Stem cell therapy compared to Hyaluronic acid in experimentally induced osteoarthritic rats

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Abstract— Osteoarthritis (OA) is a degenerative joint disease mostly occurring in the knee. Bone marrow-derived mesenchymal stem cells are multipotent stromal cells which can provide a promising treatment in tissue regeneration. Intra-articular injection of Hyaluronic acid (HA) has been widely used for treatment of knee OA. The purpose of this study was to compare the therapeutic effect of mesenchymal stem cells (MSCs) and (HA). Fifty albino rats were divided into five groups; group (1) served as control, groups (2,3,4,5) served as arthritic rats and given single intra-articular injection of 1 mg of monosodium iodoacetat. Group 3 injected with single dose of 2 x 106 stem cell (intraarticulary) while group 4 treated with the same dose but Interaperteonialy and group 5 treated with therapeutic dose of (HA). Radiological, biochemical, histopathological and histochemical analysis were performed before and after treatment. Biochemically there were highly significant decrease in C-reactive protein and osteocalcin. Radiological, histopathological and histochemical results showed marked improvement in MSCs treated group, the animals injected with HA revealed less degree of improvement compared to animals treated with stem cells. MSCs are an effective option for treating OA.

Key words — Osteoarthritis, Mesenchymal stem cells, Hyaluronic acid

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1 INTRODUCTION

ARTHRITIS is a form of joint disorder that involves inflammation of one or more joints (Nadia et al., 2011). There are different forms of arthritis such as osteoarthritis, rheumatoid arthritis (Agarwal and Malaviya 2005).Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain and a progressive loss of articular cartilage (Rosenberg, 2002). It can affect both large and small joints of the body, including the hand, feet, back, hip and knee (Witter and Dionne, 2004). It has been suggested that biochemical alterations occur within the articular cartilage resulting in imbalances between synthetic and degradative pathways (Goldring, 2000).Concurrent with these biochemical alterations are changes in the joint cartilage and bone. Changes that occur within the cartilage include fibrillation and splitting of the noncalcified cartilage with subsequent thinning of the cartilage layer. The underlying bone is characterized by increased osteoclast and osteoblast activity, resulting in altered bone contour and formation of subchondral cysts (Felson et al., 2001) Despite the high prevalence and morbidity of osteoarthritis (OA), an effective treatment is currently lacking (Nöth et al., 2008). Animal models of osteoarthritis (OA) include spontaneous models in aging animals, genetically modified mice, as well as surgically, enzymatically or chemically induced models (Ameye and Young, 2006).

Cartilage degeneration can also be induced by administration of quinolone antibiotics or by intra-articular injection of iodoacetates (Bendele et al., 1990) Mono-iodoacetate (MIA) is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and therefore an inhibitor of glycolysis shown to induce chondrocyte death in vitro (Cournil et al., 2001). Intra-articular injection of MIA induces chondrocyte death in the articular cartilage of rodent and non-rodent species (Dunham et al., 1993). When used in rodents, the model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human OA (van der Kraan PM et al., 1989) cartilage, lesions are characterized by chondrocyte

necrosis, cell cloning (chondrones), fibrillation, loss of stainable proteoglycan matrix, and erosion with exposure of subchondral bone. Reported bone lesions include remodeling and sclerosis of subchondral bone with osteophyte formation (Guingamp et al., 1997).

Mesenchymal stem cells (MSCs) have the capacity to differentiate into a variety of connective tissue cells including bone, cartilage, tendon, muscle, and adipose tissue (Colter et al., 2001). These cells may be isolated from bone marrow and expanded in culture through many generations, while retaining their capacity to differentiate when exposed to appropriate signals (Toma, 2002). Multipotent cells have been isolated from various mesenchymal tissues in adults, including skeletal muscle, fat, and synovial membrane as well as hematopoietic, neural, and hepatic tissues (Weissman, 2000). These MSCs secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities. These secreted bioactive factors suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells. Adult stem cells may represent units of active regeneration of tissues damaged as a result of trauma or disease (Caplan and Dennis, 2006). In certain degenerative diseases such as osteoarthritis (OA), stem cells are depleted and have reduced proliferative capacity and reduced ability to differentiate .The systemic or local delivery of stem cells may therefore enhance repair or inhibit the progressive loss of joint tissue. The use of MSCs for cell therapies relies on the capacity of these cells to home and engraft long-term into the appropriate target tissue (Murphy et al., 2002).

MSC therapy has been applied in bone and cartilage repair and in the treatment of osteoarthritis (Barry, 2003). Hyaluronic acid (HA), a high molecular weight polymer of glucosamine and glucuronic acid residues is one of the key components of the articular cartilage matrix. The molecular weight is approximately 2 to 3 millions in normal synovial fluid. HA is also a ma-

jor ingredient of synovial fluid which aids in the absorption of mechanical impact, joint lubrication, and preservation of articular cartilage. Moreover, recent studies have revealed that HA modulates the function of various types of cells, including articular chondrocytes and macrophages. Previous reports have revealed that HA concentration and molecular weight in the synovial fluid are significantly lower in arthritic joints (Dahl1985). Intra-articular injections of (HA) have been widely used for a number of years in the treatment of osteoarthritis. HA is relatively free of side effects and provides relief of symptoms in some patients. There is also some evidence from animal studies that HA can modify disease activity and protect articular cartilage from further degeneration (Goldberg and Buckwalter, 2005).

