Molecular Mechanism of Cartilage Turnover in Monosodium iodoacetate induced Knee Osteoarthritis

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Abstract: Osteoarthritis (OA) is characterized by a progressive degradation of cartilage and loss of joint function. A simple and reproducible animal model that mimics molecular and pathological changes in the articular cartilage of knee joint OA would help in understanding its pathophysiological mechanism and aid in the preclinical assessment of disease modifying drugs. The aim of our study is to evaluate changes in Aggrecan gene expression as a part of molecular events underlying cartilage turnover in monosodium iodoacetate (MIA)-induced knee OA through determination of aggrecan mRNA expression and immunostaining and its relation to disease chronicity and severity. OA was induced by single intra-articular injection of MIA into knee joints of male rats. Histologic and molecular changes in the knee cartilage were evaluated by light microscopy, real-time PCR and immunohistochemistry. Our results showed that injection of an MIA induced typical OA-like lesions in the knee joint within 3 weeks. Furthermore, there was a significant down-regulation of aggrecan gene expression in MIA-induced OA in a time dependent manner, and that reflects the severity of OA in terms of Mankin score. These results were confirmed through estimation of aggrecan content in cartilage by immunohistochemical analysis, which revealed a progressive decrease in aggrecan immunoexpression. In conclusion, intra-articular MIA injection is an animal model that presents reliable profiling of the sequence, chronic and complex nature of OA. This would help studying the OA modifying drugs specially those suggested having anabolic effect and enhancing the PG contents both at early and late stages of the disease.

Keywords: Monosodium iodoacetate, Osteoarthritis, Cartilage, Aggrecan, Real time PCR.

INTRODUCTION:

Osteoarthritis (OA) is one of the most common forms of degenerative joint disease and a major cause of pain and disability affecting the aging population [1]. It is a significant burden in terms of cost as well as the health of society and individuals [2]. OA causes moderate to severe disability in 43.4 million people [3]. Globally, about 250 million people (3.6% of the population) have OA of the knee [4].

Although pathologic changes occur in all joint tissues, the articular cartilage has received much of the attention in OA studies because the gross articular cartilage damage is the most obvious pathologic feature leading to joint dysfunction [5]. The articular cartilage loses its homogeneous nature and becomes disrupted with pitting, clefts, and ulceration [6].

Aggrecan is the major proteoglycan (PG) of hyaline cartilage where it is present at very high concentrations in the form of aggregates [7]. It is the second most abundant molecule in cartilage with an estimated half-life of months to a few years [8]. Aggrecan exhibits a bottlebrush structure, in which glycosaminoglycan (GAG) negatively charged linear polysaccharides such as chondroitin sulfate and keratan sulfate side chains are attached to an extended protein core. In the presence of hyaluronic acid
(HA) and a link protein, aggrecan molecules self-organize into a secondary bottlebrush superstructure with as many as 100 aggrecan monomers attached as side chains or bristles on the hyaluronan core, aggrecan-HA complex. The strong electrostatic repulsive forces arising from the high charge density on the bottlebrush bristle favor chain extension, thus generating gel-like structures that are highly swollen in three dimensions. In cartilage, the aggrecan-HA complexes occupy the pores of the collagen matrix of bone articulation and provide osmotic resistance to de-swelling under compressive load with minimum deformation, even for prolonged periods. It also contributes to the nearly frictionless lubrication of joints [9].

In OA, aggrecan is one of the first matrix components to undergo measurable loss that ultimately leads to a loss of cartilage function. Therefore, it is considered to be a crucial initial event in the development of arthritis, which is followed by essentially irreversible collagen degradation [10]. Depletion of aggrecan renders the cartilage less resistant to mechanical compression and may be a prerequisite for subsequent matrix metalloproteinase (MMP)-driven destruction of the collagen network [11].

Several arthritis animal models have been established [12]. The disease develops in several strains of mice or can be induced surgically in other species. Injection of the metabolic inhibitor monosodium iodoacetate (MIA) into the joint, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and glycolysis shown to induce OA-like changes in the articular cartilage of rodents [13].

Comparing the mRNA expression and protein levels of cartilage-specific proteins should provide information about cellular events in the OA disease progression. In this study, we tested the hypotheses that the severity, as evidenced by Mankin score, and chronicity, by testing different time points, of MIA induced knee OA is correlated with aggrecan mRNA expression and that differences in expression can occur within an affected joint along with histopathological and immunohistochemical studies. This was to evaluate MIA induced knee OA as a model suitable to study severity, chronicity, and being applicable for further drug application.

