

1 **Original research paper**

2 **Surgery**

3 **Title: Efficacy of Adipose and Bone Marrow Derived Mesenchymal Stem Cells for**  
4 **Treatment of Surgically Induced Knee Osteoarthritis in Rats**

5 **Running title: Stem cell therapy for Knee Osteoarthritis in Rats**

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26 **ABSTRACT**

27 **Objective:**To evaluate the efficacy of direct intra articular (IA) injections of adipose derived  
28 mesenchymal stem cells(ADSCs) and bone marrow derived mesenchymal stem cells (BMSCs) for  
29 regenerating articular cartilage in surgically induced knee osteoarthritis (OA) in sprague-dawely  
30 rats.

31 **Design:** A randomized-controlled study.

32 **Animals:** thirty Sprague dawely rats were divided into 5 groups (6 rats in each group).

33 **Procedures:**OA was surgically induced by medial collateral ligament (MCL) transection and  
34 medial meniscal tear of the left knee joints to induce joint destabilization of the left knee. 4 weeks  
35 after the operation, single dose of ADSCs, BMSCs and phosphate buffer saline (PBS) was  
36 delivered to the operated knee by direct aseptic IA injection in each treated group (III), (IV) and  
37 (V) respectively. The healing was assessed clinically, grossly and microscopically at 10 weeks  
38 postoperatively.

39 **Results:** rats that received ADSCs grossly showed better efficiency in regenerating of articular  
40 surface and closed to the normal knee morphology without any noticed defects and showed better  
41 cartilage quality and lower degree of cartilage degeneration histopathologically and there was a  
42 trend towards higher scores for all parameters in treated groups, and demonstrated that  
43 chondrocytes and chondroid matrix regained their histological architecture upon ADSCs more than  
44 BMSCs.ADSCs is mimicking the normal articular cartilage, the BMSCs alleviated inflammatory  
45 process and increased the lay down of chondroid matrix and the superficial cracks remains for  
46 some degree. ADSCs exhibited mild Immunohistochemical expression against Matrix  
47 Metalloproteinase 9(MMP9) in comparison to other treated groups, at the time the control group  
48 showed no expression at all.

49 **Conclusion and clinical relevance:**

50 The results of the present study demonstrated that ADSCs and BMSCs can alleviate knee OA via  
51 their strong regenerative capacity on chondrocytes and increased chondroid matrix deposition. So,for  
52 such positive results, ADSCs are more effective and easily available compared to BMSCs.

53

54 **Key words :** OA, MCL, ADSCs, BMSCs, Rat.

55

## 56 1. INTRODUCTION

57 Osteoarthritis is the most commonly diagnosed joint disease in both human and veterinary medicine  
58 [1]. OA is a chronic, degenerative process characterized by progressive cartilage deterioration,  
59 subchondral bone remodeling, and loss of joint space, marginal osteophytosis, and loss of joint function.  
60 Although the etiology of OA may differ across species or among individuals within a species, some  
61 components of the pathophysiology of the disease are consistent [2, 3]. OA is a condition not only  
62 associated with focal damage of the articular cartilage, but also with other intraarticular structures. It is  
63 characterized by inflammation and catabolic joint metabolism and biochemical changes which affect the  
64 results of chondrocyte-based treatments [4].

65 Mesenchymal stem cells (MSCs) have been proposed instead of mature chondrocytes for OA  
66 treatment, because of their paracrine effects, anti-inflammatory and immunomodulatory  
67 properties, beside their capability to differentiate in cells of chondrocyte lineage within the lesion  
68 site [5]. Different cell sources exist but currently the most investigated cells for cartilage  
69 regeneration and OA treatment are BMSCs and ADSCs [6].

70 ADSCs have shown numerous advantages, including the high number, easier availability, and the  
71 evidence to be able to differentiate toward osteoblasts and chondrocytes [7]. Intra-articular (IA) injection  
72 of ADSCs reduced synovitis, osteophyte formation, cartilage degeneration [8], and improved the cartilage  
73 degradation and reduced knee synovitis in OA rabbit model [9].

74 BMSCs have multipotent differentiation potential (could differentiate into numerous tissues, such as  
75 bone, cartilage and fat), self-renewal capacity and immunomodulatory properties, has great potential for  
76 use in stem cell-based articular cartilage diseases [10]. BMSCs also have shown desirable effects in the  
77 treatment of OA, probably via the secretion of bioactive trophic factors to exert potent anti-inflammatory,  
78 immunomodulatory, and antifibrotic effects [11].

79 Pain and disability are the primary symptoms for patients who suffer from OA.  
80 relationship between a specific molecular, cellular or pathological event and OA pain, requires  
81 therapeutic or prophylactic modification of that factor, severity of the pain defining the key changes  
82 that cause OA pain needs to be investigated in pre-clinical models where such factors can be  
83 therapeutically targeted [12].

84 This study aimed to evaluate the effectiveness of ADSCs and BMSCs derived MSCs in regeneration of  
85 surgically induced osteoarthritic knee in sprague-dawley (SD) rats.

86 **2. MATERIALS AND METHODS**

87 *2.1. Animals, housing and feeding*

88 This study was carried out on a total number of 30 male Sprague–Dawley (SD) rats (body weight 200–  
89 250 gram). Rats were housed at temperature 20°C-25°C in the animal house of Medical Experimental  
90 Research Center (MERC), Faculty of Medicine, Mansoura University. They were retained under  
91 constant conditions and supplied with standard diet and water ad libitum. The experimental protocol of  
92 this work was approved by the Local Ethical Committee, Faculty of Medicine, Mansoura University in  
93 accordance with the Ethics committee of national research Center-Egypt with registration number  
94 (09/189).

95 *2.2. Study design*

96 All rats were divided into 5 groups: group (I) (negative control group, 6 rats). Surgical induction of OA  
97 was performed in 25 rats which were divided into group (II) (positive control), group (III): osteoarthritic  
98 rats were treated with BMSCs, group (IV) osteoarthritic rats were treated with ADSCs,  
99 group (V) osteoarthritic rats were treated with PBS. Single dose of a million cells derived from bone  
100 marrow & adipose tissue and suspended in 100 µl of PBS was delivered to the operated knee by direct  
101 aseptic IA injection 4 weeks postoperative [13].