The aim of the present study was to evaluate the effect of (intraarticular and intraperitoneal) mesenchymal stem cell (MSc) injection compared to Hyluronic acid (HA) of induced osteoarthiritis in experimental animal model (rat).

2 MATERIALS AND METHODS

Fifty adult male albino rats weighing (170-200) gm. were obtained from the animal house of National Organization for Drug Control and Research (NODCAR), Egypt. Albino rats were allowed to acclimatize for one week before the induction of osteoarthritis (OA). The animals were kept on a 12 h light/dark cycle at room temperature and fed on standard pellets diets and water adlibitum.

2.1 Induction of osteoarthritis

For induction of MIA induced arthritis, rats were anesthetized with diethyl ether (fine–chem limited) and given single intraarticular injection of 1 mg of monosodium iodoacetate (MIA; Sigma, St. Louis, MO, USA; cat #I2512) through the infrapatellar ligament of the right knee and sacrificed at 28, days postinjection (Creamer et al., 1996). MIA was dissolved in physiologic saline and administered in a volume of 50 µl.

2.2 Preparation of bone marrow- derived mesenchymal stem cells from rats

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll / Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL).Cells were incubated at 37 °C in 5% humidified CO2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1ml EDTA (GIBCO/BRL) for 5 min at 37 °C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures (Alhadlaq and Mao, 2004) mesenchymal stem cell (MSC) in culture were characterized by their adhesiveness and fusiform shape (Rochefort et al.,

2005).We also detected CD29 gene expression by (RT-PCR) as a marker of MSC (Munoz et al ., 2006).

2.3 Experimental design

50 albino rat divided into five groups each one 10 rats as follow:

1- Control group: animals received intraaticular injection of 50 µl of physiologic saline

2-Arthritic group: rats given single intraarticular injection with 1 mg/joint of MIA and scarified after 28 days.

3- Treated groups: subdivided into:

A-Stem cell (Intraarticular) (SC-IA) group:- - Arthritic rats treated with a single recommended dose of 2 x 106 cells/0.5 ml of MSCs intraarticularly and scarified after one month. (Meng et al., 2013).

B- Stem cell (Interaperteonial) (SC-IP) group: Arthritic rats treated with a single dose of 2 x 106 cells/0.5 ml of MSCs intrapertioneally and scarified after one month (Meng et al., 2013).

C-Hyaluronic acid (HA) group: - arthritic rats treated with therapeutic dose intraarticulary of HA)10mg/ml) and scarified after one month. The dose calculated for rat with reference to the human as suggested by Paget and Barnes (1964).

2.4 Radiography

The radiographic examination was performed in X-ray unite, Surgery, Anaesthesiology & Radiology Department, Faculty of Veterinary Medicine, Cairo University. The experimental animals were anaesthetized before radiographic examination using Xylazine HCl 5-10 mg/kg.b.wt (Xyla-Ject®, ADWIA-Egypt) and Ketamine HCl 40-80 mg/kg.b.wt (Ketamine®, SIGMA-Egypt) that were administered intramuscularly (Gaertner et al., 2008). The examination was carried out via a mobile X-ray machine (Ficher Machine, Eureka X-ray tube/ Model E-Merald-125, 1985, U.S.A).The used exposure factors were 38-40 kVp, 0.1 mAs and 90 cm focal film distance.

All the animals were examined in dorsal recumbency with using of anterioposterior radiographic projection for the stifle (knee) joint. The examination was carried out at 4 weeks post osteoarthritis induction, and one month post stem cells and HA injection. The femoral condyle and tibial epiphysis contour regularity with joint space radiodensity were the points of the injected stifle joint radiographic evaluation compared with the contralateral limb.

2.5 Biochemical parameters

C–reactive protein (CRP) level was detected by latex agglutination test, (Young, 1995) and osteocalcin level was estimated by Enzyme-linked Immune Sorbent Assay (ELISA) kit (Demiaux and Arlot, 1992).

Blood was withdrawn in sterilized tubes from the retro-orbital plexuses puncture by sterile heparinzed capillaries from each rat of all groups, and then centrifuged at 4,000 rpm (round per minute) for 20 minutes. Plasma was separated and stored at -4°C.

2.6 Histological and histochemical Studies

Tissue samples were prepared for light microscopy using standard procedures. Briefly, samples were fixed in 10%

phosphate-buffered formalin and subsequently decalcified in 5% formic acid for 72 hours. The decalcified specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness and stained with haematoxylene and eosin (Al-Saffar et al., 2010).Alcian Blue stain was used for staining of the proteoglycan the appearance of the blue color in the areas of cartilage histologically it indicates newly formed cartilaginous tissue (Bancroft et al., 1996).