**MATERIALS AND METHODS:**

**Ethics statement:** Rats were obtained from the Animal House of the Faculty of Medicine, Assiut University. All animal procedures were approved by the Animal Research Ethics Committee, Faculty of Medicine, Assiut University.

**Animals:** A total of 40 adult male albino rats weighing 200-220 g at the start of the study was purchased from Animal House of the Faculty of Medicine, Assiut University. They were housed in groups under conventional health conditions in a natural light-dark cycles. Animals were fed standard rat chow with free access to water, and were acclimatized 2 weeks before the experiment.

**Animal grouping:** Rats were assigned randomly into 5 groups, 8 rats in each group, as follows:

1. **Control group:** rats kept fed on normal healthy conditions.
2. **Vehicle group:** rats given single intra-articular injection of sterile saline in the left knee joint (the vehicle of MIA).
3. **MIA-induced osteoarthritis group (MIA$_3$):** rats given single intra-articular injection MIA in the left knee joint then sacrificed 3 weeks post injection.

4. **MIA-induced osteoarthritis group (MIA$_6$):** rats given single intra-articular injection of 5 MIA in the left knee joint then sacrificed 6 weeks post injection.

5. **MIA-induced osteoarthritis group (MIA$_9$):** rats given single intra-articular injection of MIA in the left knee joint then sacrificed 9 weeks post injection.

**Induction of OA in the rat:** OA was induced by a single intra-articular injection of MIA (Sigma-Aldrich, Cat.NO.I2512, USA) into the left knee joint of rats. Each rat was positioned on its back and the left leg was flexed 90° at the knee. The patellar ligament was palpated and the injection was made into the region below the patella. Each rat received 50 µl sterile 0.9% saline containing 3 mg of MIA using 0.3 mL insulin syringe [14, 15]. Care was taken not to advance the needle too far into the cruciate ligaments [16].

**Gross Observation:** After MIA injection, all experimental rats were carefully inspected every 2 days to assess the knee joint swelling and gait disturbances under natural conditions in the cages, where they moved freely.

**Tissue preparation:** By the end of the specified induction period, rats were sacrificed by cervical dislocation. After that, left knee joints were dissected from adhering muscles and connective tissues, washed in ice-cold isotonic saline, the articular cartilages were scraped and snap frozen in liquid nitrogen and stored separately at -80 °C for subsequent qRT-PCR.

A total of 2 joints per group at each time point was dissected and fixed in 10% neutral buffered formalin for 48 h decalcified in 10% EDTA for 4 weeks during which the solution was renewed every 2 days until the tissues had softened. The decalcified knee joints were cleaved longitudinally in a sagittal plane along the central portion. They were dehydrated in graded alcohols, cleared in xylene, embedded in paraffin for subsequent histopathological and Immunohistochemical analysis [17].

**Histological staining:** Paraffin-embedded knee joint blocks were sagittally cut in serial sections at a 5µm thickness. Sections were stained with hematoxylin and eosin (H&E) for routine histological evaluation and Toluidine blue (TB) stain to evaluate proteoglycans in the cartilage matrix. The proximal tibia was selected to measure the thickness of the articular cartilage in 10 fields of each hematoxylin and eosin-stained specimens. Morphometric analysis was carried out using computerized image analyzer system software (Leica Q 500 MCO; Leica, Wetzlar, Germany) connected to a camera attached to a Leica universal microscope at the Histology Department, Faculty of Medicine, Assiut University. The measurements were performed using a ×40 objective lens.

Histological scoring was done by examining the H&E and toluidine blue-stained sections. Grading was performed according to Mankin grading system for scoring of articular cartilage damage in OA on a scale of 0–14 [18]. The total Mankin score is a sum of the scores for cartilage structure, cellular abnormalities, tideline and matrix staining with toluidine blue and total score of 14 means severe damage of cartilage while 0 means normal cartilage.
**Aggrecan immunostaining:** Paraffin sections were mounted on poly L-lysine coated slides to prevent section loss. They were deparaffinized in xylene and then rehydrated in descending grades of ethanol, down to distilled water. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide for 30 minutes. After blocking with Ultra V Block at room temperature for 5 minutes to block non-specific background staining, the sections were incubated with monoclonal anti-aggrecan antibody, (Novous Company, U.S.A, NB 110-6852,) at room temperature for 60 minutes.

