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Research Article

Therapeutic Effects of Sodium Hyaluronate and Diclofenac Sodium in Chronic Arthritis

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ABSTRACT

The present study was designed to compare the anti-inflammatory effect of sodium hyaluronate, which is similar to the lubricant fluid that found naturally in the capsule of the healthy joint with diclofenac sodium, a member of NSAIDs commonly used in treatment of Osteoarthritis (OA), separately and in combination on an experimental model of osteoarthritis in rats induced by monosodium-iodoacetate (MIA). Twenty-five male albino rats weighing at the beginning of the experiment 160 ± 20 gm were used in this study. Rats were housed in cages at $25 \pm 0.5^\circ\text{C}$. The rats were divided into 5 main groups.

Key Words: Sodium Hyaluronate, Diclofenac Sodium, Chronic Arthritis**ARTICLE INFO:** Received 06 May 2019; Review Completed 22 July 2019; Accepted 31 July 2019; Available online 15 August 2019**Cite this article as:**

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INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis. It is a group of chronic painful, disabling conditions affecting the synovial joints. It results from cartilage failure induced by a complex interplay of genetic, metabolic and biomedical factors with secondary components of inflammation. The process involves interactive degradation and repair of cartilage, bone and synovium⁽¹⁾. Osteoarthritis is not limited to articular cartilage but also considered "a whole-joint disease" with involvement of subchondrial bone and cartilage changes⁽²⁾. All of these drastic changes are most likely to play an important role in the complex cascade of pathologic changes during OA development leading to loss of joint functions and the impaired quality of life⁽³⁾. The most used options including analgesics (such as acetaminophen) and non-steroidal anti-

inflammatory drugs (NSAIDs) are focused on pain relief and improvement of joint function⁽⁴⁾. Diclofenac sodium is the most popular prescribed non-steroidal compound with pronounced anti-rheumatic, anti-inflammatory, analgesic, and antipyretic properties. It is known that diclofenac, as other non-selective NSAIDs, is able to impair prostaglandin synthesis by the inhibition of the cyclooxygenase (COX) enzymes in the injured tissues and the central nervous system. Thus, chondrio-protectives agents are used nowadays matching with the main goal of osteoarthritis (OA) in delaying cartilage degeneration and helping to regenerate the cartilage structure⁽⁵⁾. Such agents were classified as symptomatic slow-acting drugs for OA (SYSADOAs), including cartilaginous matrix precursors (glucosamine, chondroitin sulfate and hyaluronic acid) and cytokine modulators (diacerein)⁽⁴⁾. Hyaluronic acid provides an option

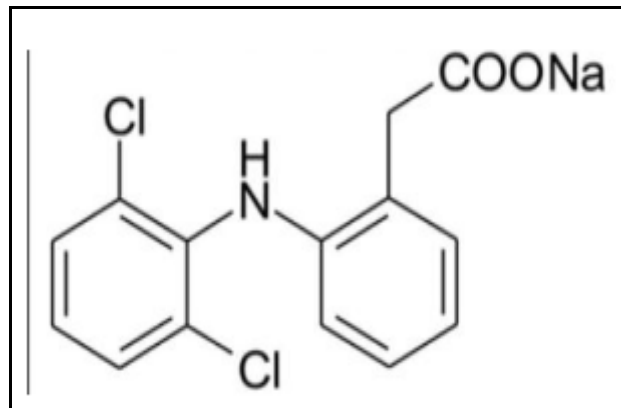
for people whom probably at increased risk for upper gastrointestinal (GI) complications, such as peptic ulcer or upper GIT bleeding. HA also may be considered in patients with kidney failure⁽⁶⁾.

The aim of this study:

This study was carried out to compare the anti-inflammatory effect of sodium hyaluronate, which is similar to the

lubricant fluid that occur naturally in the articular capsule of the healthy joint with diclofenac sodium, a member of NSAIDs commonly used in treatment of OA, separately and in combination on an experimental model of osteoarthritis in rats induced by monosodium-iodoacetate (MIA).

Structural Formula:



Structural formula of Diclofenac Sodium⁽⁸⁾.

Chemical name: [2- (2, 6-dichloroanilino) phenyl] acetic acid.

Dose of voltaren: I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month)⁽⁹⁾

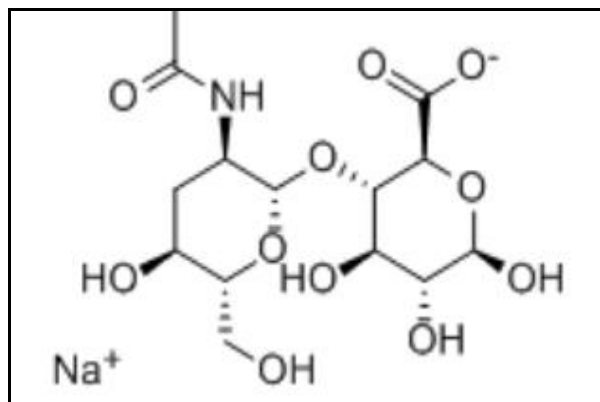
MATERIALS AND METHODS

Materials

I) Drugs

A) Sodium Hyaluronate (Hyalubrix®):

Structural Formula:



Structural formula of Sodium Hyaluronate⁽⁷⁾.

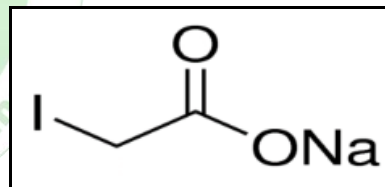
Chemical name: Hyaluronic Acid, Sodium salt.

Dose of Hyalubrix®: Intra-articular injection of (0.036 ml) once weekly/month (Paget and Barnes, 1964).

II) Chemical used for induction of OA:

Monosodium-iodoacetate (MIA):

Structural Formula:



Structural formula of MIA⁽¹⁰⁾

Molecular Formula: C₂H₂INaO₂ (Roberto et al., 2003).

Dose: Single dose of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) intra-articular injection in to the right stifle joint⁽¹¹⁾.

III) Experimental animals:

Twenty-five male albino rats weighing at the beginning of the experiment 160 ± 20 g. Rats were housed in cages at 25±0.5°C, under a 12:12 light/dark cycle, with free access to feed and water. Rats of all groups were kept under similar environmental conditions of temperature, illumination, acoustic noise, and ventilation, and received the determined feed during the course of the experiment. Feed and water were kept in special open containers fixed in the walls of the cages. Cleaning and changing water and feed were done for all rats twice daily. The experimental protocols were

approved by the Faculty of Vet. Medicine Zagazig University. Egypt.

Methods

I) Mono Sodium Iodo Acetate (MIA) Induced osteoarthritis: Prior to induction of OA, all rats except control group were anesthetized by 1ml/kg body weight of a mixture of ketamine (50mg/ml) and xylazine (20mg/ml) at a ratio of 2:1 intra muscularly into the gluteal region⁽¹²⁾. Single dose of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) intra-articular injection in to the right stifle joint⁽¹¹⁾.

