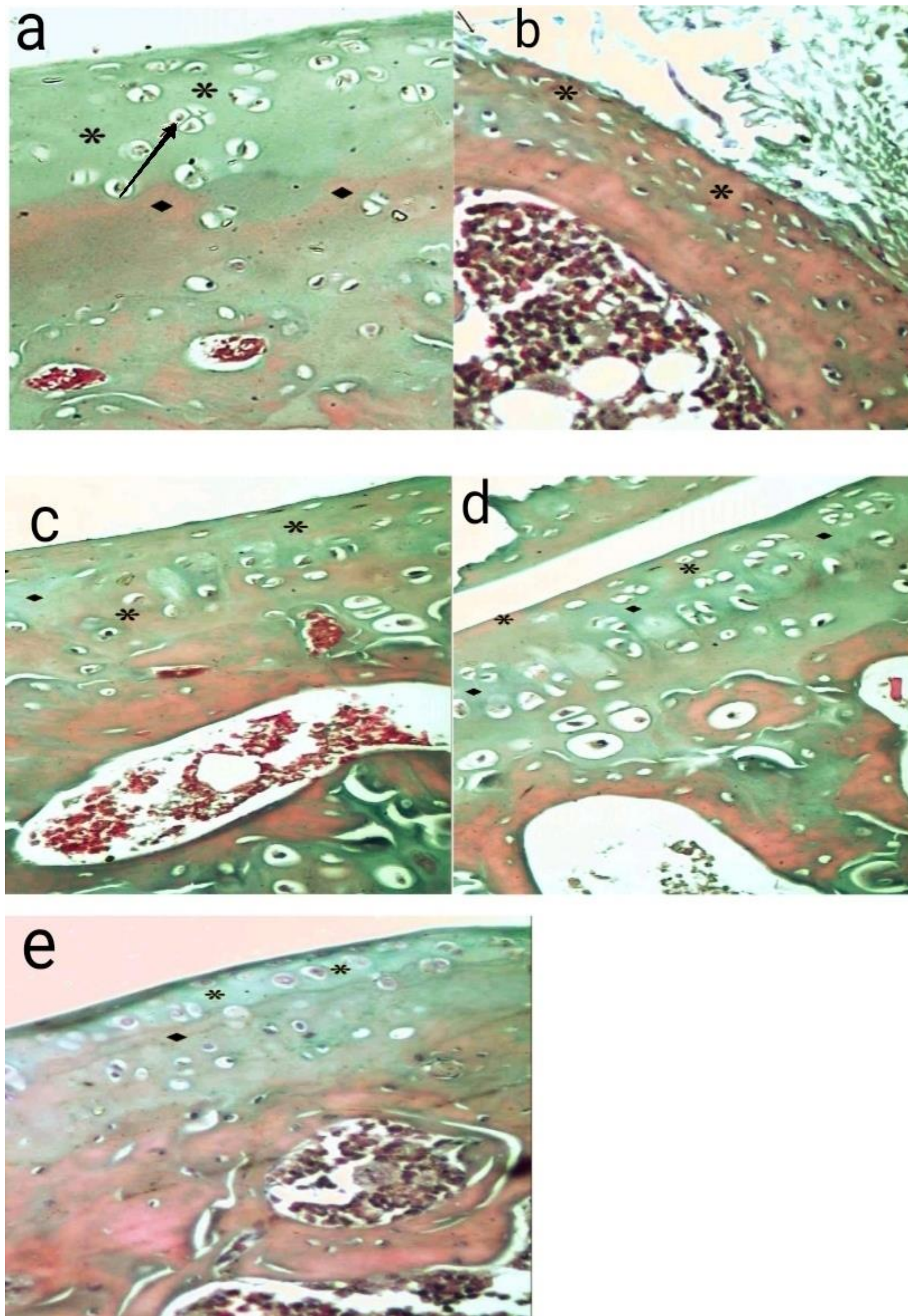

Fig. (3): A photomicrograph of Masson's trichrome–stained sections of articular cartilage of: **a) Control** group showing that the matrix of articular cartilage is well stained with Masson's trichrome (green color (*). The nuclei were stained dark blue (↑). **b)** Osteoarthritis group showing marked reduction of Masson's trichrome–stained area for collagen with appearance of an extensive red color (*). Minimal erosion of the articular cartilage surface is observed (↑). **c)** Glucosamine treated group showing mild increase of Masson's trichrome–stained area; with reduced red color (*). **d)** BM-MSCS treated group showing moderate increase of Masson's trichrome–stained area; with reduction of red color (*). **e)** Glucosamine and BM-MSCS treated group showing marked increase of Masson's trichrome–stained area (*) with normal appearance of red color in calcified zone; as in the control group. **(Masson's trichrome X 200).**