2.7 Detection of homing of injected cells at rat joint tissue

After forty days, joint tissue was examined with a fluorescence microscope to detect the cells stained with PKH26 dye to ensure homing and trace the injected cells in the joint tissue after injection.

2.8 Mankin score

The articular cartilage injuries found in the rats' knees were evaluated and recorded using the Mankin score referred to by Armstrong. (Armstrong et al., 1994). In this system, the higher the score, the higher the level of OA.

Variable	Score
Surface	0 = normal
	1 = irregular
	2 = fibrillation / vacuoles
	3 = blisters and erosion
Hypocellularity	0 = normal
	1 = small decrease in chondrocytes
	2 = large decrease in chondrocytes
	3 = no cells
Clones	0 = normal
	1 = occasional duos
	2 = duos or trios
	3 = multiple nested cells
Alcianophilia	0 = normal
	1 = small decrease in color
	2 = large decrease in color
	3 = no color

2.9 Statistical analysis

The data obtained from different groups were compared for statistical significance by one-way analysis of variance (ANOVA) adopting SPSS statistics program (version 16,SPSS Inc. Chicago, USA) followed by post hoc multiple comparison using the least square difference (LSD). Differences between series were considered to be significant at $p \leq 0.05$.

3 RESULTS

3.1 Radiology

The radiographic changes were observed in right stifle (knee) joint with induction of OA. After one month post OA induction (Fig.1A); increasing joint radiopacity with osteophyte formation, decreasing joint space, dystrophic changes in articular surfaces with irregularity.
In case of SC-IA treated group; the radiographic changes were

encountered as; (Fig.1B); joint space, radiodensity was almost normal with low osteophyte reaction and irregular articular surface with femoral condyle erosions were more or less observed.
In SC-IP treated group. (Fig.1C); the joint space radiodensity appeared almost normal with little dystrophic changes in both femur and tibia articular surfaces.
In HA intra-articular treated group;. (Fig.1D); the joint space radiodensity was decreased and the sclerotic changes in both femur and tibia were still appeared.
1ACraniocaudal radiographs of right knee (stifle) of Control rat showed normal femro-tibial joint space with no marginal osteophytes.
1B-Craniocaudal radiographs of right knee (stifle) one month after induction of (OA) showing decreased joint space, dystrophic changes with irregular articular surfaces in femoral condyle and tibia with appearance of osteophytes (arrow)
1C- Craniocaudal radiographs of right knee (OA induced) one month post (SC-IA) injection showed changing in the joint space radiodensity (decreased) and decreased sclerotic changes in both femur condyle and tibia
1D- Craniocaudal radiographs of right knee (OA induced) one month post (SC-IP) injection showed different degrees of radiographic changes; the joint space radiodensity almost decreased and little sclerotic changes in both femur condyle and tibia articular surfaces.
1E- Craniocaudal radiographs of right knee (OA induced) one month post intra-articular (HA) showed some radiographic changes in the joint space radiodensity (decreased) and some sclerotic changes in both femur condyle and tibia articular surface.
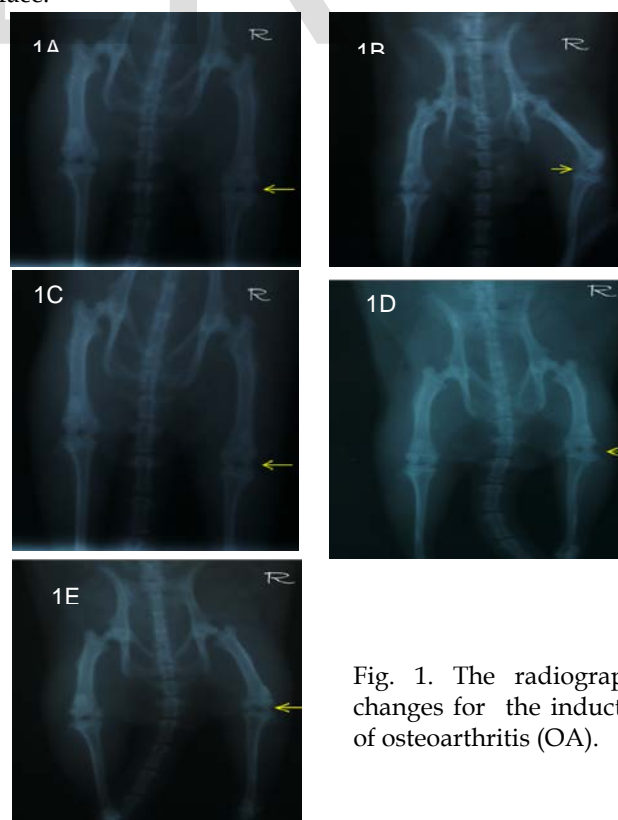

Fig. 1. The radiographic changes for the induction of osteoarthritis (OA).