Next, the sections were incubated with biotinylated goat anti-polyvalent antibody (secondary antibody) at room temperature for 30 minutes and washed 4X in phosphate buffered saline (PBS). Then, enzyme conjugate, Streptavidin-Horseradish peroxidase was applied to the slides and incubated for 10 minutes at room temperature. Finally, one drop of DAB chromogen (diaminobenzidine) was added to 2ml of DAB substrate. The mixture was mixed well and applied immediately to the sections. Slides were incubated at room temperature for 15 minutes. All images were acquired at x1000 magnification using light microscopy.

**Real-time PCR:** Cartilage tissues were scraped from the subchondral bone using a sterile surgical scalpel. A part of knee cartilage (about 50 mg) was ground in an autoclaved 70% ethanol washed mortar using liquid nitrogen (cryogenic grinding). Total RNA was isolated from rat articular cartilage using Trizol reagent (Epigentics, Cat. No.R2050-1-50, USA) in conjunction with the Pure Link® RNA Mini Kit (Ambion, Cat. NO.12183018A, USA). The concentrations were examined on Epoch Microplate Spectrophotometer (Biotek, Richmond, Virginia, USA). RNA quality was assessed using A260/280 nm and A260/230 nm ratios. Reverse transcription was performed with SensiFAST™ cDNA Synthesis Kit (Bioline, Cat. No. 65053, London) in 20 ml reaction volume containing 500 ng of total RNA.

Real-time PCR was performed on the 7500 real-time PCR machine (Applied BioSystem Germany), using SYBR Green PCR Master Mix (Bioline Lot NO # 415305, London, UK). The reaction mix contained 5ul of 1/5 diluted cDNA, 10ul SYBR Green PCR Master Mix, 500 nm of each primer, and up to 20 ul total volume using PCR grade water. The amplification was performed for 40 cycles of denaturation (95°C for 25 Sec), annealing and extension (59°C for 1 min) using the following primers: **aggrecan;** forward primer 5′-GAATGGAACGATGTCCCCTG-3′ reverse primer 5′-GCTTGTAGGTGTTGGGGTC-3′; **β actin** forward primer 5′-GATCAAGATCATTGCTCCTCCTG-3′ reverse primer 5′-ACGCAGCTCAGTAACAGTCC-3′.

The gene expression levels were assessed using the comparative cycle threshold (CT) method (ΔΔCT method). ΔCT was calculated using the formula: ΔCT = CT (target gene) − CT (endogenous reference gene, β-actin). ΔΔCT method was calculated using the formula ΔΔCT method = ΔCT target−ΔCT control. The relative fold-change in expression was calculated by $2^{-\Delta\Delta CT}$ where ΔΔCt = ΔCt diseased – ΔCt control [19].

**Statistical Analysis:** The statistical analysis was performed with the statistical package for the social science (SPSS) version 20 software. All results are presented as mean ± SD. One way analysis of variance (ANOVA) was used to compare between all groups followed by post hoc analysis (Tukey test). Results of cartilage thickness were analyzed statistically by Mann-Whitney t test. A difference was considered to be statistically significant at $P < 0.05$. 
RESULTS:

Gross observations: In the present study, the MIA injected rats showed limping and slight swelling of left knee joint that were first observed at 7 days after the injection. These manifestations were gradually aggravated and reach maximum at 3 weeks post injection, indicating the presence of inflammatory edema. At the 6th week of the experiment, swelling and limping started to decline and disappeared at 9 weeks of the experiment.

Histopathological changes in articular cartilage: Histological changes were assessed to detect progression of cartilage degeneration of the knee joints of OA rats by analyzing the isolated knee joints from the five groups microscopically. H&E and toluidine blue staining of both, the control and vehicle control groups, showed a smooth surface of the articular cartilage, normal cellularity, and intensive toluidine blue staining. By contrast, the joints from MIA-induced OA rats showed decrease in cartilage thickness, with irregular fibrillated surface, abnormal matrix intensity, reduced chondrocyte number and marked depletion of proteoglycan. A detailed description is provided in (Figure I).