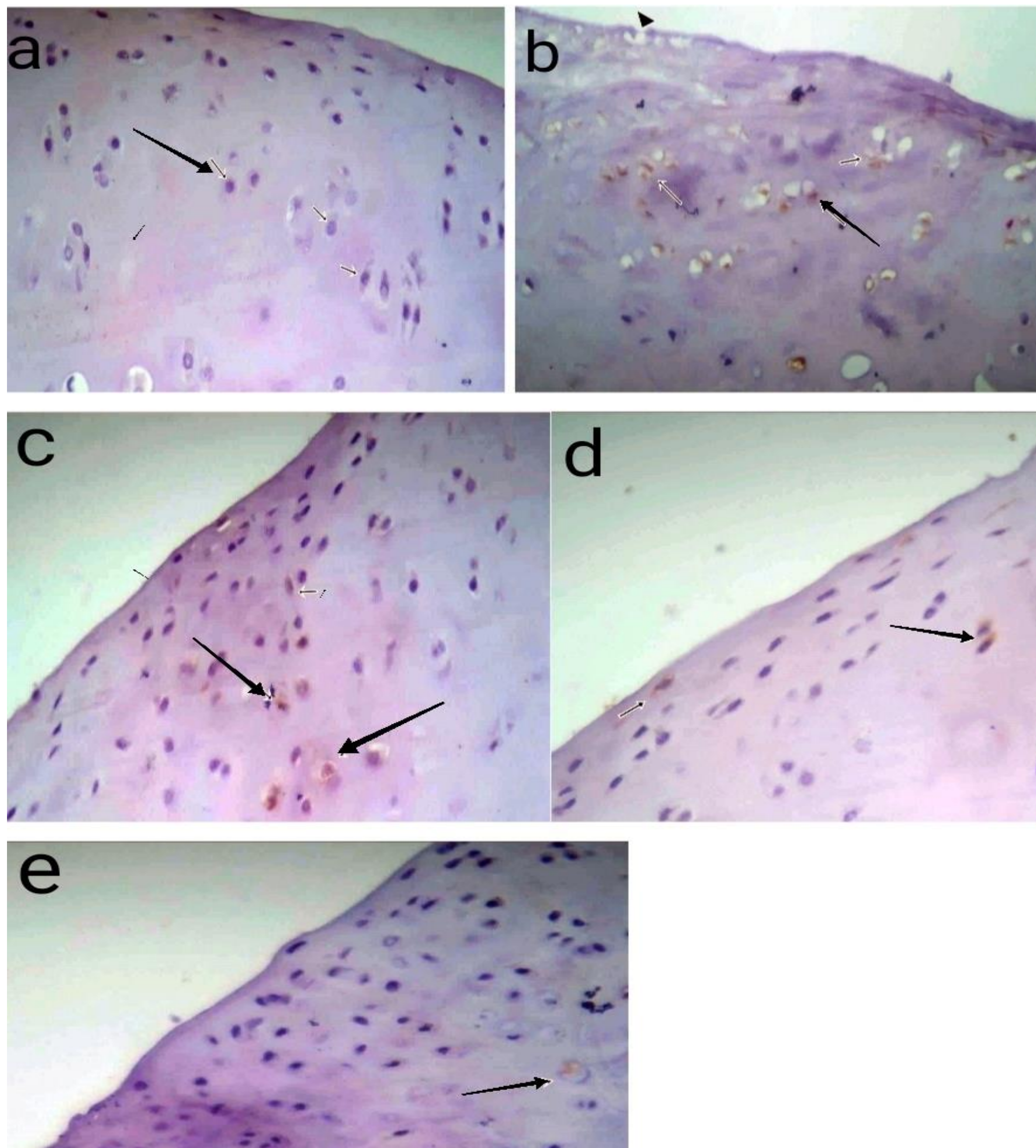

Fig. (4): A photomicrograph of iNOS immuno-staining sections of **a)** control group showing negative iNOS immuno-staining of chondrocytes**.** **b)** Osteoarthritis group showing erosion of the surface of articular cartilage and many iNOS-positive (brown staining) chondrocytes (↑). **c)** Glucosamine treated group showing few iNOS-positive chondrocytes (↑). **d)** BM-MSCS treated group showing very few iNOS-positive chondrocytes (↑). **e)** Glucosamine and BM-MSCS treated group showing scattered iNOS-positive chondrocyte (↑). **(iNOS immunostaining ×400).**