102 All rats were anaesthetized by intra-peritoneal (IP) injection of a mixture Xylazine HCl in dose 10  
103 mg/kg (Xylajet (20mg/ml), ADWIA, Egypt) and ketamine HCl in dose 75mg/kg  
104 (Ketamax(50mg/ml), Troikaa Pharmaceuticals Ltd, Gujarat, India) according to [14].

105 The skin over the medial aspect of the left knee joint was aseptically prepared. The rats were fixed in  
106 dorsal recumbency in plate of wax. A 2-3 cm medial parapatellar incision was performed. Blunt  
107 dissection was performed to expose the medial collateral ligament (MCL), which was next transected to  
108 expose the medial meniscus. The medial meniscus was cut across its full thickness to induce joint  
109 destabilization of the left knee. Following transection of the meniscus, the joint capsule and s/c tissues  
110 were sutured with simple continuous pattern using 4/0 Vicryl. The skin was opposed by simple  
111 interrupted stitches using 4/0 monofilament Nylon according to [15]. After surgery, the rats were kept in  
112 a recovery room for about 2 h under heat lamps to maintain body temperature and to monitor any adverse  
113 effects. Each rat was given amoxicillin (E-MOX, EIPICO, Egypt) in dose (20 mg/kg IM), and analgesic

114 Meloxicam (MOBITIL, MUP, Egypt) in dose (5 mg/kg SC) once a day for the first 3 days, and once  
115 animals regained complete consciousness, permitted free cage activity until the end of the study .

### 116 2.3. BMSCs isolation and culture

117 Rat BMSCs were isolated by flushing morselized femurs and tibia from 4-week old, male, SD rats. The  
118 harvested cells were cultured in BMSCs culture medium, consisting of low-glucose Dulbecco's  
119 modified Eagle's medium (L-DMEM) containing 10% fetal bovine serum (FBS, Lonza, Verviers,  
120 Belgium), 50 µg/ml gentamicin and 1.5 µg/ml fungizone. All media were renewed twice a week. These  
121 primary cells were referred to as passage 0 (P0). The confluent cells were dissociated with 0.25% trypsin  
122 and 0.01% EDTA, and subcultured in new six-well culture dishes at a plating density of  $5 \times 10^4$  cells/well.  
123 These procedures were repeated four times and the cultures were referred to as P1, P2, P3, P4 and P5  
124 according to method of [16].

### 125 2.4. ADSCs isolation and culture

126 The steps of ADSCs preparation were performed under complete a septic condition in a biosafety  
127 cabinet, while wearing appropriate personal protective equipment. Place adipose tissue in a  
128 preweighed sterile petri dish and weigh to get a final tissue weight . Mince the fat into small pieces  
129 with a sterile scalpel and blade until tissue is no longer fibrous. Place tissues into a 15-mL tube  
130 with an equal volume of prewarmed PBS and agitate for 45 s. Allow the mixture to separate into  
131 phases for 3–5 min and then remove the infranatant. Continue the process until the infranatant is  
132 clear for successive 4 washes. Add an equal volume of collagenase solution to the adipose solution,  
133 clean the outside of the tube with 70% EtOH, cover the top with para-film, and place in a 37°C  
134 shaking water bath at 75 rpm for 1.5 min or until the tissue becomes homogenous. Vortex for 15 s  
135 to thoroughly mix cells and then centrifuge at 1,200 rpm ( $300 \times g$ ) for 5 min. Vortex solution for  
136 10 s to get as many cells out into the media as possible. Then centrifuge again at 1,200 rpm ( $300$   
137  $\times g$ ) for 5 min., carefully, remove the supernatant consisting of lipids, primary adipocytes, and  
138 collagenase solution, leaving the pelleted stromal vascular fraction . Resuspend the pellet in 1%  
139 BSA solution. Transfer solution to a new 15-mL centrifuge tube, centrifuge cells at 1,200 rpm ( $300$   
140  $\times g$ ) for 5 min and remove the supernatant. When aspirating, keep the tip of the pipette very  
141 superficial so the majority of liquid is removed. Resuspend the pellet in 1 mL of stromal

142 media. Centrifuge a 20- $\mu$ L aliquot in a microcentrifuge tube at 1,200 rpm ( $300 \times g$ ) for 5  
143 min. Remove the supernatant and resuspend the pellet in 20  $\mu$ L of red cell lysis buffer. Incubate for  
144 5 min at room temperature. Add 20  $\mu$ L of Trypan Blue and count the cells with a  
145 hemocytometer. Plate the cells at the appropriate density in complete stromal media and incubate  
146 at 37°C and 5% CO<sub>2</sub>. Change the media after 24 h to remove nonadherent cells. Media should be  
147 changed about every 3 days according to method of [17].

## 148 2.5. Evaluation of OA

### 149 2.5.1. Clinical assessment

150 Subjective assessment of pain was performed by observing changes in general locomotor activity (e.g.,  
151 guarding a specific area or avoiding weight-bearing on an injured limb and changes in food and water  
152 intake and body weight [18].

153 Mechanical hyperalgesia was assessed by the Randall–Sellito analgesiometer (Ugo Basil,  
154 Varese, Italy). Briefly, the rats were maintained in a normal/horizontal position. The left hind paw  
155 was placed in an analgesiometer, which was composed of a cone-shaped paw-pressor with a  
156 rounded tip that was used to apply linear increasing force to test paw. The withdrawal threshold  
157 was taken as the point at which the rat vocalized or struggled vigorously, expressed as the force in  
158 grams (g). The withdrawal threshold (gram) was recorded at the first, second, third and fourth  
159 week respectively after treatment and compared to the control group [19].

160 All rats in each group were euthanized 10 weeks after surgery with IP injection of thiopental  
161 sodium in a dose of 120 mg/kg [20]. Assessment of joint stiffness by measurement of maximum  
162 extension angle of the knee. The left knee of each animal was dissected and the articular cartilage  
163 was left intact. After the dissection, the maximum extension angle of each knee was measured  
164 with zero degree represent as the maximum possible extension, so the lower the angle value, the  
165 better the knee function [21].

### 166 2.5.2. Gross evaluation

167 The femoral condyle and tibial plateau were collected, and surfaces of the cartilage were examined  
168 macroscopically and photographed using a digital camera. Cartilage lesions were evaluated by two  
169 examiners who were blinded to treatments [22].

