ijpbs 2023;doi10.22376/ijpbs.2023.14.3.b43-53

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

Original Research Article

Histopathology of Bone Marrow Mesenchymal Stem Cells

Effects of Bone Marrow Mesenchymal Stem Cells in Damaged Knee Articular Cartilage of Wistar Albino Rats- A Histopathological and Radiological Anatomy Study.

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Abstract: Knee osteoarthritis (OA) is a joint disease that can limit mobility and affect an individual's life quality. As empirical evidence significantly reported that there is no complete cure for OA knee in human beings, this study aimed to find the effectiveness of Bone marrow mesenchymal stem cells (BMMSCs) in treating the damaged articular cartilage of knee joint in Wistar albino rats. 15 male Wistar albino rats were divided into 3 groups. Control group (n=4 rats)- Normal rats, Experimental Group A (n=5 rats)- Rats with damaged articular cartilage deprived of BMMSCs treatment, and Experimental Group B (n= 5 rats)- Rats with damaged articular cartilage treated with BMMSCs. Trauma mimicking OA was created in the left knee joint of experimental rats. One rat was used for BMMSCs culture. Cultured BMMSCs were transplanted into the left knee joint of group B rats. On the 7th, 14th, and 28th day, histopathological and radiological analysis of articular cartilage was performed in all group rats. Hematoxylin and Eosin (H and E) stains were used for histopathological investigation, and a Modified Mankin score was used to grade it. Administration of BMMSCs drastically reduced knee swelling and ameliorated the alterations in articular cartilage in group B rats histopathologically and radiologically compared to group A rats. This may be due to the paracrine and chondroprotective effects of BMMSCs. BMMSCs could be considered a recovery treatment for OA knee in Wistar albino rats. However, this study achieved its aim not in humans. So, this work advises researchers to examine BMMSCs' effects in healing damaged articular cartilage in higher animal models before moving on to humans.

Keywords: Articular cartilage, Cell culture, Knee joint, Osteoarthritis, Stem cells, Wistar albino rats

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Date of Receiving 2 June, 2023

Date of Revision 17 June, 2023

Date of Acceptance 24 June, 2023

Date of Publishing 3 July, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors

Citation Dr Nirmal Kumar K, Dr Kalyana Panchakshari P and Dr Srikanth K , Effects of Bone Marrow Mesenchymal Stem Cells in damaged knee articular cartilage of Wistar Albino Rats- A Histopathological and Radiological Anatomy study..(2023).Int J Pharm Sci.14(3), b43-53 http://dx.doi.org/10.22376/ijpbs.2023.14.3.b43-53

1. INTRODUCTION

Osteoarthritis (OA) is the most common type of arthritis, which affects almost 303 billion people globally.[1] Its high prevalence, especially in older people, made them disabled and have deformities.[2] The primary concern in OA is pain which occurs due to progressive articular cartilage damage, formation of osteophytes, sclerosis of subchondral bone, biochemical changes like the release of inflammatory mediators (cytokines & chemokines) by synovium, fibrous capsule, and chondrocytes.[3-5] OA mainly affects weight-bearing joints yet spares others. Commonly affected joints include the cervical spine, lumbar spine, hip, knee, and first metatarsal phalangeal joint.[6] Aetiology of OA is not yet clear, but the imbalance of microbial community in the gut (Dysbiosis) is considered a predisposing factor.[7] Dysbiosis increases body mass index, insulin resistance, and systemic inflammation, resulting in OA pathogenesis.[8] Medical treatments available are targeted to ease OA symptoms rather than cure the disease.[9] As the effects of new drugs for treating OA are initially tested in animal models, it is vital to make animal models that ideally depict OA pathogenesis and its response to treatment will give a useful biochemical, radiological, and histological assessment of OA.[10] Empirical evidence reported that BMMSCs have the potential to protect, and regenerate the damaged articular cartilage and restore the pathogenesis of OA.[11-13] BMMSCs can differentiate into either bone, cartilage, muscle, tendon, ligament, etc. Chondrocytes from BMMSCs develop, maintain and repair the extracellular matrix (ECM), produce proteoglycans, and replace the degenerated chondrocytes with new ones (cartilage homeostasis) in the joint's articular cartilage.[14] Cartilage homeostasis is a complex interaction between metabolic, inflammatory, and apoptotic pursuit. [15] The potential of BMMSCs to differentiate into functional chondrocytes and produce growth factors in the damaged articular cartilage has drawn our attention.[9,16-18] So, this study aimed to determine the effectiveness of BMMSCs in treating traumatized knee articular cartilage in Wistar albino rats and to evaluate the results by radiographic and histopathological analysis.