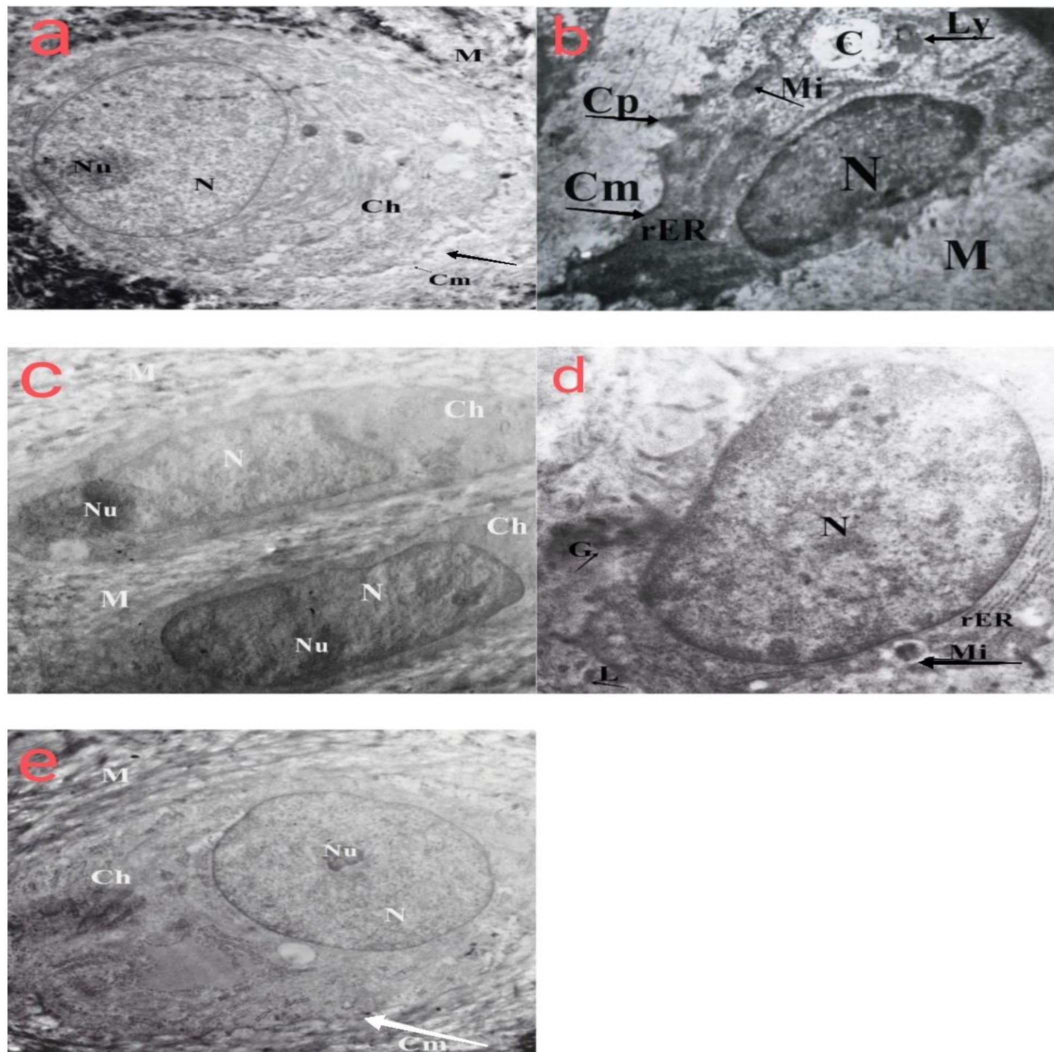

Fig. (5): Transmission electron micrograph of knee joint articular cartilage of:

a) Group I: (control group) showing spherical chondrocyte (Ch), with rounded, eccentrically located nucleus (N), with nucleolus (Nu), intact cell membrane (Cm) and surrounding matrix (M). **(TEM X3000)**

b) Group II: (osteoarthritis group) showing: Shrunken chondrocyte (Ch), dark vacuolated nucleus (N), cytoplasm containing cavitation (c) rough endoplasmic reticulum (rER) , mitochondria (Mi) and lysosomes (Ly). Note the cell membrane (Cm) with multiple cytoplasmicprocesses (Cp), surrounding matrix (M). **(TEM X3000)**

c) Group III: (glucosamine treated group) showing: Two parallel elongated chondrocytes (Ch) with the matrix in between (M). Note elongated dark nucleus (N) with nucleolus (Nu). **(TEM X3000)**

d) Group IV: (BM-MSCS treated group) showing: Slightly circular chondrocytes (Ch) with slightly rounded, dark, eccentric and vacuolated nucleus (N). Cytoplasm contains many granules (G), lipid droplet (L), rough endoplasmic reticulum (rER) and mitochondria (Mi). Note the intact cell membrane (Cm) and surrounding matrix (M). **(TEM X3000)**

e) Group V (glucosamine and BM-MSCS treated group) showing: Spherical chondrocyte (Ch), rounded, eccentrically located nucleus (N), with nucleolus (Nu). Note the intact cell membrane (Cm) and surrounding matrix (M). **(TEM X3000)**.

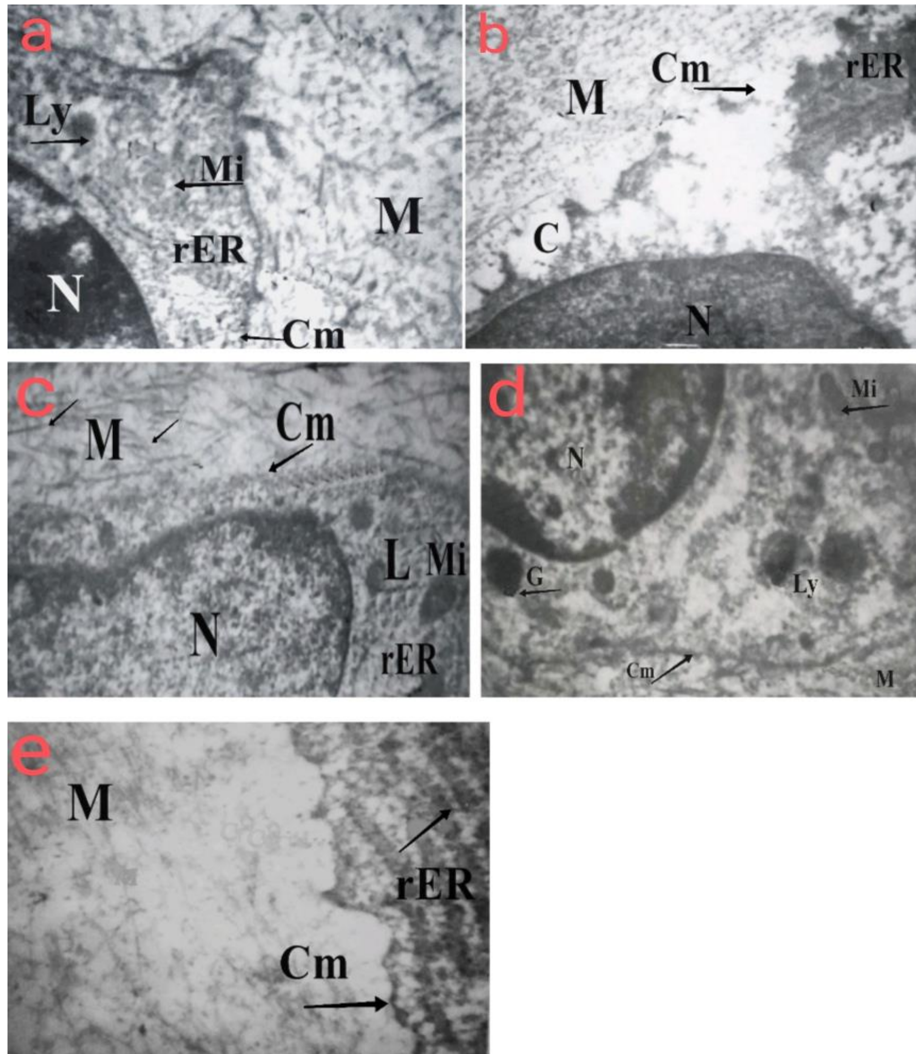

Fig. (6): A higher magnification of the previous sections showing:

a) Part of the nucleus (N), cytoplasm containing lysosome (Ly), rough endoplasmic reticulum (rER) and mitochond ria (Mi). Note the intact cell membrane (Cm) and surrounding matrix (M). **(TEM X8000)**

b) Part of dark vacuolated nucleus (N), cytoplasm containing multiple cavitations (C) and degenerated rough en doplasmic reticulum (rER). Note the ruptured cell membrane and surrounding matrix (M). **(TEM X8000)**

c) Part of elongated dark nucleus (N), cytoplasm containing lipid droplet (L), rough endoplasmic reticulum(rER) and mitochondria (Mi). Note intact cell membrane (Cm) and surrounding matrix (M) with distributed collagen fibers in different directions (↑). **(TEM X8000)**

d) Part of vacuolated nucleus (N), cytoplasm containing multiple granules (G), lysosome (Ly) and mitochondria (M i). Note the intact cell membrane (Cm) and surrounding matrix (M). **(TEM X8000).**

e) Part of the cytoplasm containing rough endoplasmic reticulum (rER). Note intact cell membrane (Cm) and s urrounding matrix (M). **(TEM X8000)**

Morphometric and statistical results:

Mean values of the thickness (mm) of the hyaline articular cartilage (table 1):

Group ll (osteoarthritis group) showed significant decrease ($P < 0.05$) with group I, group III, group IV and group V.

Group lll (glucosamine group) showed significant decrease ($P < 0.05$) with group I, group IV, group V and significant increase ($P < 0.05$) with group II.

Group lV (mesenchymal stem cell group) showed significant decrease ($P < 0.05$) with group I and group V. It showed significant increase ($P < 0.05$) with group II and group III.

Group V (glucosamine + mesenchymal stem cell group) showed significant decrease ($P < 0.05$) with group I. It showed significant increase ($P < 0.05$) with group II, group III and group IV.

Mean area percentage of collagen fiber deposition by Masson's trichrome staining (table 2):

Group II (osteoarthritis group) showed significant decrease ($P < 0.05$) with group I, group III, group IV and group V.

Group III (glucosamine group) showed significant decrease ($P < 0.05$) with group I, group IV, group V and significant increase ($P < 0.05$) with group II.

Group IV (mesenchymal stem cell group) showed significant decrease ($P < 0.05$) with group I and group V and significant increase ($P < 0.05$) with group II and group III.

Group V (glucosamine + mesenchymal stem cell group) showed significant decrease ($P < 0.05$) with group I and significant increase ($P < 0.05$) with group II, group III and group IV.

Mean percentage of number of iNOS positive stained chondrocytes (table 3):

Group II (osteoarthritis group) showed significant increase ($P < 0.05$) with group I, group III, group IV and group V.

Group III (glucosamine group) showed significant increase ($P < 0.05$) with group I, group IV, group V and significant decrease ($P < 0.05$) with group II.

Group lV (mesenchymal stem cell group) showed significant decrease ($P < 0.05$) with group II and group III and significant increase ($P < 0.05$) with group I and group V.

Group V (glucosamine + mesenchymal stem cell group) showed significant increase ($P < 0.05$) with group I and significant decrease ($P < 0.05$) with group II, group III and group IV.

Table (1) showing mean values of the thickness (mm) of the hyaline articular cartilage in different groups:

Mean ± SD	Group I (Control group)	Group II (Osteoarthritis group)	Group III (glucosamine group)	Group IV (stem cell group)	Group V (glucosamine + stem cell group)
Mean cartilage thickness (mm)	12 ± 2.5	5.5 ± 0.6	9.4 ± 0.9	10.3 ± 0.8	11.2 ± 1.5
significance ≤ 0.05		With groups I, III ,IV,V	With group I, II,IV,V	With group I, II, III ,V	With group I, II, III, IV

Table (2) showing mean area percentage of collagen fibers deposition:

Mean%±SD	Group I (Control group)	Group II (Osteoarthritis group)	Group III (glucosamine group)	Group IV (stem cell group)	Group V (glucosamine + stem cell group)
Mean area percentage of Collagen fibers deposition	43.5 ± 4.7	10.4 ± 0.7	31.5 ± 2	35.1 ± 1.6	39.6 ± 2.9
significance ≤ 0.05		With groups I, III ,IV,V	With groups I, II, IV, V	With groups I, II, III, V	With groups I, II, III, IV

Table (3) showing mean percentage of number of iNOS positive stained chondrocytes:

Mean%±SD	Group I (Control group)	Group II (Osteoarthritis group)	Group III (glucosamine group)	Group IV (stem cell group)	Group V (glucosamine + stem cell group)
INOs area%	4.8 ± 2.8	45.3 ± 1.7	28.3 ± 0.9	16 ± 1.6	7.1 ± 2.6
significance ≤ 0.05		With groups I, III ,IV,V	With groups I, II, IV, V	With groups I, II, III, V	With groups I, II, III, IV

Discussion:

In the present study, the MIA-induced arthritis rat model showed progressive decrease in the thickness of articular cartilage. Chondrocytes showed degeneration. Moreover, the matrix exhibited fibrillations and fissuring. This agreed with the researchers [5] who explained the progression and expansion of cartilage destruction by the increased production of inflammatory mediators.

The induced arthritis in rat model showed significant decrease ($P < 0.05$) in thickness of the hyaline articular cartilage and the mean area percentage of collagen fibersdeposition when compared with group I, group III, group IV and group V. For the mean percentage of number of iNOS positive stained chondrocytes, group II (ostoarthritis group) showed significant increase ($P < 0.05$) when

compared with group I, group III, group IV and group V .These results were supported by others [10] and [11].The injection of MI increased the activity of matrix metalloproteinases which were involved in the cartilage destruction [12].

Electron microscopic examinations of the articular cartilage in MIA-induced arthritis rats in the current study showed shrunken chondrocytes with dark vacuolated nuclei, irregular cytoplasmic membrane. The cytoplasm contained different sizes of cavitations, damaged mitochondria, degenerated rough endoplasmic reticulum and granules. The cell membrane had multiple cytoplasmic processes. These results were like [13] and [14] who revealed that TEM images in the animal group that received MIA showed vacuoles in the cytoplasm and damaged mitochondria, nucleus, and rough endoplasmic reticulum.

In hematoxylin and eosin-stained sections of glucosamine treated group showed slightly irregular degenerated surface and shrunken chondrocytes which appear disorganized and few in number. These results were in line with those of others [2] and [6] who revealed less degenerative changes in the articular cartilage after glucosamine treatment as compared to the osteoarthritic group.

In this current study, the articular cartilage of the group treated with glucosamine that was examined using electron microscope, showed elongated chondrocyte with elongated dark nucleus. These results were in consistent with [15] who revealed that chondrocytes treated with glucosamine in combination with other medications showed a phenotype similar to healthy articular chondrocytes in vivo.

In the current study, the glucosamine treated group showed significant decrease ($P < 0.05$) in thickness of the hyaline articular cartilage and the mean area percentage of collagen fibers deposition when compared with group I, group IV, group V and significant increase ($P < 0.05$) with group II. For the mean percentage of number of iNOS positive stained chondrocytes, group III showed significant increase when compared with group I, group IV, group V and significant decrease with group II. These results were in line with those of others [16 and 6] who explained the reduction of collagen content by an imbalance between synthesis and degeneration due to the activity of matrix metalloproteinase aggrecanase II which has a role in debasing the collagen content of articular cartilage.

In this study, Hematoxylin and Eosin-stained sections of mesenchymal stem cell treated group showed restored cartilage thickness and zonal layers arrangement, but the tidemark was still faint. These findings were similar to others [7 and 12] who explained the restoration of the normal architecture of the articular cartilage in MSC treated rats was attributed to the time needed by MSCs to proliferate and differentiate to regularly arranged chondrocytes to form proper hyaline cartilaginous matrix.

In this study, electron microscopy of MSC treated rats revealed that no clear differences that were identified between control and treated cells except for being slightly circular with slightly rounded nucleus. These results we

re in agreement with the studies that revealed that the chondrocytes were oval in shape and were distributed in the cartilage matrix, with full nuclei; chromatin was evenly distributed; and rough endoplasmic reticulum, Golgi body, and mitochondria were scattered in the cytoplasm [17 & 18].