170

171 2.5.3. *Microscopical evaluation*

172 The preparation of knee specimen for the microscopic examination at 10 weeks postoperative.  
 173 The fixation of the specimens was done in 10% buffered formalin, and then decalcified with EDTA  
 174 for 8-10 weeks till they became soft. Fixed samples were processed by the paraffin embedding  
 175 technique, sectioned, and stained by hematoxylin and eosin (H&E), Masson’s trichrome and  
 176 Safranin O stains. Cartilage changes were graded according to histopathological Mankin score in  
 177 (Table 1)[23].

178 **Table 1.** Histopathological Mankin scores

Cartilage structuring		Chondrocytes		Safranin-O Staining		Tidemark	
Score	Definition	Score	Definition	Score	Definition	Score	Definition
0	Normal	0	Normal	0	Normal	0	Intact
1	superficial fibrillation	1	Diffuse hypercellularity	1	Slight reduction	1	Tidemark infiltrated with vessel
2	Pannus and superficial fibrillation	2	Cell clusters	2	Moderate reduction		
3	Fissures to mid zone	3	Hypocellularity	3	Severe reduction		
4	Fissures to deep zone			4	No staining		
5	Fissures to calcified zone			5	Total disorganization		

179 2.5.4. *Immunohistochemical Analysis*

180 Paraffin embedded sections were rehydrated in graded alcohols, steamed in citrate buffer at pH 6 and  
 181 probed at room temperature for 2 hours using the MMP-9 (rabbit polyclonal; 1:250; Abcam [AB38898]  
 182 and processed with a polymer-HRP kit (BioGenex) with diaminobenzidine development and Mayer  
 183 hematoxylin counterstaining according to[24].

184 The Immunohistochemical expression against mmp9 for the enzyme matrix metalloproteinase to  
 185 display marked expression in the articular cartilage in the diseased group in comparison to other group  
 186 and calculate number of immunopositive cells per 1000 cells.

187

188

189 2.5.5. *Statistical analysis*

190 All data obtained from the experiment were expressed as means  $\pm$  SEM. Statistical analysis of data  
191 was carried out by software SPSS program [25] using the one-way analysis of variance ANOVA  
192 followed by post hoc tukey for testing the significant differences between variables. Results were  
193 considered significant only at the level of ( $p \leq 0.05$ ).

194 **3. RESULTS**

195 *3.1. Clinical findings*

196 All rats experienced pain and discomfort in the form of gradually decreased activity, avoiding weight-  
197 bearing on an injured limb, decreased appetite, pale appearance of conjunctiva and some osteoarthritic  
198 rats suffered from weight loss. All these clinical signs were diminished gradually.

199 In mechanical hyperalgesia, the improvement of the decreased withdrawal threshold in ADSCs  
200 treated group was significantly higher compared to all other treated groups but this improvement still  
201 significantly lower than the control normal group (Table 2).

202 Assessment of joint stiffness by measurement of maximum extension angle of the knee, ADSCs  
203 treated group showed a significant enhancement of the increased maximum angle of the knee extension  
204 found in the diseased group, but still significantly higher than the control normal group, less than PBS  
205 treated group and BMSCs treated group (Table 2).

206 *3.2. Gross finding*

207 The isolated articular cartilage surfaces from 5 groups were grossly observed. There was no gross  
208 evidence of any side effects such as infection or tumor formation throughout the observation period.  
209 Gross morphological features of the knee joints from various treatment groups were compared with  
210 control normal group. Osteoarthritic knee joint showed formation of hard, thick, yellowish fibrotic tissue  
211 and exhibited severe erosion and fibrillation (granular appearance)(Fig. 1A) over the whole articular  
212 surface compared with the gross appearance of normal group with smooth, glistening surface with no  
213 fibrillation or erosions(Fig. 1B).Also, macroscopic observations from femoral condyle in BMSCs group  
214 represented intense reddish patches distributed in the almost of articular surface resulted from  
215 diminishing in cartilage thickness reflecting the colour of red marrow of sponge bone and grossly evident  
216 of less articular cartilage erosion and fibrillation in most regions of the articular knee surface compared  
217 with the OA group(Fig.1C).In the ADSCs group, the gross observation of articular surface showing

218 homogeneity of grayish coloration near normal appearance without any noticed defects indicating better  
219 efficiency in regenerating of articular surface and is closer to the normal knee morphology(Fig.1D,E).The  
220 gross appearance of the PBS group was like OA group ,articular surface showing marked erosion of  
221 trochlear articular surface exposing underling vascularized tissue (sponge bone of distal end of femur)  
222 (Fig.1F),and partial erosion of condyloid articular surface with appearance of reddish coloration of  
223 sponge bone (Fig.1G ). In the ADSCs group, cartilage regeneration was significantly higher versus all  
224 treated groups whereas no significant regeneration was found in PBS group in comparison to other  
225 different treatment groups.

226

### 227 *3.3.Microscopical findings*

228 The histological score for the cartilage structure estimated that OA group showing significant  
229 cartilage destruction towards increasing in the fissures to the deep zone ,fissures to the calcified  
230 zone, articular degeneration ,fibrillation as dentated surface (Fig.2A;arrow) and articular surface  
231 thinning (Fig.2B;arrow) in comparison to other groups, also OA joint displayed articular capsule  
232 with exuberant fibrous proliferation protruding from capsule (Fig.2C;arrow) and hyperplasia in  
233 synovial membrane appeared as multilayers cuboidal epithelium (Fig.2D; arrow) and congested  
234 blood vessels. Meanwhile, the ADSCs group showed marked improvement of cartilage structure  
235 with only minimal superficial fibrillation and normal synovial membrane appeared as monolayer  
236 of flattened cuboidal epithelium (Fig.3B; arrow) with normal underling stromanealy similar to  
237 control group. while in BMSCs group ,the histological view displayed hyperplastic synovial  
238 membrane appears as multilayer of cuboidal to columnar epithelium (Fig.3D; arrow) which  
239 showing significant improvement in histological score as compared to the OA group and PBS  
240 group but still less than control normal (Fig.2E,F) and ADSCs treated group(Table 3) .