2. MATERIALS AND METHODS

2.1. Animal Maintenance and Handling

Standard cages were used for housing, and clean food (Standard pellet) and water were available to Wistar albino rats at all times. Wistar albino rats were kept in ventilated plastic cages and housed in controlled animal care facility rooms with ambient temperatures of $25^0 \pm 2^0$ C and a 12-hour cycle of light and darkness was maintained. All efforts were made to minimize the animal suffering and to reduce the number of animals used. All Wistar albino rats were brought to the test environment ($25^0 \pm 2^0$ C and a 12-hour day/night cycle) during experimentation and allowed 24 hours for acclimatization to the environment. All necessary procedures recommended by the ethical committee and American Veterinary Medical Association were followed during animal maintenance and experimentation. Each cage was labelled with its group name and body weight. Out of 15 rats, one rat was used for cell culture, and the rest 14 rats were divided into the Control group (4 rats) and the Experimental group (10 rats). Experimental groups were further divided into group A (5 rats) - Rats with articular cartilage damage deprived of BMMSCs treatment and group B (5 rats) - Rats with articular cartilage damage treated with BMMSCs.

2.2. Isolation and Culturing of BMMSC

As per Ahmed et al[11] protocol, BMMSCs were isolated and cultured in this study: BMMSCs were aspirated from the rat's long bones (humerus, femur, and tibia). At room temperature, aspirated BMMSCs were centrifuged at 3000 RPM for 5 minutes. They were cultured in a T flask containing Dulbecco's Modified Eagle's Minimal essential medium (DMEM), supplemented with 0.36% sodium hydrogen carbonate, 10% fetal bovine serum, and 1% penicillin. It was kept in a 5% Co2 incubator at 37^0 C. Cultured bone marrow mesenchymal stem cells were observed up to the sixth passage. Cell growth and progress were observed during the passage. The first passage was done on 3rd day. The time interval between the subsequent passages was reduced. This showed that the cells adapted to the in-vitro environment. Around the 10th day, 6th passaged cells were collected using trypsin, and 10 µL of trypan blue (0.4%) was added to 10 µL of BM-MSCs. 6th passage cells were used for the transplant. BMMSCs were separated from in vitro culture by adding Antibodies CD44 and CD34. BMMSCs were counted and sorted by flow Cytometer. BMMSCs with higher than 95% viability were transplanted at a 5×10^6 cells/rat dose. Though wear and tear (internal trauma) of articular cartilage occurs in OA, the trauma of articular cartilage mimicking human OA was produced in the knee joint of Wistar albino rats.[19] In the operation theatre, with the help of a veterinary surgeon following procedures were carried out. To make rats anesthetized, 1.2ml of 0.9% saline was added to Ketamine 8ml and Xylazine 0.8ml. From this mixture, 0.1ml was administered intraperitoneally to rats.[11] In groups A and B left knee joint of the rat was selected uniformly. The rats' hair over the left knee joint was shaved using a sterilized blade. A surgical incision was made on the anterior aspect of the knee joint. The patella bone and ligamentum patellae were retracted using a retractor. After reaching the articular ends of the knee joint, articular cartilage in the lower end of the femur condyle was damaged with a sterile surgical blade. In the group, a rat's incision was closed and allowed for natural healing. In group B rats, 50 µL at a dose of 5×10^6 cells of BMMSCs was injected at the injured site, and the incision was closed. The incision area was sutured with black silk and dressed with betadine. Check computerized tomography was taken for the left knee joint to confirm whether articular cartilage was damaged, and this was compared with the normal right-side knee joint. The rate of recovery was observed in both groups. Articular cartilage of the left knee joint was excised from control and experimental groups rats for histopathological examination on the 7th day, 14th day, and 28th day. For 24 hours, the excised cartilage was fixed in 10% buffered formalin for preservation and in 10% formic acid solution for decalcification. Later it was dehydrated and embedded in paraffin wax. Using microtome, 5 µm sections were made and mounted on the slide. Slides were stained with hematoxylin and eosin stain (H&E) for examining the tissue under light microscopy. Modified Mankin score was followed to grade the histopathology of OA (refer to Table 1).[20-21] Radiologic analysis was done on the 7th day, 14th day, and 28th day for control and experimental groups rats. Using these findings structure of articular cartilage was analysed. After completion of this study, the Wistar albino rats used were given rehabilitation.

Table 1: Modified Mankin Score for histological observation of OA Articular Cartilage [20-21]

Category	Subcategory	Score
Structure	Normal	0
	Surface Irregularities	1
	Complete disorganization	2
	Clefts to the noncalcified layer	3
	Clefs to calcified layer	4
Cells	Normal	0
	Hypercellularity	1
	Hypocellularity	2
	Pyknosis	3
Tidemarks	Intact	0
	Damaged	1

2.3. Ethical Statement

After obtaining the institutional animal ethical committee approval from Saveetha medical college, Chennai (IAEC/SMC/I/03/2013),15 adult Wistar albino rats (weighing 100-200g) were utilized in this study. Institutional animal ethical committee rules and procedures were followed during this study in our animal experimentation.