II) Experimental protocol: After two weeks of adaptation, the rats were randomly allocated into 5 equal groups each of 5 rats as shown in table (1). **Group I (control):** non-arthritis, non-treated rats. **Group II (arthritic non-treated):** Rats received single dose of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) intra-articular injection in to the right stifle joint. **Group III (sodium hyaluronate treated**

rats): Rats received sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), after 2 weeks from I/A injection of MIA. **Group IV (diclofenac sodium treated rats):** Rats received diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) after 2 weeks from I/A injection of MIA. **Group V (combination):** Rats received sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) and diclofenac sodium 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA.

III) X-ray: At the end of study, all rats were anesthetized by 1ml/kg body weight of a mixture of ketamine (50mg/ml) and xylazine (20mg/ml) at a ratio of 2:1 intra muscularly into the gluteal region for applying X-ray⁽¹²⁾. X-ray on the stifle joint (latero-medial view) was done by using x-ray machine. The exposure factors: 40 KVP, 2.3 MAs.

IV) Collection of samples: A- Serum samples: At the end of experiment, all rats were sacrificed, blood samples were collected from rats, centrifuged at 8000 r.p.m. for 15 minutes, the resulting supernatant serum were collected and used for estimation of: 1- Some pro-inflammatory and inflammatory cytokines (TNF α , IL-1 β and IL-6). 2- Lipid peroxidation marker as malondialdehyde (MDA) and antioxidant enzymes as glutathione peroxidase activity (GPx) and superoxide dismutase (SOD). 3- Liver function enzymes (ALT and AST). 4- Kidney function parameters (urea and creatinine).

B- Histopathological samples: 1- right stifle joint: Soft tissue was removed from the right stifle joint. The patella was removed from each stifle to facilitate thorough fixation of the joint. Tissue samples were prepared for light microscopy using standard procedures. Briefly, samples were preserved in 10% neutral-buffered formalin, and subsequently decalcified in 5% formic acid for 72 h. Samples were dehydrated in an ethanol series and embedded in paraffin. Sections were stained with either Hematoxylin & Eosin and examined microscopically according to⁽¹³⁾. 2- Liver and kidney were kept in 10 % neutral formalin and processed in paraffin wax. Sections of 5 microns thickness were stained with Hematoxylin and Eosin and examined microscopically⁽¹⁴⁾.

V) Biochemical analysis: A- Some pro-inflammatory and inflammatory cytokines (TNF- α , IL-6 and IL-1 β). 1-. Determination of serum TNF- α by rat TNF- α ELISA kit according to Yu , (2012), 2-. Determination of serum IL-6 using rat IL-6 ELISA Kit according to Boulanger, (2003), 3. Determination of serum IL-1 beta using Rat IL-1 beta ELISA kit: RayBio® Rat IL-1 beta ELISA Kit for cell and tissue lysates. B- Lipid peroxidation marker as MDA and antioxidant enzymes as GPx and SOD. 1- Marker of Lipid peroxidation (MDA): Determined according to the method adapted by⁽¹⁵⁾. 2- Superoxide dismutase (SOD): Super oxide dismutase activity in serum was determined according to the method⁽¹⁶⁾. 3- Glutathione peroxidase (GPX): Determined

according to the method⁽¹⁷⁾. C- Liver function tests: 1- Alanine aminotransferase (ALT): Principles of reaction:- The serum activity of alanine aminotransferase (ALT) was determined colorimetrically according to the method⁽¹⁸⁾ using readily made kits. 2-Determination of Aspartate aminotransferase (AST): Principles of reaction: The serum activity of Aspartate aminotransferase (AST) was determined calorimetrically according to the method⁽¹⁸⁾ using readily made kits. D- Kidney function tests: 1-Determination of serum urea: Serum urea was determined according to the method⁽¹⁹⁾.

VI) Statistical analysis: In order to assess the influence of Sodium Hyaluronate, Diclofenac Sodium and their combination on some hematological, and biochemical parameters, one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (Tukey's HSD) test as post hoc test was used. Analysis was done using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Results were reported in means \pm SEM (Standard Error of Mean). The value of $P < 0.05$ was used to indicate statistical significance.

RESULTS

(I) Clinical signs: On the second day after injection of single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, right stifle joint revealed signs of acute arthritis; hyperemia and severe swelling as depicted in Fig (1).

(II) X-ray findings of right stifle joint rats: Group I (control): Radiography on normal rat's right stifle joint of control group showing normal radiopacities, smooth articular surfaces with clear radiolucent synovial fluid (Fig 2). Group II (osteoarthritic non-treated): Radiography on right stifle joint of osteoarthritic rats injected with single dose of 50 μ l of MIA solution intra-articularly, showing complete joint space disappearance with osteophyte formation and irregularity in articular surface (Fig 3). Group III (sodium hyaluronate treated rats): Radiography on right stifle joint of osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA showing improvement in the form of minimal changes in the articular surface and the joint space appeared normal (Fig4). Group IV (diclofenac sodium treated rats): Radiography on right stifle joint of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA showing increased joint space radiopacity, osteophyte formation with irregular articular surface and erosions at the femoral condyles (Fig 5). Group V (combination): Radiography on right stifle joint of osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium,

after 2 weeks from I/A injection of MIA showing osteophyte formation with irregular articular surface (Fig 6).

(III) Biochemical Parameters: (A) Pro-inflammatory cytokines serum levels:

1) Serum TNF- α level (pg/ml): As shown in Table (2) and Fig. (7), Osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intraarticularly, showed a significant ($P<0.05$) increase in TNF- α levels (53.55 ± 3.3 pg/ml) when compared with (11.46 ± 0.82 pg/ml) for control group. Serum of osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA revealed a significant ($P<0.05$) decrease in TNF- α levels (19.58 ± 1.8 pg/ml) when compared with (53.55 ± 3.3 pg/ml) for osteoarthritic (nontreated) group. On the other hand, osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA showed a significant ($P<0.05$) increase in TNF- α levels (74.89 ± 2.16 pg/ml) when compared with (53.55 ± 3.3 pg/ml) for osteoarthritic (non-treated) group. Combined treatment with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) and diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), 2 weeks from MIA injection, evoked a significant ($P<0.05$) increase in TNF- α levels (75.63 ± 2.29 pg/ml) when compared with (53.55 ± 3.3 pg/ml) for osteoarthritic (nontreated) group. There is non-significant difference in TNF- α level of combination treated group when compared with diclofenac sodium treated group, while there is a significant increase in TNF- α level when compared with sodium hyaluronate treated group. 2) Serum IL-1 β Levels (pg/ml). Serum of osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a significant ($P<0.05$) increase in IL-1 β levels (62.77 ± 2.12 pg/ml) when compared with (12.77 ± 1.69 pg/ml) for control group (Table, 1 and Fig., 8). Treatment of osteoarthritic rats with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA evoked a significant ($P<0.05$) decrease in IL-1 β levels (22.54 ± 2.83 pg/ml) when compared with (62.77 ± 2.12 pg/ml) for osteoarthritic (non-treated) group. There was a significant ($P<0.05$) increase in IL-1 β levels of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA (80.35 ± 5.41 pg/ml) when compared with (62.77 ± 2.12 pg/ml) for osteoarthritic (nontreated) group. Combined treatment with sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection, induced a significant ($P<0.05$) increase in IL-1 β levels (81.17 ± 3.93 pg/ml) when compared with (62.77 ± 2.12 pg/ml) for osteoarthritic (nontreated) group. There is