241 The histological examination for the chondrocytes within the various groups demonstrated that  
242 OA group showing significant chondrocyte pathological alterations, including hypocellularity and  
243 destruction of the lacunae and cloning of chondrocytes forming clusters (Fig.2A;arrow head) in  
244 comparison to other groups, in the contrary, the ADSCs group showed marked significantly  
245 improvement of chondrocytes histological architecture with normal chondrocytes inside its  
246 lacunae and normal basophilic chondroid matrix (Fig. 3A;arrow) which only displayed mild  
247 hypercellularity. while The histological examination for the chondrocytes in BMSCs group

248 displayed focal loss of chondrocytes appears and chondroid matrix as fade basophilic devoid from  
249 lacunae (Fig.3C;arrow) showing significant improvement in histological score as compared to  
250 PBS group which displayed fused lacunae (Fig.3E;arrow) and loss of chondroid matrix in articular  
251 surface which appeared pale esinophilic(Table 3) .

252 The histological examination for cartilage stained with safranin O stain explore that OA group  
253 showing Severe reduction in the staining of chondroid matrix which appears faint from red safranin  
254 stain at which indicates marked loss of chondrocytes and loss of proteoglycan at chondroid matrix  
255 (Fig.4A;arrow) in comparison to other groups, in the contrary, the ADSCs group showed slight  
256 loss of proteoglycan at chondroid matrix(Fig.4B;arrow) while the remaining articular surface  
257 appears normal red stained against safranin stain in comparison to other treated groups. while The  
258 histological examination for cartilage in BMSCs treated group showing articular degeneration  
259 represented by fissured surface and uneven distribution of safranin O stain with loss of  
260 chondrocytes(Fig. 4C;arrow),The histological score for cartilage stained with safranin O stain in  
261 BMSCs treated OA group and PBS treated group (Fig.4D; arrow) but still significantly higher than  
262 normal group (Fig.4E;arrow) and no significant improvement in histological score as compared  
263 to ADSCs treated group(Table 3).

264 The histological findings in OA group showing decrease length of articular surface with tidemark near  
265 to articular surface (Fig.2B;arrow head) it was significantly destructed and penetrated blood vessels,more  
266 significantly in OA group in the comparison to all other groups(Table 3).

267 Using masson trichrome stain which is specific stain for the collagen fiber in the chondroid  
268 matrix revealed loss in OA group(Fig.5A) and PBS group (Fig.5D), meanwhile in the ADSCs  
269 (Fig.5B) and BMSCs(Fig.5C)displayed marked restoration.The normal group was normal  
270 histological appearance (Fig.5E).

271 The Immunohistochemical expression against MMP9 for the enzyme matrix metalloproteinase  
272 displayed strong marked expression in the articular cartilage in the OA group (Fig.6A) in  
273 comparison to other groups. ADSCs treated group exhibited mild expression of it (Fig.6B) in  
274 comparison to other treated groups at the time the control normal group showed no expression at  
275 all (Fig.6E) while in the BMSCs group displayed moderate expression in the articular cartilage  
276 (Fig.6C) that showed significantly increase in expression in the articular cartilage as compared to  
277 the control group and ADSCs treated group .But in the PBS treated group showed significantly

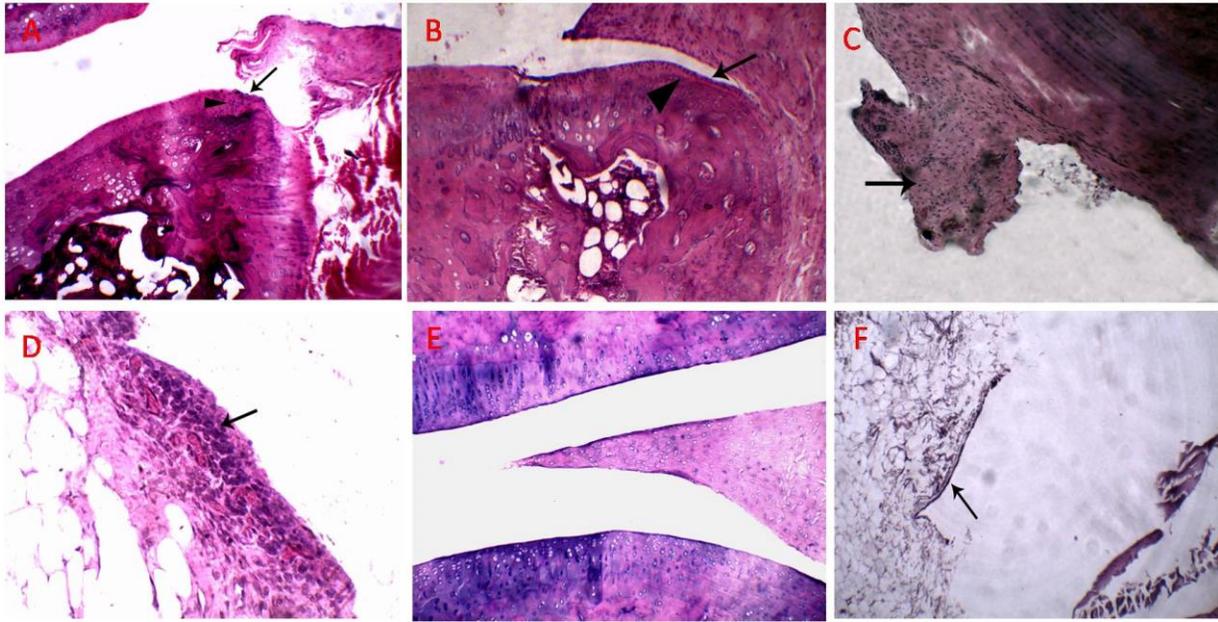
278 increase in expression in the articular cartilage (Fig.6D) as compared to all treated groups but  
279 showed no significant improvement in histological score in expression in the articular cartilage as  
280 compared to OA group(Table 4).