3. RESULTS

3.1. Histopathology result

3.1.1. Control group rats: Normal rats

On days 7th, 14th, and 28th, the Modified Mankin score for the control group rats was 0 (structure, cells, and tidemarks of articular cartilage were normal), indicated by the black arrow in Figure. 1

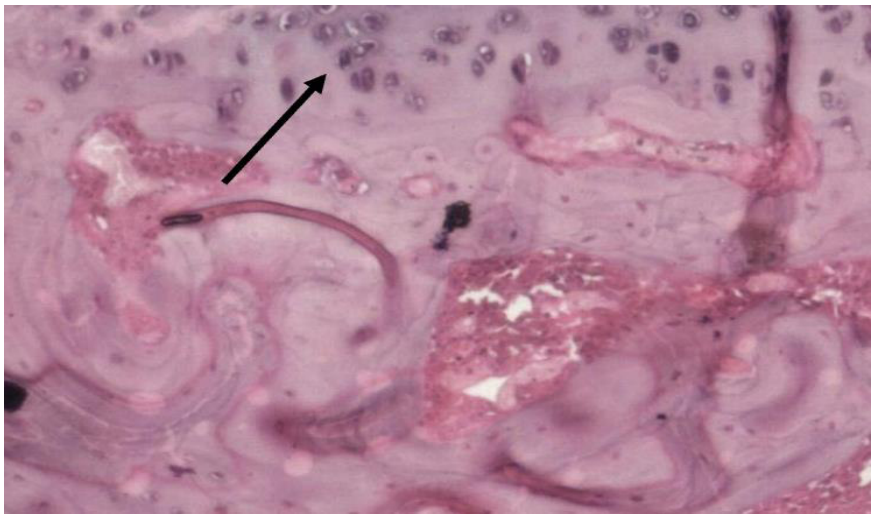

Fig 1: Histopathology of Articular cartilage on 7th, 14th and 28th day (H and E stain)

3.1.2. Experimental Group A

For rats with articular cartilage damage deprived of BMMSCs treatment on the 7th day, the Modified Mankin score for the structure was 1 (surface irregularities), and for cells was 2 (hypocellularity). Tidemarks of articular cartilage was 1 (damaged), which was indicated by the black arrow in Figure. 2

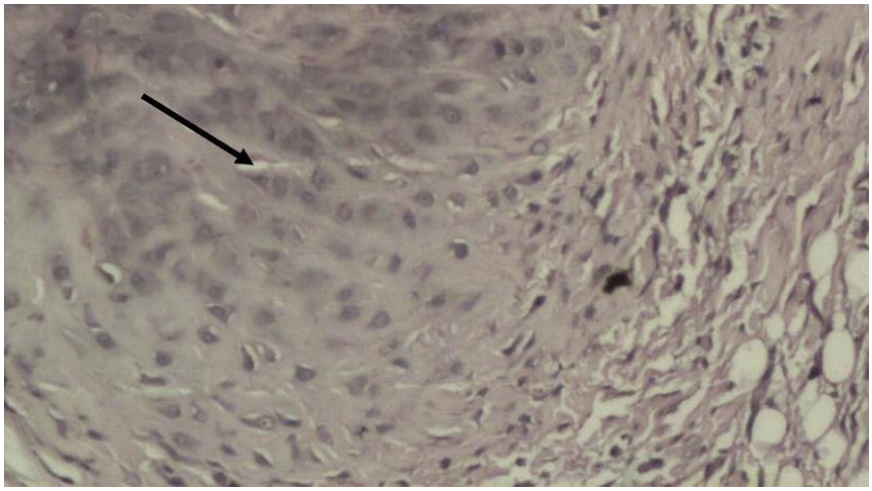

Fig 2: Histopathology of Articular cartilage on 7th day (H and E stain)

On the 14th day, the Modified Mankin score for the structure was 3 (Clefts to the noncalcified layer), and for cells was 2 (Hypocellularity). Tidemarks of articular cartilage was 1 (damaged), indicated by the black arrow in Figure.3