In the present study, MSC treated group showed significant increase ($P < 0.05$) in thickness of the hyaline articular cartilage and the mean area percentage of collagen fibers deposition when compared with group II, group III and significant decrease when compared with group I and group V. This group showed significant decrease in the mean percentage of number of iNOS positive stained chondrocytes with group II, group III and significant increase with group I and group V. These results were similar to others [8 and 12] who explained that the improvement of the cartilage after MSCs treatment might be either due to differentiation to tissue cells and restoration of lost morphology and function, or secretion of bioactive and growth factors such as; fibroblast growth factor.

Based on the current study, Hematoxylin and Eosin-stained sections of glucosamine and BM-MSCS treated group showed restoration of the thickness, structure, and regular surface of the articular cartilage that became as in control group. The normalization of the organization and structure of the chondrocytes into four zones was a prominent feature. These findings were consistent with other studies which revealed well-developed cartilage layer, and the chondrocytes were intact and properly oriented [(19) and (20)].

In this study, the group treated with glucosamine and BM-MSCS which was examined using electron microscopy; showed that the chondrocytes had restored normal shape and structure as in control group. These results were similar to the study that revealed that the chondrocytes restored normal shape and distribution in the cartilage matrix, with normal organelles in the cytoplasm more in combined rather than in single therapy (18).

In the present study, group treated with glucosamine and BM-MSCS showed significant decrease ($P < 0.05$) in thickness of the hyaline articular cartilage and the mean area percentage of collagen fibers deposition with group I and significant increase with group II, group III and group IV. This group showed significant increase in the mean percentage of number of iNOS positive stained chondrocytes with group I and significant decrease with group II, group III and group IV. These results were in agreement with the study which demonstrated that MSCS promoted cartilage regeneration and enhanced matrix synthesis of type II collagen [20 & 21].

Conclusions

From this study, we concluded that treatment with glucosamine showed mild improvement in the changes in articular cartilage structure of knee joint. While stem cell therapy showed moderate improvement compared with untreated osteoarthritic rats.

Considering the efficacy of combined glucosamine and stem cell therapy, the current results showed a significant ameliorative effect

on all histopathological features on the a rticular cartilage of osteoarthritic rats.

References

1- Al-Saadi HM, Pang KL, Nirvana SI, Chin KY. Multifaceted Protective Role of Glucosamine against Osteoarthritis: Review of its Molecular Mechanisms. Scientia Pharmaceutica. 2019; 87 (4), 34.

2- Gibson M, Li H, Coburn J, Moronic L, Nahas Z, Bingham C, et al. Intra-Articular Delivery of Glucosamine for Treatment of Experimental Osteoarthritis Created by a Medial Meniscectomy in a Rat Model. J. Orthop Res. 2014; 32:302–309.

3- Wang J, Zhou L, Zhang Y, Huang L. Mesenchymal stem cells - a promising strategy for treating knee osteoarthritis. Bone Joint Res. 2020; 9 (10):719–728.

4- Mancuso P, Raman S, Glynn A, Barry F, Murphy JPM. Mesenchymal Stem Cell Therapy for Osteoarthritis: The Critical Role of the Cell Secretome. Front. Bioeng. Biotechnol. 2019; 7:9.

5- Xu Q, Zhang BZ, Sun AW. Effect of Naringin on Monosodium Iodoacetate Induced Osteoarthritis Pain in Rats. Med Sci Monit. 2017; 23: 3746-3751.

6- Salman A, Shabana AI, El-ghazouly DE, Maha E. Protective effect of glucosamine and risedronate (alone or in combination) against osteoarthritic changes in rat experimental model of immobilized knee. Anat Cell Biol. 2019; 52:498-510.

7- Li F, Li X, Liu G, Gao C, Li X. Bone Marrow Mesenchymal Stem Cells Decrease the Expression of RANKL in Collagen-Induced Arthritis Rats via Reducing the Levels of IL-22. Journal of Immunology Research. 2019; Article ID 8459281, pages.1-9.

8- Mohammed SA, Makhlouf NA, Baher W, Aboud SM. The Effect of Bone Marrow Mesenchymal Stem Cells Versus Methotrexate on the Knee Joint in A Rat Model of Rheumatoid Arthritis. A Histological Study. JMH. 2018; 2 (1):45-56.