281



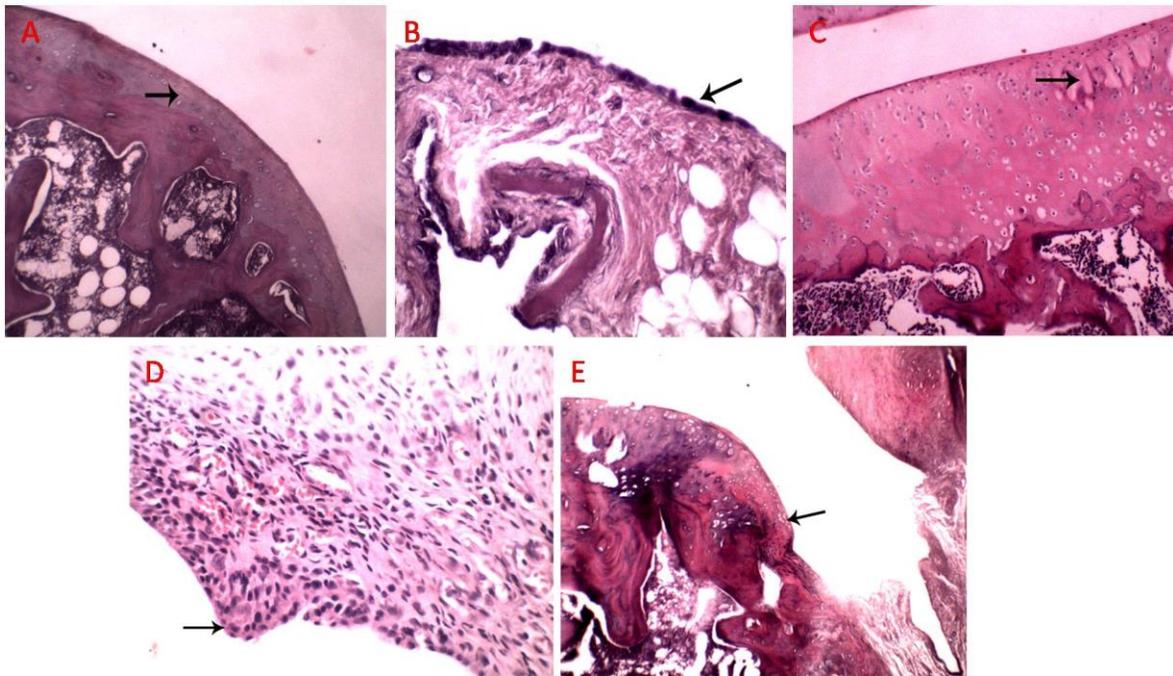
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283 **Fig.1.**Gross morphological observations of articular cartilage in the knees of the rat show severe erosion with granular  
284 (fibrillation) appearance in joint surface (black arrow; A) in The OA group;smooth and glistering articular surface  
285 with no fibrillation or erosions (black arrow;B) in normal group; congestion and intense red spots distributed in the  
286 almost of articular cartilage (black arrow;C) in BMSCs group; better efficiency in regenerating of articular surface  
287 (black arrow; D,E) in ADSCs group; and marked erosion of cartilage exposing underlying vascularized tissue(black  
288 arrow;F,G) in PBS group.



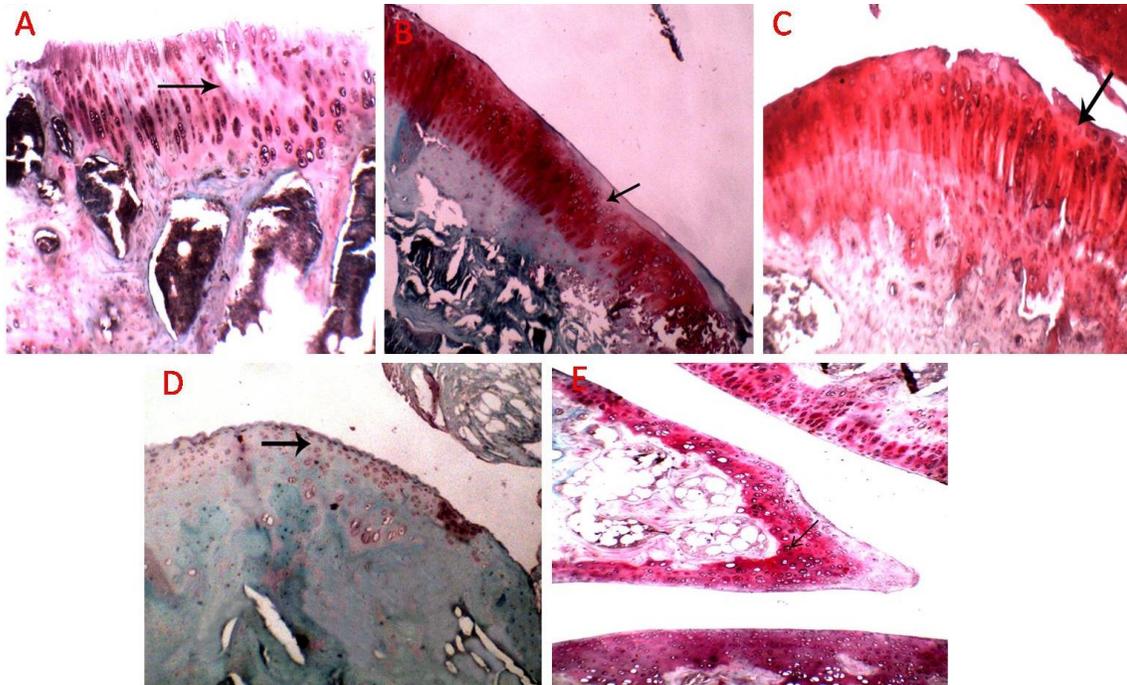
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290 **Fig.2.** Histological view of femoral condyles at week 10 after surgery. H&E staining .The OA group at week 10 A,B,C  
 291 (HE, 100x) ,D (HE, 400x) ; The control group E (HE, 100x),F (HE, 400x).