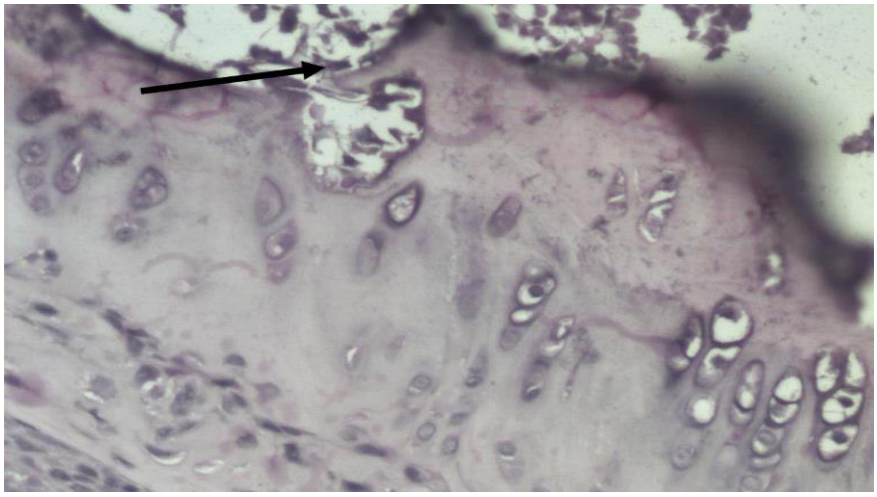

Fig 3: Histopathology of Articular cartilage on the 14th day (H and E stain)

On the 28th day, the Modified Mankin score for the structure was 4 (Clefts to calcified layer), and for cells was 3 (Pyknosis). Tidemarks of articular cartilage was 1 (damaged), which was indicated by the black arrow in Figure. 4

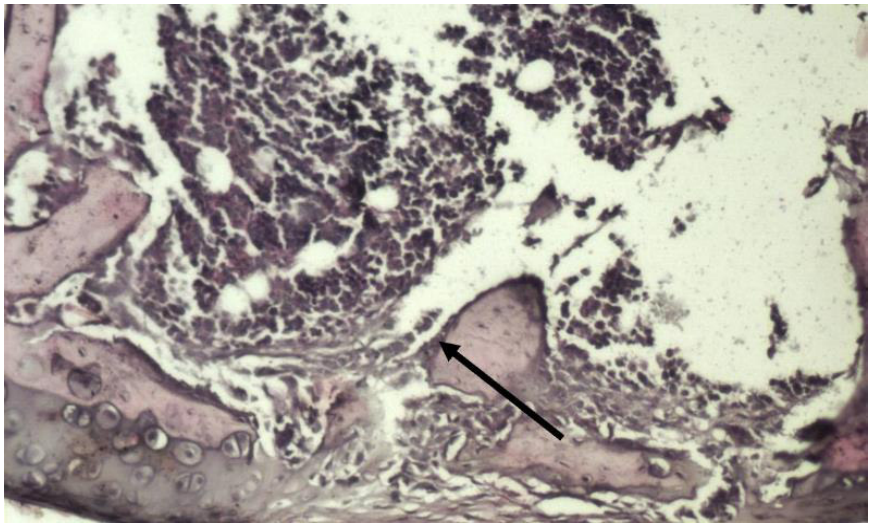

Fig 4: Histopathology of Articular cartilage on the 28th day (H and E stain)

3.1.3. Experimental Group B

For rats with articular cartilage damage treated with BMMSCs, on the 7th day, the Modified Mankin score for the structure was 1 (surface irregularities), and for cells was 1 (hypercellularity). Tidemarks of articular cartilage was 1 (damaged), which was indicated by the black arrow in Figure. 5

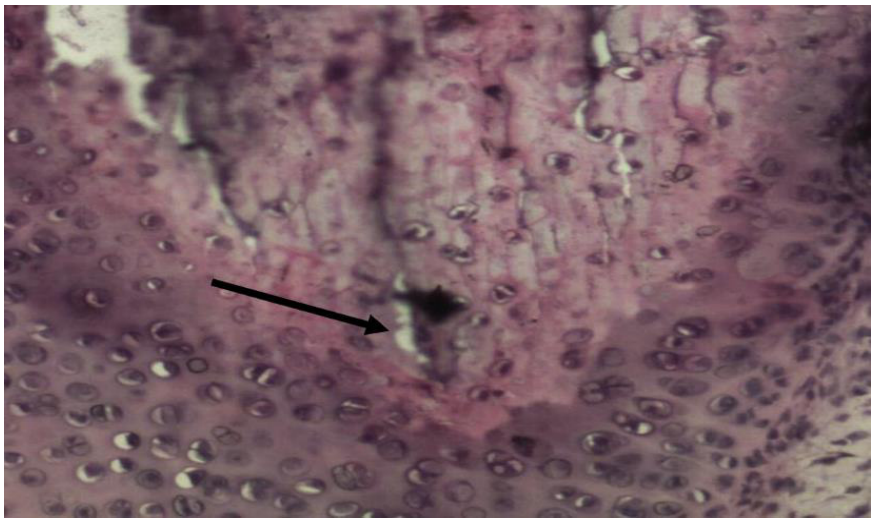

Fig 5: Histopathology of Articular cartilage on the 7th day (H and E stain)