non-significant difference in IL-1 β levels of combination treated group when compared with diclofenac sodium treated group, while there is a significant increase in IL1 β levels when compared with sodium hyaluronate treated group. 3) Serum IL-6 levels (pg/ml). Osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intraarticularly, showed a significant ($P<0.05$) increase in IL-6 levels (252.55 ± 25.05 pg/ml) when compared with (22.81 ± 5.48 pg/ml) for control group (Table, 1 and Fig., 9). Treatment of osteoarthritic rats with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA elicited a significant ($P<0.05$) decrease in IL-6 levels (59.78 ± 9.61 pg/ml) when compared with (252.55 ± 25.05 pg/ml) for osteoarthritic (non-treated) group. A significant ($P<0.05$) increase in IL-6 levels was noted in osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA (420.71 ± 46.84 pg/ml) compared with (252.55 ± 25.05 pg/ml) for osteoarthritic (nontreated) group. Combined treatment with sodium hyaluronate and diclofenac sodium induced a significant ($P<0.05$) increase in IL6 levels (379.63 ± 30.01 pg/ml) compared with (252.55 ± 25.05 pg/ml) for osteoarthritic (non-treated) group. There is non-significant difference in IL-6 levels of combination treated group when compared with diclofenac sodium treated group, while there is a significant increase in IL-6 levels when compared with sodium hyaluronate treated group. (B) Serum lipid peroxidation marker; (MDA) and antioxidant enzymes activity; (GPx and SOD): 1) Serum MDA level (μ mol/l) As shown in Table (2) and Fig (10). Osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intraarticularly, showed a significant ($P<0.05$) increase in MDA serum levels (27.63 ± 4.81 μ mol/l) when compared with (2.64 ± 0.57 μ mol/l) for control group. Serum of osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA revealed in a significant ($P<0.05$) decrease in MDA levels (4.83 ± 0.95 μ mol/l) when compared with (27.63 ± 4.81 μ mol/l) for osteoarthritic (nontreated) group. On the other hand serum of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly/month), after 2 weeks from I/A injection of MIA showed a significant ($P<0.05$) increase in MDA levels (48.39 ± 5.05 μ mol/l) when compared with (27.63 ± 4.81 μ mol/l) for osteoarthritic (non-treated) group. Combined treatment with sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection revealed a significant ($P<0.05$) increase in MDA serum levels (47.31 ± 3.11 μ mol/l) when compared with (27.63 ± 4.81 μ mol/l) for osteoarthritic (non-treated) group. There is non-significant difference in serum MDA levels of combination treated group when compared

with diclofenac sodium treated group, while there is a significant increase in MDA levels when compared with sodium hyaluronate treated group. 2) Serum SOD level (U/ml) Serum of osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a significant ($P<0.05$) decrease in SOD levels (44.07 ± 1.94 U/ml) when compared with (103.98 ± 3.41 U/ml) for control group (Table, 2 and Fig., 11). Treatment of osteoarthritic rats with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA evoked a significant ($P<0.05$) increase in SOD levels (83.6 ± 5.16 U/ml) when compared with (44.07 ± 1.94 U/ml) for osteoarthritic (non-treated) group. Serum of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA revealed a significant ($P<0.05$) decrease in SOD levels (29.64 ± 3.04 U/ml) when compared with (44.07 ± 1.94 U/ml) for osteoarthritic (non-treated) group. Concerning to osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection revealed a significant ($P<0.05$) decrease in SOD serum levels (29.5 ± 3.39 U/ml) when compared with (44.07 ± 1.94 U/ml) for osteoarthritic (non-treated) group. There is non-significant difference in SOD serum levels of combination treated group when compared with diclofenac sodium treated group, while there is a significant decrease in SOD levels when compared with sodium hyaluronate treated group. 3) Serum GPX level (U/ml) As shown in Table (2) and Fig (12). Osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a significant ($P<0.05$) decrease in GPX levels (53.65 ± 4.07 U/ml) when compared with (113.73 ± 5.29 U/ml) for control group. Treatment of osteoarthritic rats with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA elicited a significant ($P<0.05$) increase in GPX levels (87.69 ± 2.27 U/ml) when compared with (53.65 ± 4.07 U/ml) for osteoarthritic (non-treated) group. A significant decrease in GPX level was noted in Serum of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA (35.8 ± 2.03 U/ml) when compared with (53.65 ± 4.07 U/ml) of osteoarthritic (non-treated) group. On the other hand, OA rats treated with combination of sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection, revealed a significant ($P<0.05$) decrease in GPX levels (36.69 ± 1.85 U/ml) when compared with (53.65 ± 4.07 U/ml) for osteoarthritic (non-treated) group. There is non-significant difference in GPX serum levels of combination group when compared with diclofenac sodium treated group, while there is a significant decrease in GPX serum levels when

compared with sodium hyaluronate treated group. (C) **Liver function enzymes:** (1) Serum ALT level (U/l) As shown in Table (3) and Fig (13). Serum of osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly showed a non-significant difference in ALT levels (35.55 ± 1.55 U/l) for control group. U/l) when compared with (34.23 ± 1.96 U/l) for Osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA evoked a non-significant difference in ALT levels (36.52 ± 1.98 U/l) when compared with (34.23 ± 1.96 U/l) for control group. On the other hand osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA revealed a significant ($P<0.05$) increase in ALT levels (105.95 ± 5.07 U/l) when compared with (34.23 ± 1.96 U/l) for control group. Concerning to osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection, revealed a significant ($P<0.05$) increase in ALT serum levels (106.9 ± 5.46) when compared with (34.23 ± 1.96 U/l U/l) for control group. There is non-significant difference in ALT levels of combination treated group when compared with (105.95 ± 5.07 U/l) of diclofenac sodium treated group, while there is a significant ($P<0.05$) increase in ALT serum levels when compared with (36.52 ± 1.98 U/l) for sodium hyaluronate treated group. (2)- Serum AST level (U/l): Serum of osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a non-significant difference in AST levels (37.38 ± 1.73 U/l) for control group (Table, 3 and Fig, 14). U/l) when compared with (37.85 ± 2.56 U/l) for control group. Osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a non-significant difference in AST levels (37.38 ± 1.73 U/l) for control group (Table, 3 and Fig, 14). U/l) when compared with (37.85 ± 2.56 U/l) for control group. Osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA showed a non-significant difference in AST levels (38.81 ± 1.52 U/l) when compared with (37.85 ± 2.56 U/l) for control group. Osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA elicited a significant ($P<0.05$) increase in AST levels (120.23 ± 8.83 U/l) when compared with (37.85 ± 2.56 U/l) for control group. Combined treatment with sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection revealed a significant ($P<0.05$) increase in AST serum levels (117.21 ± 9.2 U/l) when compared with (37.85 ± 2.56 U/l) for control group. There is non-significant difference in AST levels of combination treated group when compared with (120.23 ± 8.83 U/l) of diclofenac sodium treated group, while there is a significant ($P<0.05$) increase in AST levels when compared with (38.81 ± 1.52 U/l) for sodium hyaluronate treated group. (D)- **Kidney function tests:** (1)- Serum urea levels: As shown in Table (4) and Fig (15). Serum of osteoarthritic rats injected