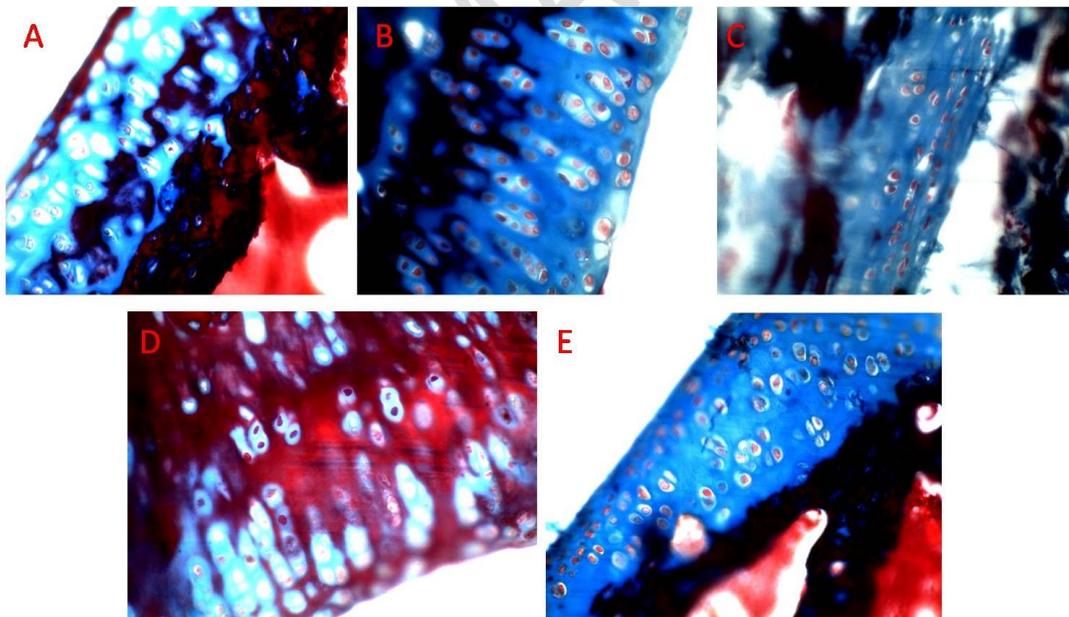
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 293

294 **Fig.3.** Histological view of femoral condyles at week 10 after surgery. H&E staining the ADSCs group at week 10 A  
 295 (HE, 100x), B (HE, 400x) ;The BMSCs group at week 10 C (HE, 100x), D (HE, 400x);The PBS group E (HE,  
 296 100x).



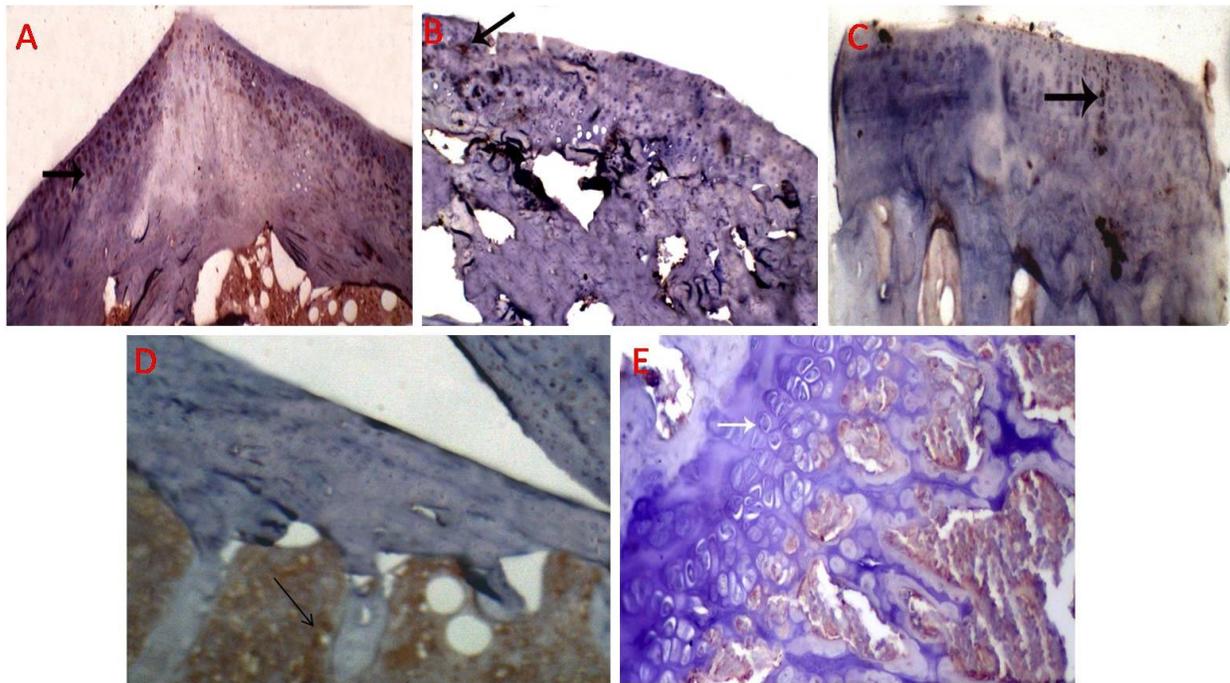
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298 **Fig.4.** Histological view of femoral condyles at week 10 after surgery. The OA group at week 10 A (safranin O stain,  
 299 100x); the ADSCs group at week 10 B (safranin O stain, 100x); The BMSCs group at week 10 C (safranin O stain,  
 300 100x) ;The PBS group D (safranin O stain, 100x); The control group E (safranin O stain, 100x).



301

302 **Fig.5.** Histological view of femoral condyles at week 10 after surgery. The OA group at week 10 A (Masson trichrome,  
 303 400x); the ADSCs group at week 10 B (Masson trichrome, 400x); The BMSCs group at week 10 C (Masson trichrome,  
 304 400x); The PBS group D (Masson trichrome, 400x); the control group E (Masson trichrome, 400x).



305

306 **Fig.6.** Histological view of femoral condyles at week 10 after surgery.The OA group at week 10 A (IHC, DAB  
 307 immunostain, hematoxylline as counter stain, 100x); the ADSCs group at week 10 B (IHC, DAB immunostain,  
 308 hematoxylline as counter stain, 100x);The BMSCs group at week 10 C (IHC, DAB immunostain, hematoxylline as  
 309 counter stain, 100x) ;The PBS group D (IHC, DAB immunostain, hematoxylline as counter stain, 100x); The control  
 310 group E (IHC, DAB immunostain, hematoxylline as counter stain, 100x).  
 311

312 **Table 2.** Mean  $\pm$  SD of assessment of secondary mechanical hyperalgesia using an analgesiometer and assessment  
 313 of joint stiffness by measurement of maximum extension angle of the knee.