On the 14th day, the Modified Mankin score for the structure was 0 (normal), and for cells was 1 (Hypercellularity). Tidemarks of articular cartilage were 0 (intact), which was indicated by the black arrow in Figure. 6

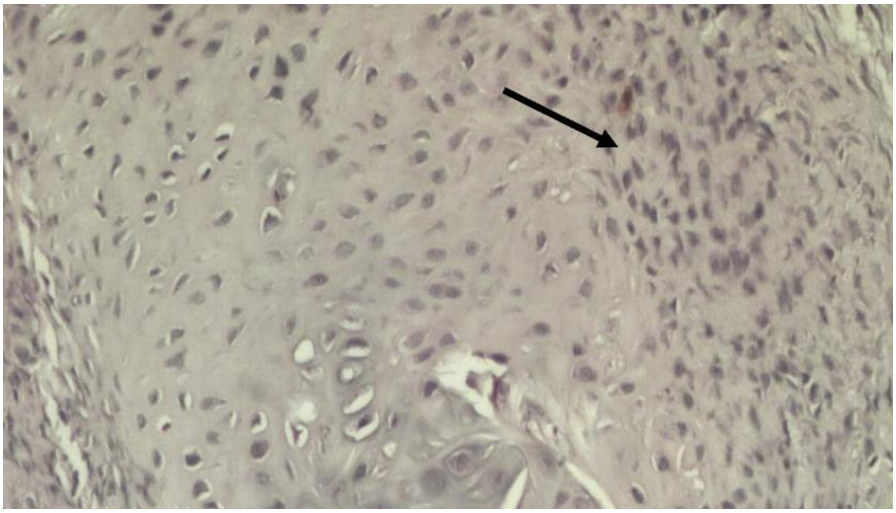

Fig 6: Histopathology of Articular cartilage on the 14th day (H and E stain)

On the 28th day, the Modified Mankin score for the structure was 0 (normal), and for cells was 0 (normal). Tidemarks of articular cartilage were 0 (intact), indicated by the black arrow in Figure. 7

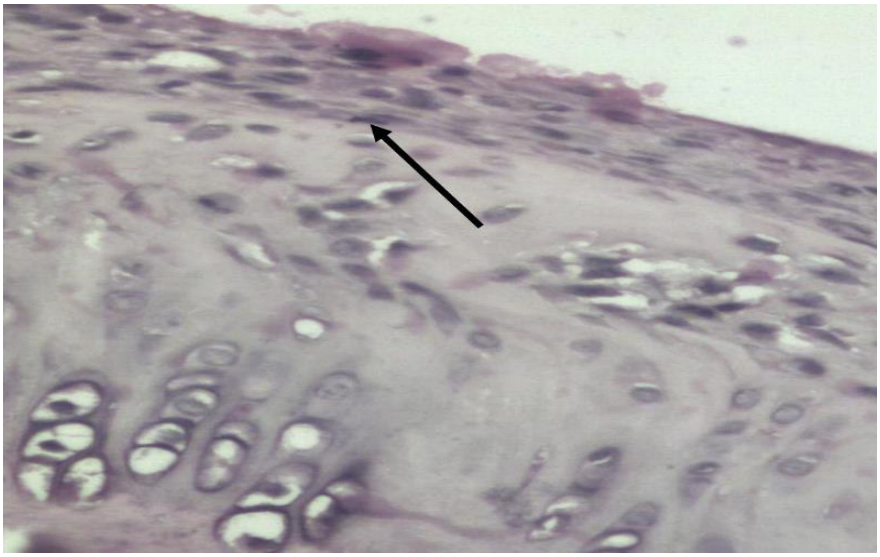

Fig 7: Histopathology of Articular cartilage on the 28th day (H and E stain)

3.1.4. Computerized Tomography Results Control group: Normal rats

On the 7th, 14th, and 28th day normal architecture of the articular cartilage of the knee joint was observed (Refer to Figure.8)