with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a non-significant difference in Urea Levels (26.11 ± 1.71 mg/dl) when compared with (22.83 ± 0.87 mg/dl) for control group. Osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA evoked a non-significant difference in urea levels (23.69 ± 1.27 mg/dl) when compared with (22.83 ± 0.87 mg/dl) for control group. On the other hand osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA revealed a significant ($P < 0.05$) increase in urea serum levels (66.63 ± 1.77 mg/dl) when compared with (22.83 ± 0.87 mg/dl) for control group. Concerning to osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection, elicited a significant ($P < 0.05$) increase in urea serum levels (67.67 ± 2.54 mg/dl) when compared with (22.83 ± 0.87 mg/dl) for control group. There is non-significant difference in serum urea levels of combination treated group when compared with (66.63 ± 1.77 mg/dl) of diclofenac sodium treated group, while there is a significant ($P < 0.05$) increase in urea levels when compared with (23.69 ± 1.27 mg/dl) for sodium hyaluronate treated group. (2)- Serum creatinine levels: As shown in Table (4) and Fig (16). Serum of osteoarthritic rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly, showed a non-significant difference in creatinine Levels (1.4 ± 0.17 mg/dl) when compared with (1.37 ± 0.13 mg/dl) of control group. Treatment of osteoarthritic rats with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA showed a non-significant difference in creatinine serum levels (1.39 ± 0.17 mg/dl) when compared with (1.37 ± 0.13 mg/dl) of control group. Osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA elicited a significant ($P < 0.05$) increase in creatinine serum levels (5.58 ± 0.23 mg/dl) when compared with (1.37 ± 0.13 mg/dl) of control group. On the other hand, OA rats treated with combination of sodium hyaluronate and diclofenac sodium, 2 weeks from MIA injection, revealed a significant ($P < 0.05$) increase in creatinine serum levels (5.75 ± 0.65 mg/dl) when compared with (1.37 ± 0.13 mg/dl) for control group. There is non-significant difference in creatinine serum levels of combination treated group when compared with (5.58 ± 0.23 mg/dl) for diclofenac sodium treated group, while there is a significant ($P < 0.05$) increase in creatinine levels when compared with (1.37 ± 0.13 mg/dl) for sodium hyaluronate treated group.

(IV)- Histopathological finding: (A) Macroscopical examination: Rats of control group, osteoarthritic (non-treated) group and sodium hyaluronate treated group revealed no lesions in internal organs as depicted in Fig (17). Diclofenac sodium treated OA rats I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA showed ascites and swollen inflamed internal organs (Fig 18). Osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium, after 2 weeks from I/A injection of MIA showed ascites and swollen inflamed internal organs (Fig 19). **(B) Microscopical examination:** Group I (control): Stifle joint: Section of stifle joint from control group revealed normally distributed chondrocytes in parallel rows with pale basophilic intercellular matrix and the subchondral bone consists of osteocytes nanaliculli surrounding bone marrows filled with bone forming elements (Fig 20). Liver: showed normal histological finding (Fig 21). Kidney: showed normal histological architecture and structures of glomerular tuft and renal tubules (Fig 22). Group II osteoarthritic (non-treated): Rats injected with single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly. Stifle joint (Fig 23) showing: (a) Replacement of subchondral bone marrow with fibrous connective tissue. (b) Infiltration of bone marrow with large numbers of mononuclear cells. (c) Pyknosis and karyorrhexis of chondrocyte with necrosis of numerous other chondrocytes. (d) Severe degeneration in the bone matrix. Liver: Normal. Kidney: Normal. Group III (sodium hyaluronate treated rats): Osteoarthritic rats treated with sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) after 2 weeks from I/A Injection of MIA Stifle joint (Fig. 24) showing: (a) Widening of the joint space. (b) High mitotic activity in chondrocytes. Liver: Normal. Kidney: Normal. Group IV (diclofenac sodium treated rats): Osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA. Stifle joint (Fig. 25) showing: (a) Prevascular oedema and mild leukocytic infiltration in the joint capsule. (b) Irregular joint surface and loss of chondrocytes. Liver (Fig. 26) showing: (a) Perihepatitis, thickening of hepatic capsule by fibrous tissue and extensive leukocytic infiltration. (b) Apoptotic hepatocytes with proliferation of Von-Kupffer cells. Kidney (Fig. 27) showing: (a) Severe congestion with perivascular leukocytic infiltration. (b) Severe hydropic degeneration with individual coagulative necrosis of the tubular epithelium and peritubular congestion. Group V (combination): Osteoarthritic rats treated with combination of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month) and diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA. Stifle joint (Fig. 28) showing: Disorganizations (irregularity

of chondrocytes) with nuclear pyknosis and mild cell loss. Liver (Fig 29) showing: (a) Severe congestion in the portal blood vessels with pyknosis and individualization of almost all hepatocytes. (b) Perihepatitis, thickening of hepatic capsule by fibrous tissue and extensive leukocytic

infiltration. Kidney (Fig 30) showing: (a) Congestion in cortical blood vessels with peritubular hemorrhages. (b) Necrotic and collapsed glomeruli with hydropic degeneration and necrosis of tubular epithelium.

Table 1: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from injection single dose of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) intra-articularly on pro-inflammatory cytokines serum levels (TNF- α , IL-1 β and IL-6). (Means \pm SE) n=5

	TNF α (pg/ml)	IL1 β (pg/ml)	IL-6 (pg/ml)
Control	11.46 \pm 0.82 ^c	12.77 \pm 1.69 ^c	22.81 \pm 5.48 ^c
Osteoarthritic	53.55 \pm 3.3 ^b	62.77 \pm 2.12 ^b	252.55 \pm 25.05 ^b
Sodium hyaluronate	19.58 \pm 1.8 ^c	22.54 \pm 2.83 ^c	59.78 \pm 9.61 ^c
Diclofenac Sodium	74.89 \pm 2.16 ^a	80.35 \pm 5.41 ^a	420.71 \pm 46.81 ^a
Combination	75.63 \pm 2.29 ^a	81.17 \pm 3.93 ^a	379.63 \pm 30.01 ^a

Means within the same columns carrying different superscripts are significant at (P<0.05).