3.2 Biochemical parameters

Table (1) demonstrated that there were very highly significant increase in (CRP and osteocalcin) (30±14.69 mg/L, 0.635±0.26 ng/ L) was found in arthritic induced group when compared to control group (4.66±1.03, 0.035±0.003) respectively ($P<0.005$)
After injection with (SC-IA) there were very highly significant decrease in (CRP) (9±3.28) ($P<0.005$) and highly significant decrease in osteocalcin level (0.058 ±0.025) ($P<0.01$) in comparison with arthritic induced group

Also, the results revealed that there were very highly significant decrease in (CRP) (8±3.09) ($P<0.005$) and highly significant decrease in osteocalcin (0.075±0.008) ($P<0.01$) when animals treated with (SC-IP group) comparing to arthritic induced group.

By using of (HA) causing highly significant decrease in (CRP) (18±6.57, ($P<0.01$) and very highly significant decrease in osteocalcin (0.039±0.006) ($P<0.005$) when compared to arthritic induced group.

Table 1. Plasma in CRP and osteocalcin.

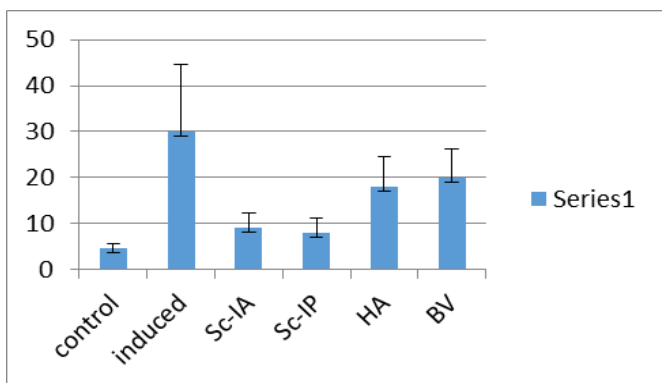| Gr	CRP	Osteocalcin
Control	4.66±1.03	0.035±0.003
Diseased	30±14.69***	0.635±0.26***
Sc-IA	9±3.28***	0.058 ±0.025**
Sc-IP	8±3.09***	0.075±0.008**
HA	18±6.57**	0.039±0.006***

Fig (2). Histogram of plasma CRP levels in all experimental groups
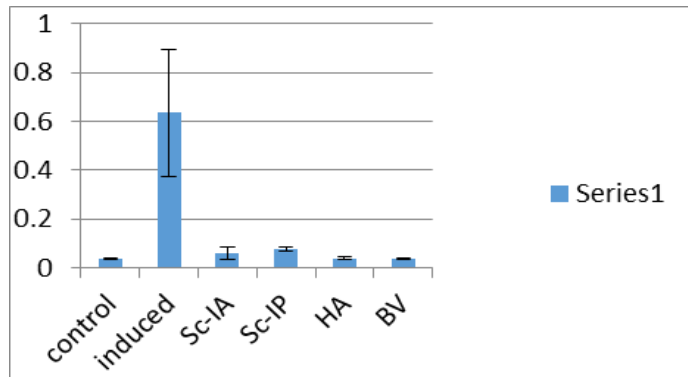

Fig (3). Histogram of plasma Osteocalcin experimental in all groups

3.3 Histopathology

Control group: Figure (4) show normal structures of the articular and subchondrol bone. It present smooth articular cartilage surface with the underneath layer of flattened chondrocytes in the tangential zone. Chondrocytes were normally distributed in parallel rows in the transitional zones of the noncalcified part of the articular cartilage, subchondrol bone revealed normal distribution of trabeculae composed of osteocytes and canailiculli surrounding the bone marrows filled with blood forming elements.

The histopathological examination of joint tissues of rats from OA induced group showed extensive areas of chondrocytes degeneration, chondrocytes were shrunken with hypereosinophilic cytoplasm and fragmented pyknotic nuclei, also extensive areas of cartilage loss with degenerative changes in the form of irregular surface, multifocal erosion hyopcellurity, fibrillation, fissures and replacement of articular cartilage with fibrous tissue. Subchondral bone with thinned trabeculae and surrounded by numerous osteoclast some adjacent trabeculea were lined by single to several rows of large osteoblast. The synovial membrane was expanded with sever edema and inflammatory cells with dilated blood vessels.

When OA induced animals treated with (SC-IA and SC-IP) they revealed marked improvement where femro-tibial joint, synovial membrane and subchondrol bone with the trabeculae and bone marrow are similar to normal, chondrocytes and cartilage are almost intact and preserved.