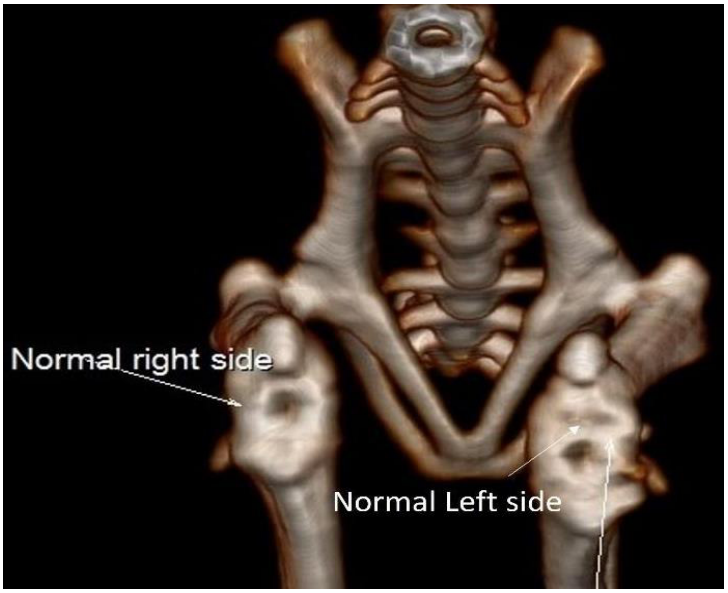

Fig 8: Wistar albino rat- Computerized Tomography image of the knee joint

3.1.5. Experimental Group A

For rats with articular cartilage damage deprived of BMMSCs treatment, no signs of healing in articular cartilage were observed on the 7th, 14th, and 28th day. Moreover, irregularities in the joint articular surface and increased joint space were observed on the 28th day (Figure 9-11).

- **On 7th day**

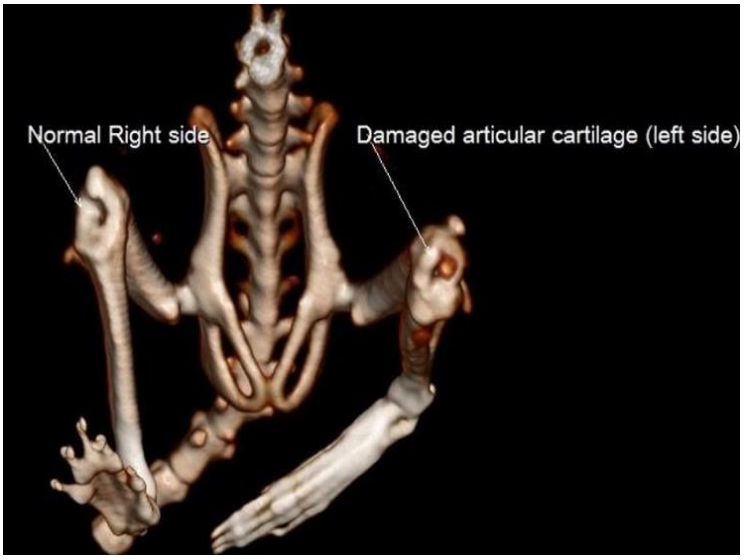

Fig 9: Wistar albino rat -Computerized Tomography image of the damaged knee joint (Left side)

- On 14th day

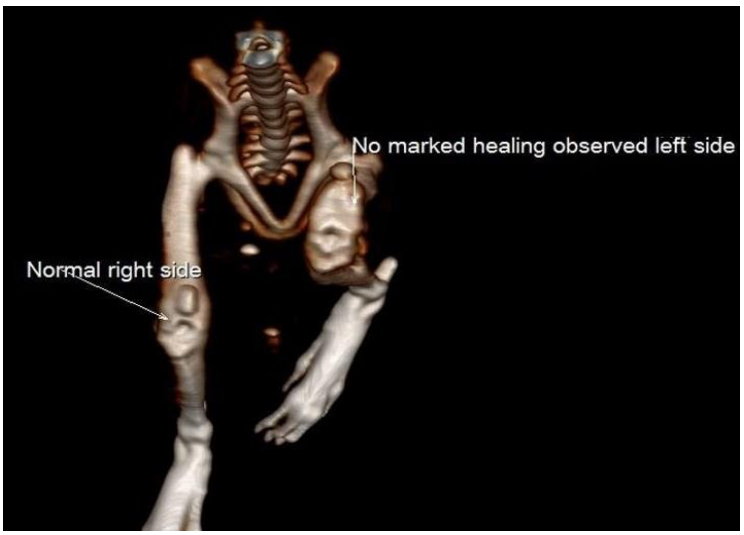

Fig 10: Wistar albino rat -Computerized Tomography image of the unhealed knee joint (Left side)

- On 28th day

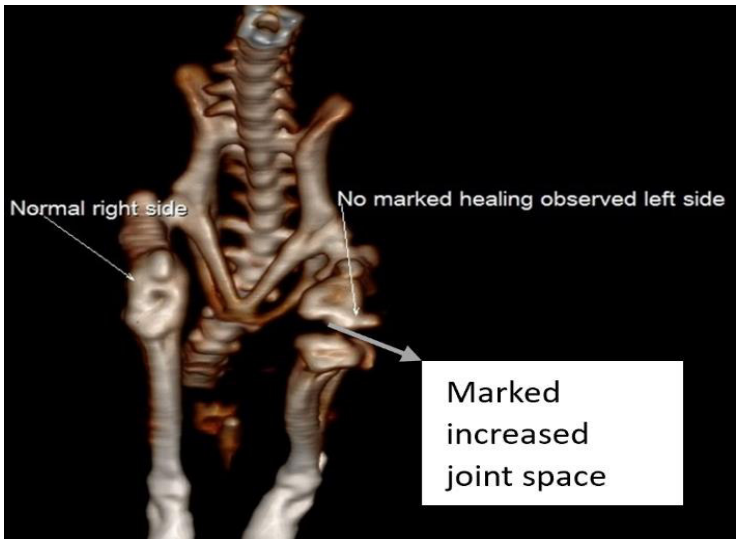

Fig 11: Wistar albino rat -Computerized Tomography image of the unhealed and deformed knee joint (Left side)