Table 2: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) on MDA and antioxidant enzymes activity; GPX and SOD: (Means \pm SE) n=5

	MDA(µmol/l)	SOD(U/ml)	GPX(U/ml)
Control	2.64 \pm 0.57 ^c	103.98 \pm 3.41 ^a	113.73 \pm 5.29 ^a
Osteoarthritic	27.63 \pm 4.81 ^b	44.07 \pm 1.94 ^c	53.65 \pm 4.07 ^c
Sodium hyaluronate	4.83 \pm 0.95 ^c	83.6 \pm 5.16 ^b	87.69 \pm 2.27 ^b
Diclofenac Sodium	48.39 \pm 5.05 ^a	29.64 \pm 3.04 ^c	35.8 \pm 2.03 ^d
Combination	47.31 \pm 3.11 ^a	29.5 \pm 3.39 ^c	36.69 \pm 1.85 ^d

Means within the same columns carrying different superscripts are significant at (P<0.05).

Table 3: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination, after 2 weeks from injection single dose of 50µl of MIA solution intra-articularly on liver function enzymes (ALT and AST): (Means \pm SE) n=5.

	ALT (U/l)	AST(U/l)
Control	34.23 \pm 1.96 ^b	37.85 \pm 2.56 ^b
Osteoarthritic	35.55 \pm 1.55 ^b	37.38 \pm 1.73 ^b
Sodium hyaluronate	36.52 \pm 1.98 ^b	38.81 \pm 1.52 ^b
Diclofenac Sodium	105.95 \pm 5.07 ^a	120.23 \pm 8.83 ^a
Combination	106.9 \pm 5.46 ^a	117.21 \pm 9.2 ^a

Means within the same columns carrying different superscripts are significant at (P<0.05).

Table 4: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50µl of MIA solution on kidney function tests (urea and creatinine) (Means \pm SE) n=5.

	Urea (mg/dl)	Creatinine (mg/dl)
Control	22.83 \pm 0.87 ^b	1.37 \pm 0.13 ^b
Osteoarthritic	26.11 \pm 1.71 ^b	1.4 \pm 0.17 ^b
Sodium hyaluronate	23.69 \pm 1.27 ^b	1.39 \pm 0.17 ^b
Diclofenac Sodium	66.63 \pm 1.77 ^a	5.58 \pm 0.23 ^a
Combination	67.67 \pm 2.54 ^a	5.75 \pm 0.65 ^a



Figure 1: Rats injected with a single dose of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) intra- articularly showing signs of acute arthritis, hyperemia and severe swelling.

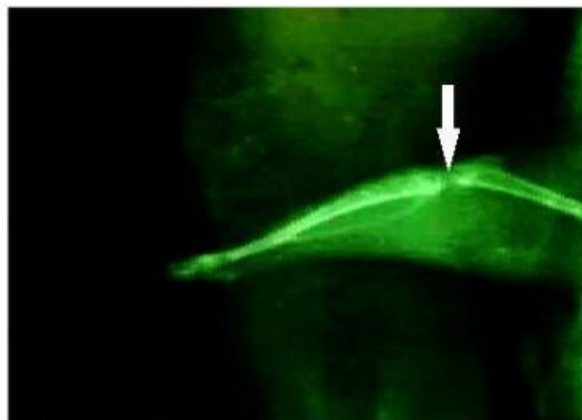


Figure 2: Radiography on normal rat's right stifle joint of control group showing normal radiopacities, smooth articular surfaces with clear radiolucent synovial fluid (arrow).

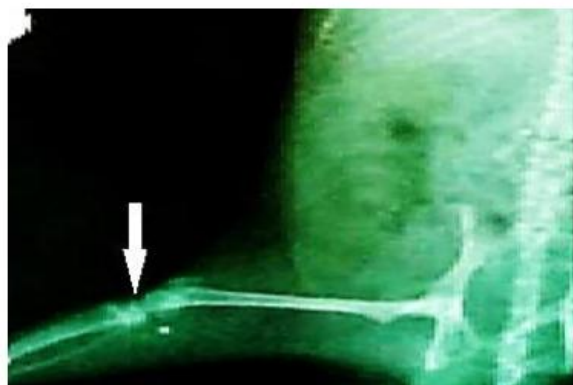


Figure 3: Radiographic changes in rat's right stifle joint of osteoarthritic rats showing complete joint space disappearance with osteophyte formation and irregularity in articular surface (arrow).



Figure 4: Radiographic changes in rat's right stifle joint in sodium hyaluronate treated osteoarthritic rats showing improvement in the form of minimal changes in the articular surface and the joint space appeared normal (arrow).

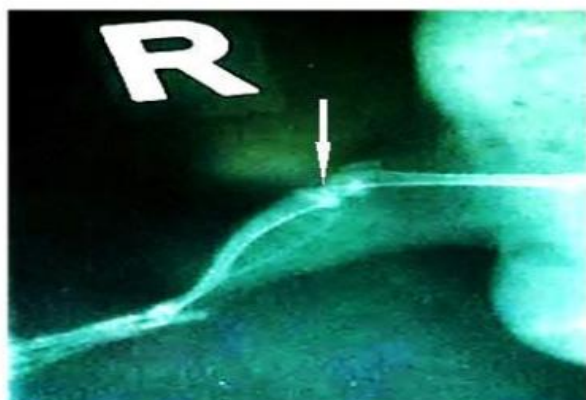


Figure 5: Radiographic changes in right rat's stifle joint in diclofenac sodium treated osteoarthritic group showing increased joint space radiopacity, osteophyte formation with irregular articular surface and erosions at the femoral condyles (arrow).

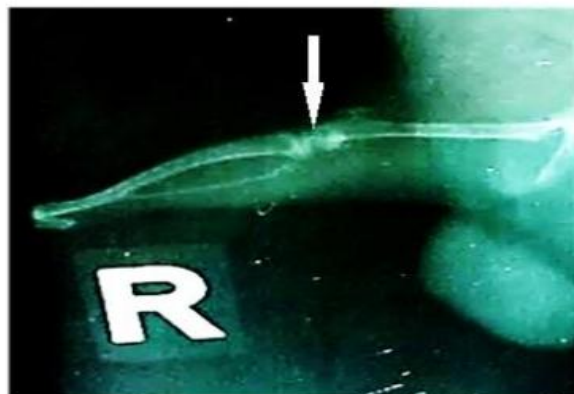


Figure 6: Radiographic changes in rat's right stifle joint in combination of sodium hyaluronate and diclofenac sodium treated osteoarthritic group showing osteophyte formation with irregular articular surface (arrow).