Sections of OA induced animals treated with HA revealed less degree of improvement compared to animals treated with stem cells, where there was some surface irregularity and hypocellurity could be detected.
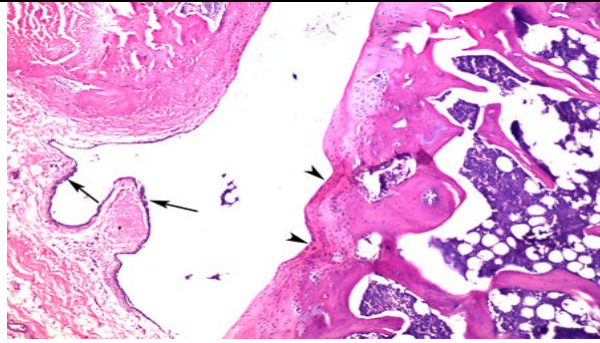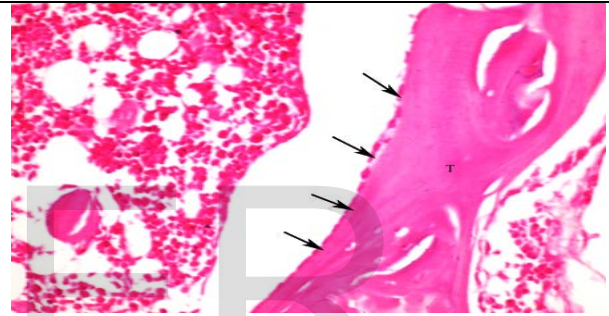
(1): Photomicrograph of joint tissue of OA induced rat show-ing synovial membrane with inflammatory cells (arrow) and irregularity or articular surface (arrow head) (H&E, X: 200).
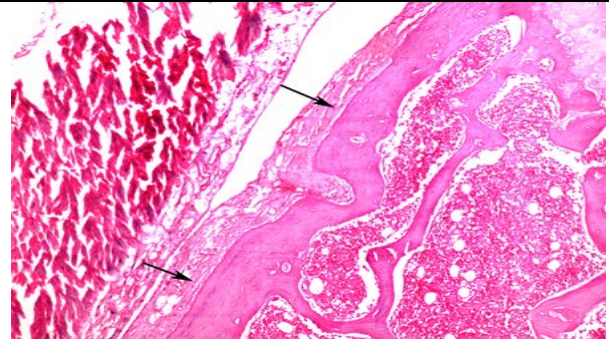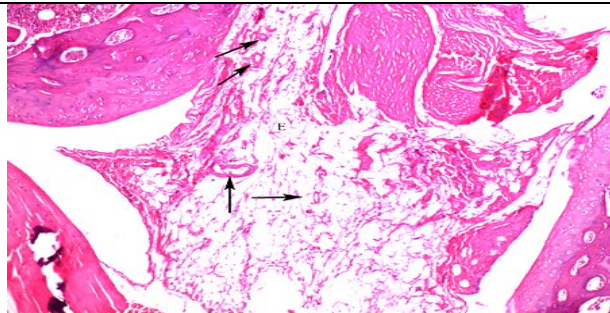

(2): Photomicrograph of joint tissue of OA induced rat show-ing nearly total replacement of articular cartilage with fibrous tissue and loss of chondrocytes (arrow) (H&E, X: 100).
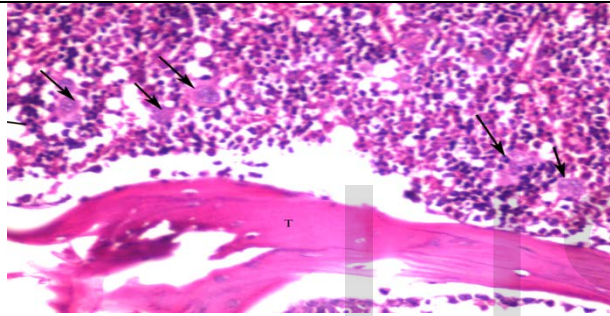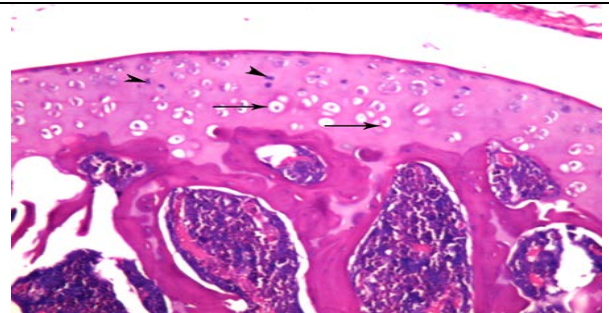

(3):Photomicrograph of joint tissue of OA induced rat showing numerous osteoclast (arrow) (H&E, X: 200)

(4):Photomicrograph of joint tissue of OA induced rat show-ing bone trabeculae (T) lined by osteoblast(arrows) (H&E, X: 200)

(5):Photomicrograph of joint tissue of OA induced rat showing synovial membrane with edema (E) and dilated blood vessel (arrows) (H&E, X: 100)

(6):Photomicrograph of joint tissue of OA induced rat treated with SC-IA showing intact chondrocytes(arrow), few closed chondrocytes (arrow head),well formed bone trabeculae(T) with regular surface (H&E, X: 400).

Fig. 4. Photomicrograph of joint tissue of OA induced rat.