3.1.6. Experimental Group B

For rats with articular cartilage damage treated with BMMSCs, on the 7th day, no signs of healing in articular cartilage were observed (Refer to Figure.12)

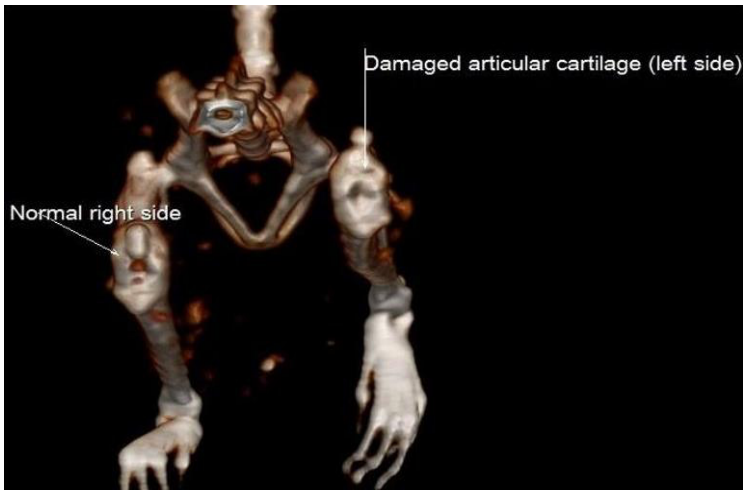

Fig 12: Wistar albino rat -Computerized Tomography image of the damaged knee joint (Left side)

On the 14th day, reduced irregularities in the articular surface and reduced joint space were observed (Refer to Figure.13).

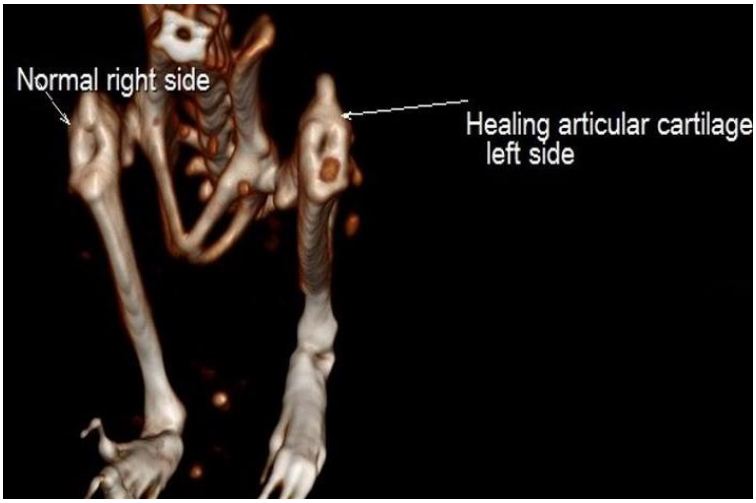

Fig 13: Wistar albino rat -Computerized Tomography image of the healing knee joint (Left side)

On the 28th day, completely healed articular cartilage was observed with a smooth surface and normal joint space (Refer to Figure.14).

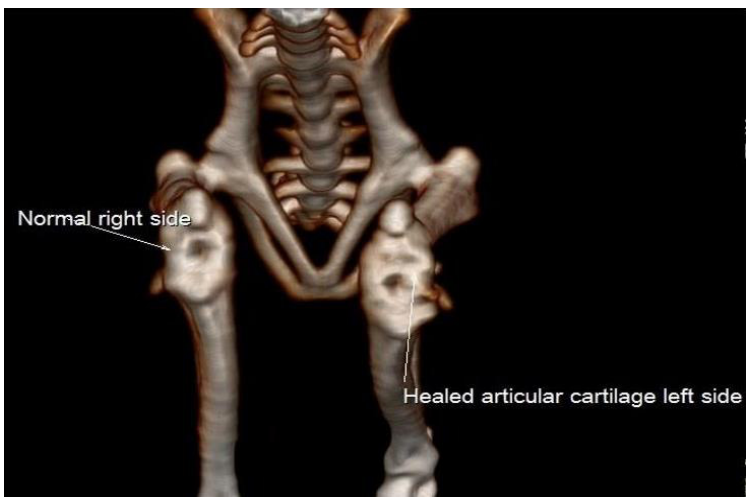

Fig 14: Wistar albino rat -Computerized Tomography image of the healed knee joint (Left side)