Groups	Withdrawal threshold (g)	Maximum extension angle of the knee
<b>Control group</b>	23.94 $\pm$ 0.45 <sup>a</sup>	22.50 $\pm$ 2.26 <sup>b</sup>
<b>Diseased group</b>	12.07 $\pm$ 3.57 <sup>c</sup>	53.83 $\pm$ 3.06 <sup>a</sup>
<b>ADSCs treated group</b>	16.59 $\pm$ 1.12 <sup>b</sup>	27.26 $\pm$ 2.14 <sup>b</sup>
<b>BMSCs treated group</b>	13.37 $\pm$ 0.97 <sup>bc</sup>	39.15 $\pm$ 2.26 <sup>a</sup>
<b>PBS group</b>	14.30 $\pm$ 1.42 <sup>bc</sup>	51.50 $\pm$ 2.26 <sup>a</sup>

314 Means with different superscript litters for each parameter are significantly different at  $P \leq 0.05$ .

315 **Table 3.**Median and range of histopathological evaluation of articular cartilage in the knees of the rat using  
 316 Histopathological Mankin score.

Histopathological Mankin score				
Groups	Cartilage structure	Chondrocytes	Safranin-O Staining	Tidemark
<b>Control group</b>	0.17 <sup>d</sup> (0.00-1.00)	0.00 <sup>c</sup> (0.00-0.00)	0.00 <sup>c</sup> (0.00-0.00)	0.11 <sup>b</sup> (0.00-0.00)
<b>Diseased group</b>	3.50 <sup>a</sup> (3.00-4.00)	2.50 <sup>a</sup> (2.00-3.00)	3.33 <sup>a</sup> (3.00-4.00)	1.00 <sup>a</sup> (1.00-1.00)
<b>ADSCs treated group</b>	1.33 <sup>c</sup> (1.00-2.00)	1.00 <sup>b</sup> (1.00-1.00)	1.00 <sup>b</sup> (1.00-1.00)	0.00 <sup>b</sup> (0.00-0.00)
<b>BMSCs treated group</b>	2.33 <sup>b</sup> (2.00-3.00)	1.67 <sup>ab</sup> (1.00-3.00)	1.33 <sup>b</sup> (1.00-2.00)	0.00 <sup>b</sup> (0.00-0.00)
<b>PBS treated group</b>	3.33 <sup>a</sup> (3.00-4.00)	2.33 <sup>a</sup> (2.00-3.00)	3.17 <sup>a</sup> (3.00-4.00)	0.67 <sup>a</sup> (0.00-1.00)

317 Median and range with different superscript litters for each parameter are significantly different at  $P \leq 0.05$ .

318 **Table 4.**Median and range of the Immunohistochemical expression against MMP9 for the enzyme matrix  
 319 metalloproteinase.

Groups	The MMP9 relative expression
<b>Control group</b>	0.00 <sup>d</sup> (0.00-0.00)
<b>Diseased group</b>	111.20 <sup>a</sup> (105.00-116.00)
<b>ADSCs treated group</b>	23.00 <sup>c</sup> (18.00-29.00)
<b>BMSCs treated group</b>	45.00 <sup>b</sup> (43.00-47.00)
<b>PBS treated group</b>	105.60 <sup>a</sup> (102.00-112.00)

320 Median and range with different superscript litters for each parameter are significantly different at  $P \leq 0.05$ .

321

322

323 **4. DISCUSSION**

324 Osteoarthritis is the most common degenerative joint disorder, characterized by progressive  
325 articular cartilage degradation, subchondral bone thickening, osteophyte formation, synovial  
326 inflammation, and calcification of ligaments [26]. Its treatment is still a great challenge so in this study  
327 we have tried to find an effective use of ADSCs and BMSCs for treatment surgically induced OA  
328 that may be a promising future clinical application.

329 It is known that OA treatment is usually symptomatic and non-curative, only aiming for pain  
330 reduction and symptoms control [27], like analgesics and non-steroid anti-inflammatory  
331 drugs [28]. Even surgical interventions, such as total knee or hip arthroplasty, could relieve pain and  
332 deformity, but also induce postoperative complications [29]. So the main target of this study, is  
333 therapeutic effect targeting reconstitution of articular surface and alleviating inflammation and pain to  
334 optimal state which mimick the normal condition. In the present study, the ADSCs and BMSCs treated  
335 rats showed a significant increase in clinical parameter, while OA-induced rat demonstrated marked  
336 decrease in mechanical hyper-algesia and increased joint maximum extension. This indicates  
337 decreased pain sensation and improves the state of the joint to higher degree. It was noticed that ADSCs  
338 induced the best improvement and was time dependent, where rats after four weeks of treatment were  
339 greatly resembling the others in the normal group, this may be due to its paracrine actions by secreting  
340 various soluble and insoluble cytokines and chemokines that alleviates the pain sensation [30, 31].

341 Gross lesions show a remarkable normal cartilage covering in ADSCs and BMSCs and absence of  
342 fibrillation or minutes erosion, in spite of the lesion were somewhat more pronounced in BMSCs,  
343 which stated by [32], who confirmed that mesenchymal cells differentiate into chondrocytes and fill  
344 cartilage lesions. Additionally, they found that direct transplantation of human MSCs into the knee  
345 joints of Hartley strain guinea pigs with spontaneous OA could differentiate into chondrocytes and  
346 were found to be located in new cartilage. Also [33,22] agreed with this study in observing femoral  
347 condyle in ADSCs treated group founding that it was grossly evident of less articular cartilage erosion  
348 and fibrillation in most regions of the articular knee surface compared with the OA group .

349 Although there are a lot of differing opinions on the disease pathogenesis but the gross lesion is  
350 still the same. OA has a multifactorial etiology, including joint injury, aging, obesity, and heredity [34].  
351 The present OA induced group displayed progressive articular erosion and fibrillation with multiple

352 noticed cracking, which is considered identical signs of OA, matching with the previous results [35,  
353 36] .

354 The gold standard in evaluation and comparison between different groups in this study is  
355 histological examination. Improvements of various histopathological parameters in the adipose tissue  
356 treated group in comparison with bone marrow indicate more efficiency of adipose tissue derived stem  
357 cells. The adipose tissue treated group demonstrated minimal superficial fibrillation, normal  
358 chondrocytes inside the lacuna, which is compatible with the results of [37], who investigated the  
359 potential role of adipose tissue derived cells in alleviating OA.

360 On the contrary, BMSCs treated group showed enhancement in the histological parameters and  
361 restoration of the chondroid matrix as previously mentioned [38- 40] .