3.4 Mankin score

The histopathological analysis using mankin score showed sever level of degenerative changes in OA induced group (9.00±0.63) compared to control group which has the lowest score (0.66±0.51) reflecting normal histological appearance and proteoglycan content.

The mankain score of stem cell groups (SC-IA and SC-IP) (3.16±0.98- 3066±0.51) respectively, were very high decreased significantly as compared to OA induced group indicates marked improvement including a preserved structural integrity of continues articular surface, normal cellular architecture and normal proteoglycan content.The histological score of (HA) treated group was highly significant decrease in comparison with OA induced group (5.66±0.81) revealed moderate improvement in articular cartilage.

The change in surface, hypocelluratiy, clones of chondrocytes and reduction of articular cartilage matrix staining were graded as described in Table (2).

Table 2. The change in surface, hypocelluratiy, clones of chondrocytes and reduction of articular cartilage matrix.

Group	Score
Control	0.666±0.51***
Diseased	9.00±0.63
Sc-IA	3.16±0.98***
Sc-IP	3.66±0.51****
HA	5.66±0.81**

3.5 Alchain blue

Table (3) and Figures (5-8) illustrated a highly significance decrease in proteoglycane content in diseased group (0.209± 0.074),which reflect the content of proteoglycane compared to control group (0.574±0.1110), while there was no significance difference in proteoglycane content between control group and stem cell treated groups (SC-IA and SC-IP) (0.564±0.107).

After treatment of arthritic rats with (HA) showed significance decrease in proteoglycane content (0.430±0.070) in comparison with control group.
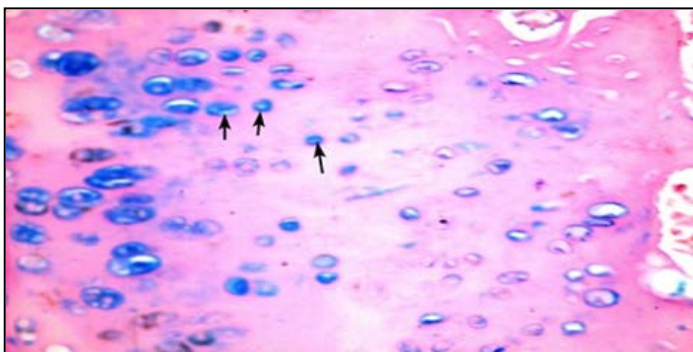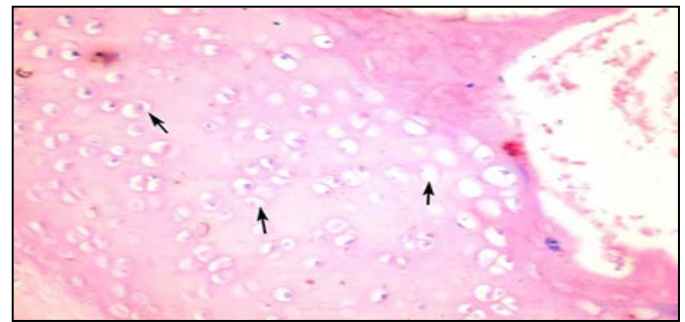

Fig. 5. A Photomicrograph of chondrocytes of articular cartilage in control group shows intense staining of proteoglycane

(arrow) (alchain blue, X 400).
Fig. 6. A Photomicrograph of chonrocytes of articular cartilage in arthritic group shows severs loss of proteoglycan content (arrow) (alchain blue, X 400).
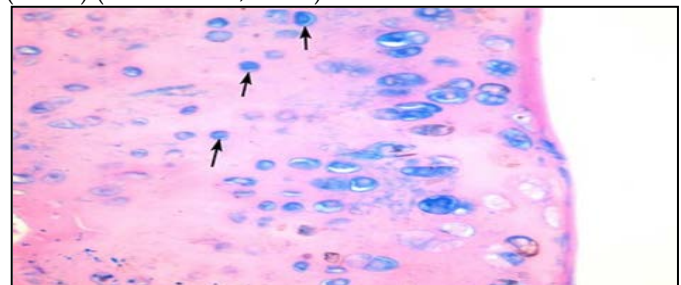

Fig 7. A Photomicrograph of chondrocytes of articular cartilage of OA induced rat treated with SC-IA shows intense staining of proteoglycane content (arrow) (alchain blue, X 400).
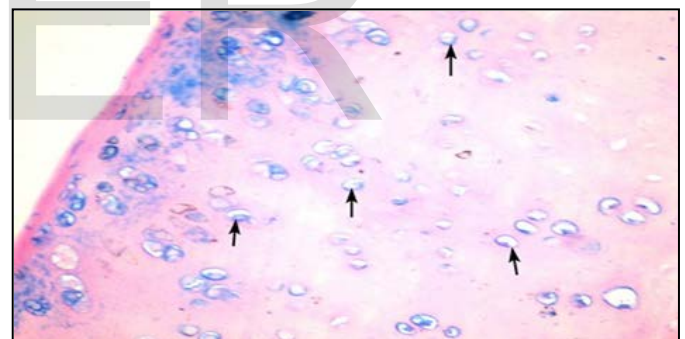
Fig. 8. A Photomicrograph of chondrocytes of articular cartilage shows moderate staining of proteoglycane of OA induced rat treated with HA content (arrow) (alchain blue, X 400).