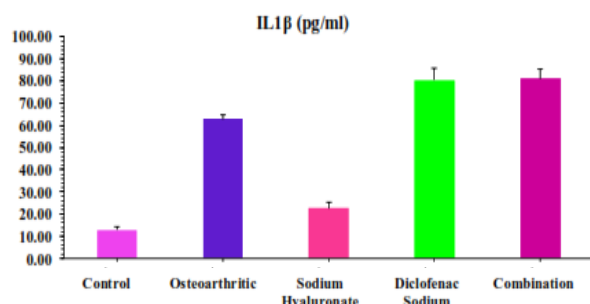


Figure 7: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50μl of MIA solution on pro-inflammatory cytokines serum levels of TNF-α.

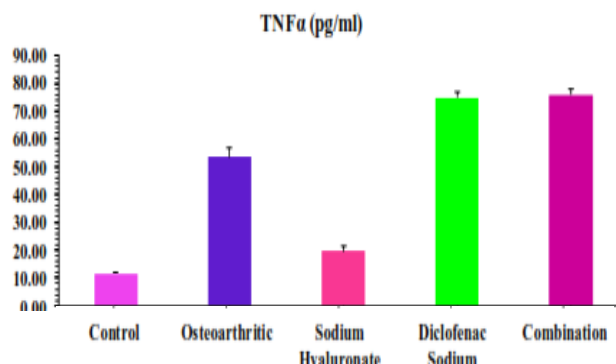


Figure 8: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from injection single dose of 50μl of MIA solution intra-articularly on pro-inflammatory cytokines serum levels of IL-1β.

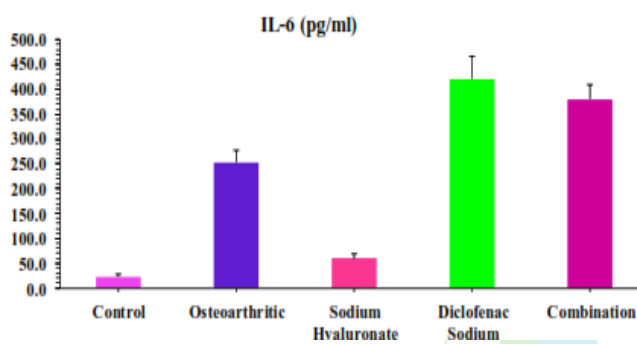


Figure 9: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50μl of MIA solution on pro-inflammatory cytokines serum levels of IL-6.

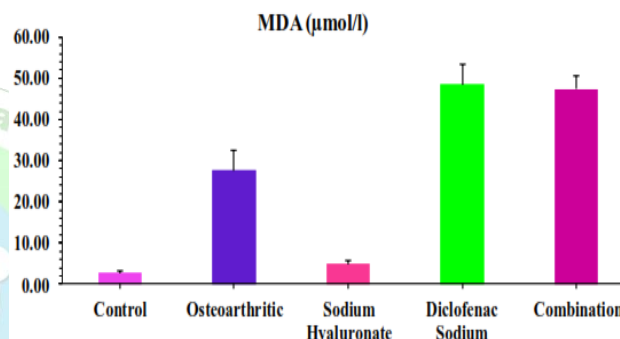


Figure 10: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50μl of MIA solution on lipid peroxidation marker (MDA).

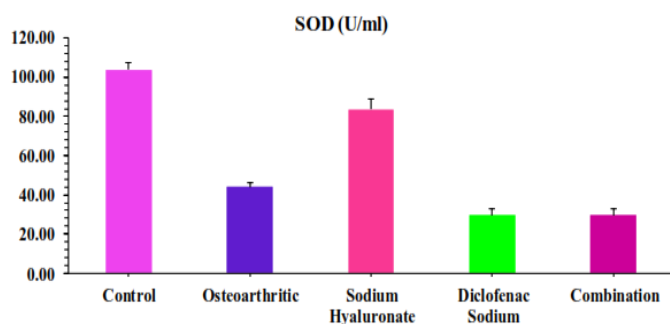


Figure 11: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination, after 2 weeks from I/A injection of single dose of 50μl of MIA solution on antioxidant enzymes activity (SOD).

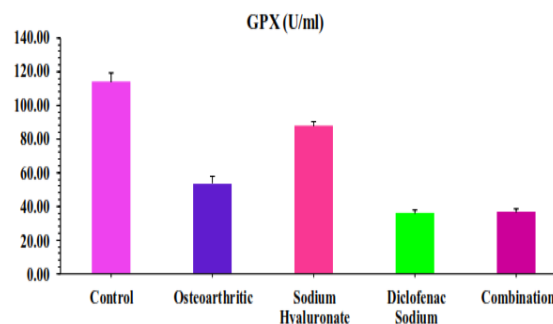


Figure 12: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50μl of MIA solution on antioxidant enzymes activity as GPx activity.

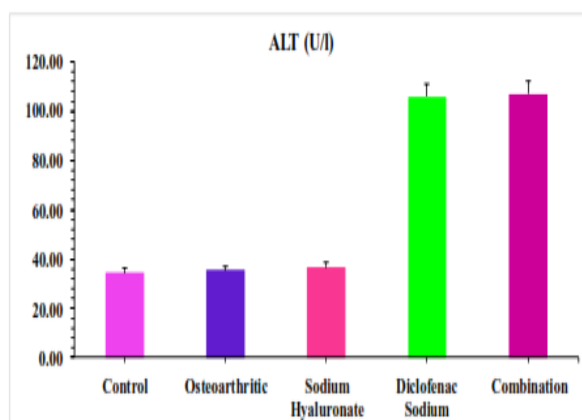


Figure 13: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination, after 2 weeks from I/A injection of a single dose of 50 μ l of MIA solution on liver enzymes (ALT).

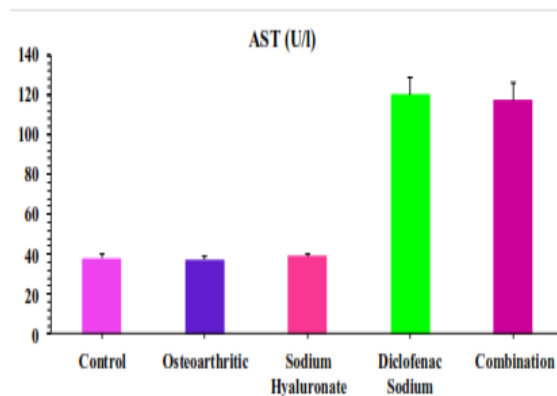


Figure14: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination, after 2 weeks from I/A injection of single dose of 50 μ l of MIA solution on liver enzymes (AST).

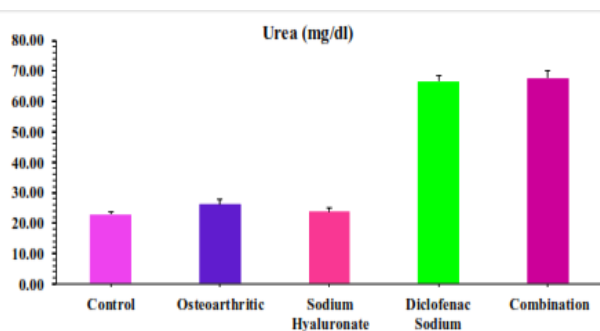


Figure 15: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50 μ l of MIA solution on kidney function tests (urea).