362 While this study results estimated that ADSCs treated group was more efficient in the restoration  
363 of the chondroid matrix. The inflammatory process was pronounced attenuated in adipose tissue group  
364 with nearly normal tidemark and normal vascularization without invasion of articular surface  
365 comparable with other group which may be attributed to the paracrine anti-inflammatory response that  
366 was initiated by adipose tissue derived cells[41- 43].

367 The chondrocytes were actively divided and surrounded by normal lacuna and well- organized  
368 pattern in ADSCs and BMSCs rather than necrosis and depletion in PBS treated group. This  
369 indicates the ability of differentiation and extremely regenerative role of MSCs which possess  
370 multilineage differentiation potential and have ability in cell therapy for OA [44, 45]. Similarly  
371 [22] agreed with this result and suggested that co cultures using ADSCs and chondrocyte  
372 possessing peri-cellular matrix (PCM) are able to produce cartilaginous tissue in-vivo.

373 Also, the chondrocytes in ADSCs was apparently more normal than BMSCs, which was approved  
374 by the most studied MSCs which were derived from bone marrows and adipose tissues[46]. It has been  
375 shown that accessibility, processing procedures, and cell yield of MSCs obtained from adipose tissues  
376 are comparable to those provided by the bone marrow [47] .

377 The cartilage covering the joint surface showed marked variability so safranin O stain was used to  
378 demonstrate the degree of cartilaginous matrix restoration and/or loss in different groups. The stem  
379 cells treated groups displayed favorable deposition of cartilaginous matrix, on the contrary of OA  
380 treated group, which displayed marked loss of cartilaginous matrix. ADSCs were more better than

381 BMSCs, implying the regenerative properties in cartilage by stem cells [48-50]. But in contrast to this  
382 study, [51] found that BMSCs have an enhanced potential for chondrogenesis compared to ADSCs.

383 The decreased safranin O stain and changes between the different groups, along with the  
384 appearance of surface fibrillation on the surface of articular cartilage in OA group and was minimized  
385 in stem cells treated groups indicates that there is a direct correlation between stem cells and  
386 proteoglycans deposition [33],The stability of matrix of articular cartilage is maintained by  
387 proteoglycans[52, 53].ADSCs has stronger effect on the proteoglycans precipitation

388 The tide mark , is the destination that separates between articular surface and underlying tissue,  
389 mostly affected by injury due to penetrating blood vessels to avascular surface cartilage, in attempts to  
390 increases blood delivery to the surface and provides the elements for the inflammatory and reparative  
391 process. The tide marks were nearly normal without penetrating blood vessels and avascular upper  
392 cartilage in ADSCs and BMSCs groups, meanwhile the OA group was with proliferating angiogenesis  
393 destructing the tide mark that implies the ability of stem cells to manage and reduce the levels of  
394 inflammatory cytokines liberated in OA. This matched the previous results that reported by [22].The  
395 regenerative effects of MSCs are attributed to its paracrine mechanisms with anti-inflammatory effects  
396 [54].

397 The synovial membrane affected by the inflammatory cytokines liberated in OA appeared  
398 proliferated and hyperplastic in OA group but decreased in thickness in ADSCs and BMSCs treated  
399 groups.This indicates that the anti-inflammatory action of stem cells extended from the articular  
400 surface to the neighboring structures. Also, they possess powerful limiting effect on liberated  
401 cytokines, counteracting the adverse effect of harmful cytokines which was approved by[8,9]who  
402 showed that ADSCs, when given at an early phase of experimental OA, inhibit synovial lining  
403 thickening and forming of enthesophytes associated with ligaments, and cartilage destruction and  
404 protect against joint destruction by both anabolic and catabolic mediators.

405 Masson trichrome is a specific stain for the collagen fiber in the chondroid matrix. It revealed loss  
406 in diseased group. on the other hand, in ADSCs and BMSCs treated groups displayed marked  
407 restoration andthis matched with previous results [40].Compared BMSCs treated group with the other  
408 groups using Masson's trichrome staining ,it was found that BMSCs-injected group was closer to  
409 normal, protected cartilage. It reduced the abnormal differentiation and proliferation and fibrosis in the  
410 knee articular cartilage, thus slow down the process of cartilage degradation.

411 Histochemical staining was done to evaluate the degree of fibrosis. The results estimated that  
412 ADSCs were the best in all the treated groups with minimal fibrosis, this means that the healing process  
413 passes in normal consequence without any accumulation of fibrotic bundles which was reflected on  
414 cartilaginous regeneration in the normal consequences and have impact on the normal movement .  
415 This was agreed by [52,53]who assessed the effect of ADSCs differentiated chondrocytes (DCs) versus  
416 ADSCs transplantation into the rat model of OA.

417 Immunohistochemical staining for MMP9 was done, in order to assess the degree of chondroid  
418 matrix destruction and the ability of Stem cell therapy to reverse it .The results was compatible with  
419 gross and histological examination where the ADSCs were the minimal expression and BMSCs was  
420 the succeeding one .The highest expression was in OA group so the MMP9 results were to confirm the  
421 degree of chondroid matrix destruction and loss of its proteoglycans. [55, 56]determined the  
422 effectiveness of ADSCs, the expression of matrix metalloproteinase and the ability of ADSCs in  
423 lowering expression in OA models.

424

## 425 **5. Conclusion**

426 The results of the present study demonstrated that ADSCs and BMSCs can alleviate knee OA via  
427 their strong regenerative capacity on chondrocytes and increased chondroid matrix deposition. So,for  
428 such positive results, ADSCs are more effective and easily available compared to BMSCs.

429

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## 434 **Conflict of interest**

435 The authors declare that there is no any conflict of interest in the current research work.

436

437 **Animal ethics committee permission**

438 This study was approved by the Local Ethical Committee, Faculty of Medicine, Mansoura University  
439 in accordance with the Ethics committee of national research Center-Egypt with registration number  
440 (09/189).

441 Authors' contribution

442 M. G., A. R. and E. M. conceived of the idea, contributed to its design and coordination, helped in  
443 acquisition of the data, performed the statistical analysis of the data and drafted the manuscript,  
444 M.H. performed and interpreted the histopathological section, G. K. and A. Z. contributed to the  
445 analysis and interpretation of the data, and revised the manuscript critically for important  
446 intellectual content. All authors read and approved the final manuscript.

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