Table 3. Demonstrated semi-quantitative measurment of proteoglycans content stained with alchain blue.

Group	M±SD
Control	0.57±0.111***
OA induced	0.209±0.074
Sc-IA	0.56±0.107***
Sc-IP	0.56±0.107***
HA	0.43±0.070**

4 DISCUSSION

The present study showed that a knee of osteoarthritic rat induced by mono-idoacetate injection exhibited histopathological changes as chondrocytes degeneration, extensive areas of cartilage loss with degenerative changes in the form of irregular surface, multifocal erosion, hyopcellurity, fibrillation, fissures and replacement of articular cartilage with fibrous tissue, subchondral bone with thinned trabeculae and surrounded by numerous osteoclast. Some adjacent trabeculea were lined by single to several rows of large osteoblast, the synovial membrane was expanded with sever edema and inflammatory cells with dilated blood vessels.

These results were in agreement with the finding of (Cournil et al., 2001) who reported that mono-iodoacetate (MIA) as inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and therefore is an inhibitor of glycolysis shown to induce chondrocyte death. Thes present also confirmed by (Van der Kraan et al., 1989) who reported that when (MIA) is used in rodents, the model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human. In cartilage, lesions are characterized by chondrocyte necrosis, cell cloning (chondrones), fibrillation, loss of stainable proteoglycan matrix and erosion with exposure of subchonrdral bone.

(Guzman et al., 2003) observed that (MIA) induced histological changed in subchondral bone and articular cartilage of rat. These changes consisted of increased osteoclastic and osteoblastic activity in the trabecular bone immediately subjacent to the areas of cartilage loss and degeneration. The changes are consistent with the initiation of bone remodeling and may have been induced by increased load in the subchondral bone due to the advanced loss of cartilage. Cartilage damage was characterized by complete loss of cellular detail of chondrocytes, with thinning and collapse of the cartilaginous matrix.

There is now general agreement that osteoarthritis (OA) involves all structures in the affected joint, culminating in the degradation of the articular cartilage, cartilage and the underlying (subchondral) bone, and characteristic changes occur in parallel in these tissues across disease progression. Changes in both the articular cartilage and the subchondral bone are mediated by the cells in these two compartments: chondrocytes in the cartilage and osteoclasts, osteoblasts and osteocytes in the bone, whose primary roles are to maintain the integrity and function of these tissues. In response to the altered mechanical and biochemical environment of a progressively diseased joint, these cells function differently and show a different profile of gene expression, suggesting direct effects of these external influences. There is also in vivo and in vitro evidence of chemical cross talk between the cells in cartilage and subchondral bone, suggesting an interdependence of events in the two compartments. It is ultimately these cellular changes that explain the altered morphology of the cartilage and subchondral bone (Findlay and Atkins, 2014)

In the current study there were very highly significant increase in (CRP and osteocalcin) was found in arthritic induced group when compared to control group. After treatment of arthritic rats with (SC-IA and SC-IP) there were very highly significant decrease in (CRP) and highly significant decrease in (OC) in comparison with arthritic induced group.

By using (HA) and for treatment of arthritic rats there was a highly significant decrease in (CRP) and very highly significant decrease in OCwhen compared to arthritic induced group.

Both in vitro and in vivo studies have demonstrated that IL-6 is the chief regulator of CRP production by hepatocytes (Gabay, and Kushner,1999). Many cell types within the joint are capable of producing (IL-6) including synovial fibroblasts (Fiorito et al., 2005). Synovial T cells and articular chondrocytes (Fan et al., 2004). Another potential stimulus of CRP production by hepatocytes is interleukin-1(IL-1).This cytokine appears to work synergistically with IL-6 to promote CRP production in vitro (Zhang et al., 1995). IL-1 is produced by synovium and cartilage of patients with OA, and is thought to play a central role in promoting articular cartilage catabolism (Abramson et al., 2001).

IL-1 and tumor necrosis factor (TNF)-α have long been known to produce a catabolic phenotype in articular chondrocytes. TNF-α increased the expression of β-catenin and matrix metalloproteinase (MMP-3), and significantly inhibited the synthesis of type II collagen and proteoglycan (Ye et al., 2011).

There was significant increase in plasma (OC) level in arthritic rats. OC is secreted solely by osteoblasts and it is implicated in bone mineralization and calcium ion homeostasis (Lee et al., 2007). The arthritic rats revealed that bone trabeculea were lined by single to several rows of large osteoblast. Evidence from animal models of OA and from human bone samples obtained at surgery indicates altered bone remodeling in OA. At the cellular level, remodeling is achieved by the actions of osteoclasts, which resorb bone, and osteoblasts, which are the bone forming cells. The activities of both of these cell types is regulated by osteocytes, embedded within the bone matrix (Findlay and Atkins, 2012 and Bonewald, 2012).The rate of bone remodeling changes across the course of the disease.