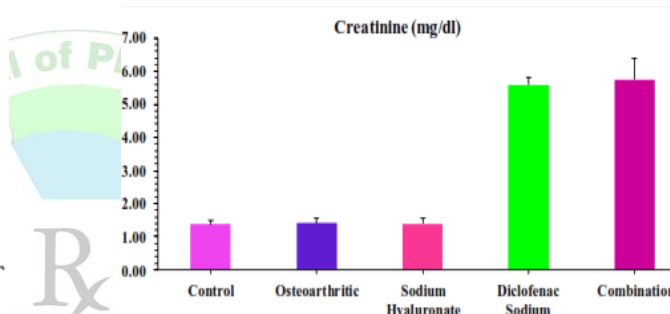


Figure 16: The effect of sodium hyaluronate (I/A injection of 0.036 ml once weekly/ month), diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month) and their combination after 2 weeks from I/A injection of single dose of 50 μ l of MIA solution on kidney function tests (creatinine).



Figure 17: PM findings of normal internal organs of control group.



Figure 18: PM findings of osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from I/A injection of MIA showing ascites and swollen inflamed internal organs.



Figure 19: PM findings of osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium, after 2 weeks from I/A injection of MIA showing ascites and swollen inflamed internal organs.

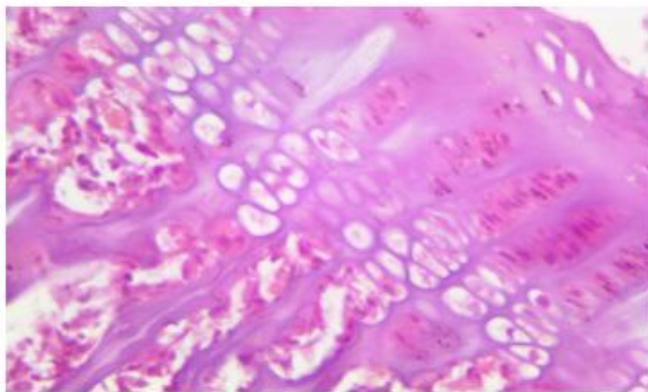


Figure 20: Photomicrograph of right stifle joint of control group showing: Normally distributed chondrocytes in parallel rows with pale basophilic intercellular matrix and the subchondral bone consists of osteocytes in canaliculi surrounding bone marrow filled with bone-forming elements.

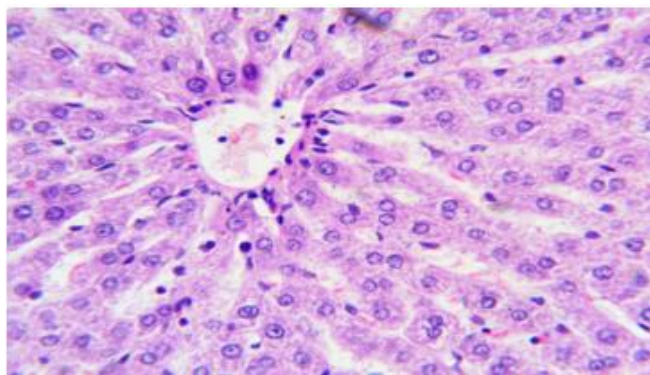


Figure 21: Photomicrograph of liver of control group showing: Normal liver, H&E (X400).

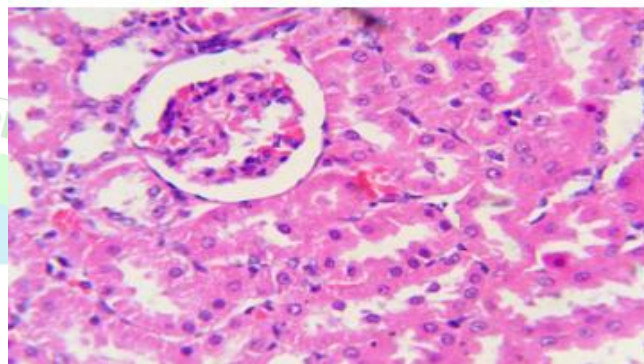


Figure 22: Photomicrograph of kidney of control group showing: Normal kidney, H&E (X400).

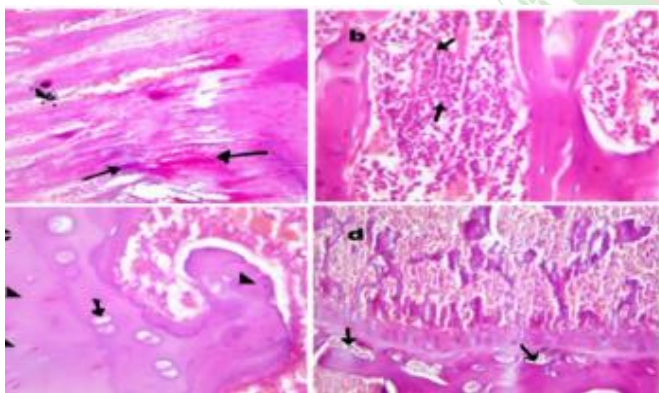


Figure 23: Photomicrograph of right stifle joint of osteoarthritic (nontreated) rats showing: (a) Replacement of subchondral bone marrow with fibrous connective tissue (Arrows), H&E (X400). (b) Infiltration of bone marrow with large numbers of mononuclear cells (Arrows), H&E (X400). (c) Pyknosis (Arrowheads) and karyorrhexis of chondrocyte with necrosis of numerous other chondrocytes (Arrow), H&E (X400). (d) Severe degeneration in the bone matrix (Arrows), H&E (X100).

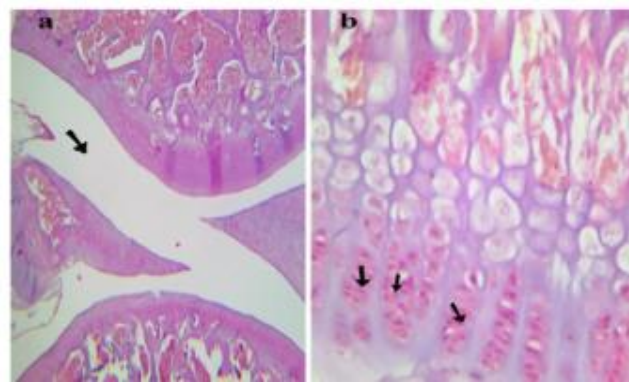


Figure 24: Photomicrograph of right stifle joint of osteoarthritic rats treated with sodium hyaluronate showing: (a) Widening of joint space (Arrow), H&E (X50). (b) High mitotic activity in chondrocytes (Arrows), H&E (X400).