9 - Tizro P, Choi C, Khanlou N. Sample Preparation for Transmission Electron Microscopy. Methods in Molecular Biology. 2019; (1897): 417-424.

10- Kwon M, Nam D, Kim J. Pathological Characteristics of Monosodium Iodoacetate-Induced Osteoarthritis in Rats. Tissue Engineering and Regenerative Medicine. 2023; 20: 435–446.

11- Ali SM, Okda AA, Dessouky IS, Hewedy WA, Zahran NM, Alamrani BA. L-Carnitine ameliorates knee lesions in mono-iodoacetate induced osteoarthritis in rats. Alexandria Journal of Medicine. 2017; (53): 61–66.

12-Abd Alhalim HI, Sarhan N, Laag EMA Histological Study on The Effect of Bone Marrow Derived Mesenchymal Stem Cells Suspended in Hyaluronic Acid on Articular Cartilage of Osteoarthritic Knee joint of Adult Male Albino Rat. The Egyptian Journal of Histology. 2020; 44 (4): 1007- 1021.

13- Hassan W, Bin-Jaliah I, Haidara MA, Eid RA, Heidar EA, Dallak M, et al. Vitamin E ameliorates alterations to the articular cartilage of knee joints induced by monoiodoacetate and diabetes mellitus in rats. Ultrastructural Pathology. 2019; (43):2-3:126-134.

14- Heidar EA, Al- Ani B, Haidara M A, Al Faya FF, Al Humayed S, Eid RA, et al. Development of a rat model of knee osteoarthritis by a combination of monoiodoacetate and streptozotocin. Int. J. Morphol. 2017; 35 (3):928-935.

15- Stoppoloni D, Politi L, Leopizzi M, Gaetani S, Basciani S, Moreschini O, et al. Effect of glucosamine and its peptidyl-derivative on the production of extracellular matrix components by human primary chondrocytes. Osteoarthritis and Cartilage. 2015; 23 (1): 103-113.

16- Martins JM, Neto LD, Sgavioli S, Araújo I C, Ries AA, Santos RS, et al. Effect of glycosaminoglycans on the structure and composition of articular cartilage and bone of broilers. Poultry Science. 2023; 102 (4):1-15.

17- Jin Y, Xu M, Zhu H, Dong C, Ji J, Liu Y, et al. Therapeutic effects of bone marrow mesenchymal stem cells-derived exosomes on osteoarthritis. J Cell Mol Med. 2021; 25? (19): 9281–9294.

18- Liu JW, Wu YL, Wei W, Zhang YL, Liu D, Ma XX, et al. Effect of Warm Acupuncture Combined with Bone Marrow Mesenchymal

Stem Cells Transplantation on Cartilage Tissue in Rabbit Knee Osteoarthritis. Evidence-Based Complementary and Alternative Medicine. 2021; Article ID 5523726, pages.1-12.

19- Hamdalla HM, Ahmed RR, Galaly SR, Ahmed OM, Nagiub IA, Alghamdi BS, et al. Assessment of the Efficacy of Bone Marrow-Derived Mesenchymal Stem Cells against a Monoiodoacetate-Induced Osteoarthritis Model in Wistar Rats. Stem Cells International. 2022; Article ID 1900403, pages.2-14.

20- AbuBakr N, Fares AE, Mostafa A, Farag DB. Mesenchymal stem cells-derived microvesicles versus platelet-rich plasma in the treatment of monoiodoacetate-induced temporomandibular joint osteoarthritis in Albino rats. Heliyon. 2022; 8 (10): e10857.

21- Allam D, Kassab A, El-Bakry N, El-Shal A. Histological and Immunohistochemical Study of the Possible Curative Effect of Intra-articular Injection of N-Acetyl Phenylalanine Glucosamine on Surgically Induced Osteoarthritis in Knee Joint of Adult Male Albino Rat. EJH. 2021; 45 (4):1000-1008.

To cite this article: Marwa Abd Elbasset, Samia M. Manawy, Saadia A. Shalaby, Amal M. Elshazly, Hanan I. Elkerdasy. The Role of Bone Marrow Mesenchymal Stem Cells and Glucosamine in the Treatment of Osteoarthritis Induced by Monosodium Iodoacetate in Adult Albino Rats. BMFJ XXX, DOI: 10.21608/bmfj.2024.305789.2142