Table I summarized cartilage thickness measurements in control, vehicle and MIA-injected rats (MIA3, MIA6 and MIA9 groups). MIA3, MIA6 and MIA9 groups showed a significant progressive decline of the cartilage thickness (47.53±3.731µm; 32.04±1.58µm and 25.27±3.27µm respectively) compared to control group (61.06±8.71 µm), (P<0.05, P<0.01 and P<0.01). Histopathological lesions score (Mankin score) results showed sustained significant increase (P<0.001) in a Mankin score of MIA3, MIA6 and MIA9 groups which reach (3.4±0.84, 7.5±1.27 and11.5±1.58 respectively) compared to control group level with a score of (0.5±0.53). There was non-significant change in Mankin score between control and vehicle groups Table II.

Effects of the MIA on aggrecan gene expression: The expression of aggrecan gene, which related to the metabolism of cartilage was examined from the knee cartilage by real-time PCR 3, 6 and 9 weeks after MIA injection. As compared with the control group, mRNA expression of aggrecan was significantly down-regulated (p<0.001) in MIA3 group with mean fold change 0.59. The decline in aggrecan gene expression was significantly maintained (p<0.001) in MIA6 and MIA9 groups to reach mean fold change (0.34 and 0.30 respectively) compared to controls (Table III and Figure II).

Immunohistochemical results: To detect the change of aggrecan content in MIA induced OA rats, immunohistochemistry was used to assess the expression of aggrecan at 3rd, 6th and 9th week after MIA injection. There was sustained decrease in immunoexpression of aggrecan in the MIA-injected joints than in the control animals (Figure III).

DISCUSSION:

OA is a degenerative joint disease characterized by deterioration in the integrity of cartilage and accompanied by pain and disability. Although multiple factors are implicated in OA etiology, several lines of suggested that OA etiology is associated with alterations of gene expression in chondrocyte with subsequent changes in the biochemical milieu of the affected cartilage leading to its degradation [20]. As chondrocytes are the sole cell type found in cartilage, any transcriptional dysregulation in OA cartilage is due to deviation of chondrocytes from homeostasis [21].
Our investigations showed that 3 mg MIA is an effective dose required for inducing OA within 3 weeks of injection. Establishment of OA was clarified by morphological and histopathological examinations. In this regard, slight swelling and limping were first observed 7 days after MIA injection, they were gradually increased at 3 and 6 weeks (MIA3 and MIA6 groups respectively). Furthermore, histopathological examination of articular cartilage revealed rough with superficial early cracking, clefting, irregular thickness and focal thinning. As expected, the decrease of the overall cartilage thickness was detected histologically by measurement thickness of the articular cartilage in 10 fields of all hematoxylin and eosin-stained specimens of MIA induced OA groups. This was correlated with a higher Mankin score of MIA3, MIA6, MIA9 groups as compared with control and vehicle group. These findings were in accordance with previous studies that established MIA-induced OA 21 days after injection of 3 mg of MIA into the knee joint of rats [22, 23]. Moreover, Kim et al (2012) observed swelling and limping 7 days after MIA injection. These symptoms subsided transiently and then reappeared at 14 days and aggravated at 21 days, then reached the most sever at 28 days [13].

Another study recorded the maximal knee diameter on day 7 post MIA injection [24]. On the other hand, previous investigators confirmed that MIA induction of OA was evident about 2 weeks of its intra-articular injection. It was also found that a second period of progressive loss of spontaneous mobility occurred more than 3 weeks of MIA injection [15, 25]. This variation in induction of OA can be explained on the basis of difference in animal race.

Aggrecan is the first matrix component that is exposed to degradation and ultimately leads to a loss of cartilage function in cases of OA. It also has a long half-life that may last for months as introduced earlier. This is why we selected this important marker to describe both short and long term consequences of intra-articular MIA injection. Our investigations showed that 3 mg led to decline in aggrecan mRNA expression in all MIA-injected groups in a time frame dependent manner. Where the decline was evident at the 3rd week and was progressive till the end of the 6th week compared to control and vehicle groups. No further deterioration was seen beyond week 6, mRNA levels were the similarities in week 6 and 9. Our results are in agreement with many studies done on MIA-induced OA rat model [15, 26, 27]. They have been shown that there is a significant down regulation of aggrecan gene expression at the onset of MIA induced OA rat model, and this finding agrees with markedly low PG synthesis, observed in human OA samples with normal appearance [28]. In addition, down regulation of aggrecan gene expression was reported in other studies done on human [6, 28]. In contrast, several authors, used molecular methods as RT-PCR and cDNA array to describe differences in the gene pattern between normal and OA cartilage, found no differences in aggrecan expression [29, 30]. This difference can be explained as these studies used cartilage from donors at autopsy, which may be of limited value due to postmortem changes.