4. DISCUSSION

Osteoarthritis (OA) was considered a non-inflammatory joint disorder characterized by wear and tear of articular cartilage. As research advances, OA is considered a joint disease with inflammation and cartilage degradation.[22] The repairing potential of degraded cartilage on its own is very limited.[17,23] Empirical evidence stated that there is no complete cure for OA in humans.[24] On the other hand, BMMSCs bear paracrine and chondroprotective effects. BMMSCs also have the potential to differentiate into chondrocytes and their matrix. So BMMSCs can be considered as alternate means of curing OA knee.[25-29] In the present study, we observed the effects of BMMSCs in healing the damaged knee articular cartilage in Wistar Albino rats. As joint swelling is one of the common symptoms in OA knee, which occurs due to the escape of fluid from blood vessels into the joint cavity. After injecting the BMMSCs in group B rats, there was a marked reduction in knee swelling. Similarly, O. Kehoe et al. proved that BMMSCs reduced knee swelling in murine.[30] Our histopathological findings revealed that in the control group rats, the Modified Mankin score was 0 on the 7th, 14th, and 28th days. This indicated that the structure, cells, and tidemarks of articular cartilage of the knee joint were normal. In experimental group A, according to the Modified Mankin score structure of articular cartilage showed irregularities in the surface (score 1) on the 7th day and the appearance of apertures in the noncalcified layer (score 3) on the 14th day, which progressed to calcified layer (Score 4) on 28th day. Hypocellularity was observed on the 7th and 14th day, followed by pyknosis of chondrocytes noticed on the 28th day. Damage in the tidemark was noticed on the 7th, 14th, and 28th day. K. Lampropoulou – Adamidou et al. and P.K. Gupta et al noticed similar findings, they concluded that the process of articular cartilage healing was delayed in rats without BMMSCs treatment compared to rats treated with BMMSCs.[31,33] In experimental group B, according to the Modified Mankin score on the 7th-day structure of the articular cartilage showed irregularities in the surface (score 1), hypercellularity (Score 1), and damage in the tidemark of articular cartilage (score 1). On the 14th day structure of the articular cartilage was normal (score 0), hypercellularity was observed (Score 1), and the tidemark of articular cartilage (score 0) was intact. On the 28th day, the Modified Mankin score was 0, indicating the structure, cells, and tidemarks of the knee joint's articular cartilage were normal. Similar findings were reported by I. Takahashi et al. and T. Ichiseki et al. 2018. However, even after a month of treatment of damaged articular cartilage with BMMSCs, hypercellularity of chondrocytes was observed.[33,34] On the 7th, 14th, and 28th days our radiological findings showed no damage in the structure of articular cartilage, joint space was normal, and no degenerative changes in the control group rats. In group, A rats, on the 7th, 14th, and 28th day there were no signs of healing, and an increase in particular surface irregularities and joint space was noticed. In group B rats, on the 7th day, there were no signs of healing, and an increase in joint space was noticed. On the 14th day, signs of articular cartilage healing were observed. On the 28th day, the normal architecture of articular cartilage was regained completely compared with the control group rats. Our radiological findings were in harmony with S.V Morais et al. and Jaleel et al.[19,35] Considering the histopathological and radiological outcomes, our results imply that BMMSCs-treated rats showed reduced inflammatory signs, delayed cartilage degradation, and cartilage regeneration was observed. Although our study achieved its aims, there were some limitations, like our study not focused on the biochemical or molecular level of assessing the potential of BMMSCs in treating OA knee in Wistar albino rats. Moreover, the sample size was also small, and the study duration was short. So, we author recommend the researchers focus on the biochemical and molecular assessment of BMMSCs effects in a large sample size with long-term study in Wistar albino rats.

5. CONCLUSIONS

BMMSCs showed marked healing effects in the damaged articular cartilage of rats treated with BMMSCs compared to rats that deprived BMMSCs treatment. Moreover, no signs of healing were observed in the damaged articular cartilage of group A rats. So according to our study BMMSCs can be considered an authentic treatment for inflammatory OA in Wistar albino rats. As there is no cure for OA knee in humans and its prevalence is increasing daily globally, this study suggests that researchers should first investigate BMMSCs' abilities to repair injured articular cartilage in higher animal models before focusing on human beings.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. Nirmal Kumar K conceptualized, designed, and prepared the original study, Dr. Kalyana Panchakshari P analyzed the data, and Dr. Srikanth K provided valuable inputs to design the manuscript. All the authors read and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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