Thus, increased remodeling, accompanied with increased vascularity, occurs in the subchondral bone in early OA, while late stage disease is characterized by reduced bone resorption with a bias toward bone formation (Kumarasinghe et al., 2010).

This study evaluates the utility of stem cell therapy for treatment of arthritic lesion that occurs following joint injury. Adult MSCs were delivered by two ways (intraarticular and intrapertioneal injection). Therefore, Arthritic rats treated with a single dose of (2 x 106) of mesenchymal stem cells and sacrified after one month, revealed marked improvement where femro-tibial joint, synovial membrane and subchondrol bone with the trabeculae and bone marrow nearly similar to normal. Chondrocytes and cartilage are almost intact and preserved. This is a very important findings that indicate the success of treatment.

MSCs secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities. These secreted bioactive factors suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells. Adult stem cells may represent units of active regeneration of tissues damaged as a result of trauma

or disease (Caplan and Dennis, 2006).

Several tissue-engineering approaches have been used for the repair of joint lesions. For example, the fixation of implanted chondrocytes beneath a sutured flap of ectopic tissue, such as periosteum, has been widely used for the treatment of cartilage defects (Brittberg et al., 2001).Other approaches have centered on the use of cells loaded on a scaffold and delivered to a lesion site. Multipotent cells have been isolated from the surface zone of articular cartilage (Archer et al., 2002) and MSCs have the capacity to repair fibrillated cartilage in vitro.

It is attractive to hypothesize that MSCs could have a role in cartilage protection by direct resurfacing of the articular cartilage or act to preserve subchondral or trabecular bone structure–associated mechanical integrity of the joint) (Li and Aspden , 1997).

MSCs can also modulate the immune response by stimulating the production of CD8+ T reg (regulatory T cells), which could inhibit lymphocyte proliferation in allogenic transplantation. However, the induction of regulatory T cells may be mediated by different factors in alloreactive and mitogen stimulated lymphocyte cultures as differences exist between the systems. MSCs also produce bone morphogenic protein-2, which mediates immunosuppression via the generation of CD8+ regulatory T cells (Maccario et al., 2005).

The present study outlines the efficacy of HA in treated rat model of MIA induced knee OA. The animals revealed less degree of improvement compared to animals treated with stem cells, where there was some surface irregularity and hypocelluarity could be detected. These results were approximately similar to (Sakamoto et al., 1984) who founded that the mechanisms of HA actions in preventing cartilage degeneration have characterized the distribution of fluorescent labeled HA injected into papain induced arthritic cartilage. Two possible mechanisms have been proposed to explain this effect: either a barrier against the diffusion of inflammatory enzymes into the arthritic cartilage, or a protective effect of HA on the cartilage proteoglycan macromolecule itself. The effects of HA on the development of early stage OA changes suggest that HA affects anabolism and catabolism of proteoglycan to prevent the progress of OA (Han et al., 1999).

A study by Maniwa (Maniwa et al., 2001) proved that HA coats the surface of the articular cartilage and shares space in the cartilage among collagen fibrils and sulfated proteoglycans. An in vitro rabbit study (Yoshiok et al., 1997) also illustrated that the rate of synovial cell migration was enhanced with HA alone, and HA increased chondrocyte migration in the presence of basic fibroblast growth factor. Hyaluronan-based polymers have been shown to enhance the natural healing process of osteochondral defects in animals (Solchaga et al., 2000). These hyaluronan-based materials possess a unique biochemical composition that recreates an embryonic-like environment, which, as hypothesized, may be favorable for the regenerative process (Toole, 1997).

Hyaluronan also contributes to the granulation phase of both fetal and adult wound healing (Chen and Abatangelo, 1999) and stimulates the migration and mitosis of mesenchymal and epithelial cells (McCarty, 1996 and Murashita et al.,1996).

The histological results were confirmed by histochemical as-sessment (staining of proteoglycans present in cartilage matrix by alchain blue stain). The histochemical results revealed sever depletion of proteoglycans content in arthritic induced group compared to control group. These results were in agreement with the finding of who reported that, the proteoglycans could be degraded by MMP-3 and other proteases. In normal cartilage, there is a close balance between the activities of MMPs and the level of (tissue inhibitor metalloproteinase) TIMPs. In OA, the protease activities are higher than the level of TIMPs, leading to a presumptive excess of there is a close balance between the activities of MMPs and the level TIMPs. In OA tissue, there was increase in active MMPs associated with an increase in plasmin in the absence of concomitant increase in TIMPs, a strong indication that cartilage degeneration in OA is related to an imbalance in the regulation of MMPs. Such an imbalance between inhibition and promotion of enzymatic activity leads to an increase in the amount of active proteases, which combined with suppression of matrix synthesis results in increased degeneration of the cartilage (Pelletier et al., 1993).

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