Loss of aggrecan was observed not only on the mRNA expression level but also on the aggrecan content of the extracellular matrix. Where, immunostaining showed progressive loss of aggrecan from the articular cartilage of the knee joint over the study period as proved by mild, moderate and marked decrease in the positive immunostaining for aggrecan in MIA3, MIA6 and MIA9 groups respectively. In the control and vehicle groups, immunostaining showed that aggrecan was diffusely present in the matrix of articular cartilage. Diminished aggrecan content was indeed part of reduced proteoglycan as a whole in response to 3 mg MIA injection. Again this single intra-articular injection triggered loss of the whole PG content of the matrix of the articular cartilage of the knee joint in time dependent manner. This was
evidenced by the mild reduction of toluidine blue associated metachromasia denoting mild reduction in the PG content, while MIA$_6$ group showed more decrease in metachromasia especially in the lower part of the articular cartilage denoting more reduction in the PG content as compared with the control group and MIA$_3$ group. In MIA$_3$ group, cartilage matrix showed a weak stainability for toluidine blue all over the matrix indicating diffuse reduction of the PG content as compared with the control group.

Loss of aggrecan expression both on gene and protein levels, was associated with an overall decrease of the cartilage thickness as detected histologically by measuring the thickness of the articular cartilage in 10 fields of all hematoxylin and eosin-stained specimens of MIA induced OA groups. Noteworthy, irregularities in the cartilage thickness was also observed at MIA$_3$ group. These has been referred to by Maldonado and Nam (2013) who observed an overall increase in cartilage thickness due to hyperproliferative chondrocytes before noticeable surface fibrillation occurs as the highly proliferating chondrocytes produce a greater amount of aggrecan that leads to cartilage thickening in dimensions as well as softening of ECM [31].

The sustained loss of ECM aggrecan was mainly attributed to stimulation of matrix metalloproteinases and other matrix degrading enzymes like aggrecanase, which degrade the aggrecan core protein [32, 33]. In mean, time loss of aggrecan at the mRNA level was attributed to the direct cellular toxicity of the MIA (that was clear by reducing cellularity of H&E sections), that is known to inhibit glycolysis and induce apoptosis of chondrocytes, and also to the transient inflammatory reaction provoked by the drug [34, 35]. The pro-inflammatory milieu has been shown to repress ECM synthesis via down regulation of transcription factor controlling type II collagen and aggrecan expression in articular chondrocytes [36]. The fact that different mechanisms are attributed to loss of aggrecan, the main PG of the cartilaginous matrix explain the why the progressive loss of this molecule was observed beyond week 6 at ECM level while the loss was maximum at 6$^{th}$ week at mRNA level. This may be due to increased catabolism irrespective of decrease expression of aggrecan, many MMPs are produced in response of various cytokines that may arise from the chondrocytes themselves or by infiltration from the inflamed synovium [32], which degrade the aggrecan core protein [33].

CONCLUSION:

Several animal models of OA have been established to study the pathogenesis of OA and to assess the efficiency of new disease modifying agents. The disease can be induced surgically by disrupting joint structures such as the cruciate ligaments or menisci [37, 38]. The utility of surgical models is limited specially that they do not mimic the molecular changes, main drug targets, of typical OA. A time dependent, consistent and progressive aggrecan gene expression and protein changes in articular cartilage were noted after intra-articular injection of MIA. These changes were correlated the typical Mankin histopathological grading of OA. The histopathological, behavioral, and molecular changes of this model support intra-articular MIA injection as an animal model that presents reliable profiling of the sequential, chronic and complex nature of OA. This would help studying the biochemical, pathological and studying the OA modifying drugs specially those suggested having anabolic effect and enhancing the PG contents both at early and late stages of the disease.
ACKNOWLEDGMENTS:

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Table I: Cartilage thickness (µm) of left knee cartilage from the studied animal groups.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>P_1</th>
<th>P_2</th>
<th>P_3</th>
<th>P_4</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>61.06±8.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>60.93±7.08</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIA3</td>
<td>47.53±3.731</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<tr>
<td>MIA6</td>
<td>32.04±1.58</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
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<tr>
<td>MIA9</td>
<td>25.27±3.27</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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</tbody>
</table>

MIA3, MIA6 and MIA9; rats given a single intra-articular injection of monosodium iodoacetate then sacrificed 3, 6, and 9 weeks post injection respectively. Data expressed as mean±SD. NS, Non-significant. P_1 vs control group, P_2 vs vehicle group, P_3 vs MIA3 group, P_4 vs MIA6 group. Statistical analyses were performed by Mann-Whitney t test.

Table II: Total histopathological lesions score (Mankin score) of knee cartilage from studied animal groups.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>P_1</th>
<th>P_2</th>
<th>P_3</th>
<th>P_4</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.5±0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.5±0.53</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>MIA3</td>
<td>3.4±0.84</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>MIA6</td>
<td>7.5±1.27</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>MIA9</td>
<td>11.5±1.58</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</table>

MIA3, MIA6 and MIA9; rats given a single intra-articular injection of monosodium iodoacetate then sacrificed 3, 6, and 9 weeks post injection respectively. Data expressed as mean±SD. NS, Non-significant. P_1 vs control group, P_2 vs vehicle group, P_3 vs MIA3 group, P_4 vs MIA6 group.
Table (III): Relative mRNA expression of aggrecan in knee cartilage of MIA-injected groups as compared to control and vehicle groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>control group</th>
<th>Vehicle group</th>
<th>MIA₃ group</th>
<th>MIA₆ group</th>
<th>MIA₉ group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 6</td>
<td>n= 6</td>
<td>n= 6</td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>1.05 ± 0.07</td>
<td>1.08 ± 0.18</td>
<td>0.59 ± 0.05</td>
<td>0.34 ± 0.02</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>P₁</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P₂</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>P₃</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P₄</td>
<td>NS</td>
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</table>

MIA₃, MIA₆ and MIA₉, rats given single intra-articular injection of monosodium iodoacetate then sacrificed 3, 6, and 9 weeks post injection respectively. NS, non-significant. P₁ vs control group, P₂ vs vehicle group, P₃ vs MIA₃ group and P₄ vs MIA₆ group.

Figure (I):

Histopathological analysis of knee cartilage from control and MIA-injected rats. The knee cartilage was stained with hematoxylin and eosin (H&E) (A-D) and by toluidine blue (E-H). By H & E, the articular cartilage of in controls appeared as a typical hyaline cartilage in structure with a regular surface. Superficially inward, this articular cartilage consisted of four poorly demarcated zones: tangential, transitional, radial, and calcified zones. In MIA₃, cartilage showed some surface irregularities (arrow). MIA₆ cartilage showed superficial erosions (fibrillations), and clefts reaching the transitional zone (Black arrow). Absence of the tidemark was seen in many areas. In addition, areas of degenerated chondrocytes are seen. MIA₉ cartilage showed marked decrease in thickness, the population of
chondrocytes also apparently reduced or is even absent, especially in the radial zone (Black arrow). By toluidine blue, the matrix of the articular cartilage appears to have homogenous metachromasia reflecting the good proteoglycan content of the controls. The cartilage matrix of MIA3 appeared with mild decrease in the metachromasia (arrow). MIA6 showed decrease in metachromasia especially in the lower part of the articular cartilage (Black arrow). MIA9 showed a weak stainability for toluidine blue all over the matrix (Black arrow) as compared with the control group indicating diffuse reduction of the proteoglycan content. MIA3, MIA6 and MIA9, rats given single intra-articular injection of monosodium iodoacetate then sacrificed 3, 6 and 9 weeks post injection respectively (magnification X400).

Figure (II): The mRNA expression of aggrecan in knee cartilage of MIA-injected groups. MIA3, MIA6 and MIA9, rats were given single intra-articular injection of monosodium iodoacetate then sacrificed 3, 6, and 9 weeks post injection respectively. Data presented as mean ± SD. *, # and Ο, p<0.05 vs control, vehicle and MIA3 groups respectively.

Figure (III): Immunostaining of knee cartilage with anti-aggrecan monoclonal antibody in control and MIA-induced OA rats. In the control a positive reaction for aggrecan was diffusely present in the matrix of articular cartilage. There was mild, moderate and marked decrease in a positive reaction for aggrecan in MIA3, MIA6, MIA9 rats respectively (magnification x 1000).
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