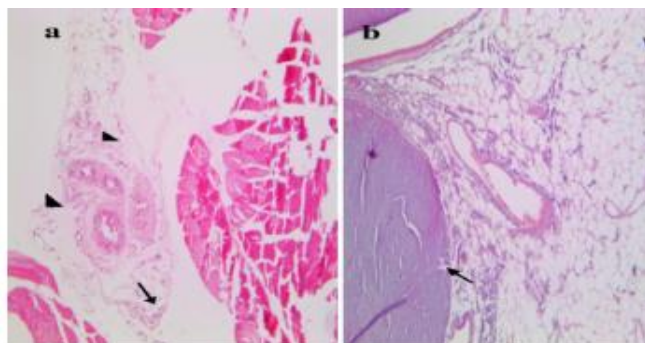


Figure 25: Photomicrograph of right stifle joint of osteoarthritic rats treated with diclofenac sodium showing: (a) Prevascular oedema (Arrow) and mild leukocytic infiltration in the joint capsule (Arrowheads), H&E (X50). (b) Irregular joint surface and loss of chondrocytes (Arrow), H&E (X100).

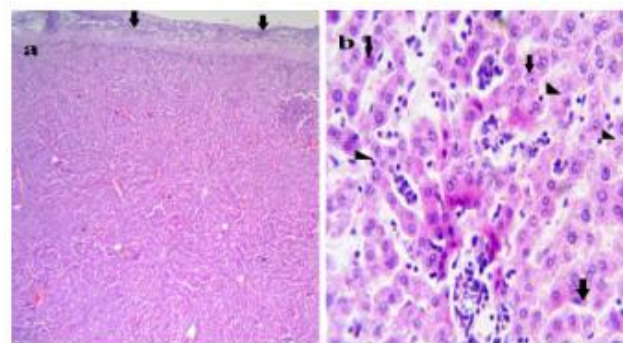


Figure 26: Photomicrograph of liver of osteoarthritic rats treated with diclofenac sodium showing: (a) Perihepatitis, thickening of hepatic capsule by fibrous tissue and extensive leukocytic infiltration (Arrows), H&E (X50). (b) Apoptotic hepatocytes (Arrows) with proliferation of Von-Kupffer cells (Arrowheads), H&E (X400).

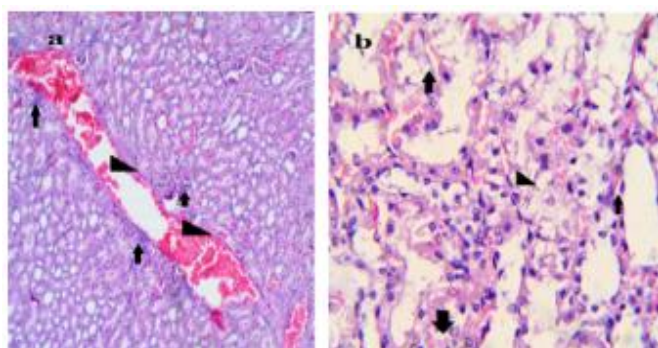


Figure 27: Photomicrograph of kidney of osteoarthritic rats treated with diclofenac sodium showing: (a) Severe congestion (Arrowheads) with perivascular leukocytic infiltration (Arrows), H&E (X100). (b) Severe hydropic degeneration (Arrows) with individual coagulative necrosis of the tubular epithelium and peritubular congestion (Arrowhead), H&E (X400).

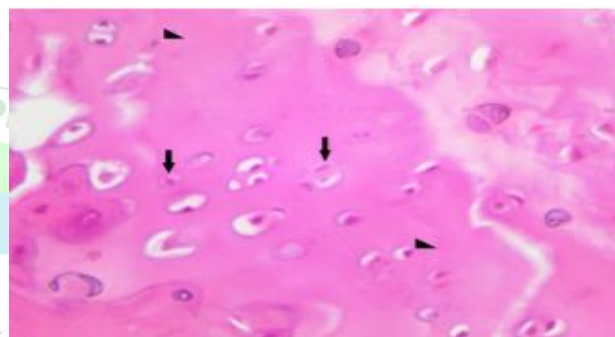


Figure 28: Photomicrograph of right stifle joint of osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium showing: Disorganizations (irregularity of chondrocytes) (Arrows) with nuclear pyknosis and mild cell loss (Arrowheads), H&E (X400).

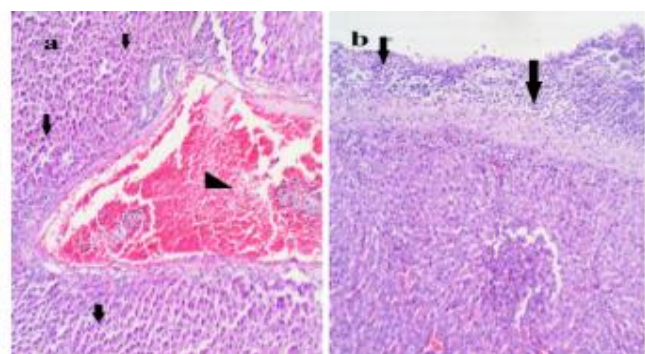


Figure 29: Photomicrograph of liver of osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium at showing: (a) severe congestion in the portal blood vessels (Arrowhead) with pyknosis and individualization of almost all hepatocytes (Arrows), H&E (X100). (b) Perihepatitis, thickening of hepatic capsule by fibrous tissue and extensive leukocytic infiltration (Arrows), H&E (X100).

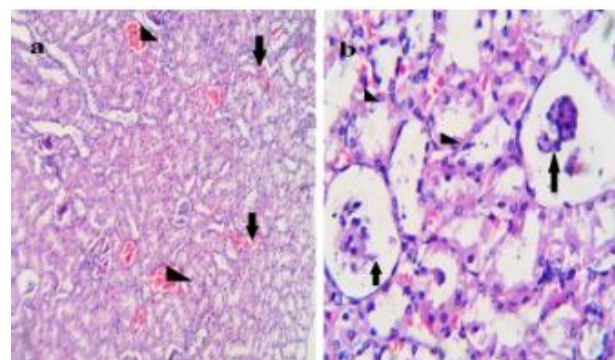


Figure 30: Photomicrograph of kidney of osteoarthritic rats treated with combination of sodium hyaluronate and diclofenac sodium at showing: (a) Congestion in cortical blood vessels (Arrowheads) with peritubular hemorrhages (Arrows), H&E (X100). (b) Necrotic and collapsed glomeruli (Arrows) with hydropic degeneration and necrosis of tubular epithelium (Arrowheads), H&E (X400).

Discussion and Conclusion

The results of the present work revealed that, osteoarthritic rats injected with a Single dose I/A injection of 50 μ l of MIA solution (40mg of MIA powder in 1ml of saline) showed a significant increase in serum pro-inflammatory cytokines (TNF α , IL-1 β and IL-6) levels when compared with control group at the end of the study period (six weeks after injection of MIA). Our results were supported by⁽²⁰⁾ who noted that several cytokines have been found in subchondral bone play major signaling roles associated with cartilage degradation including IL-1, TNF- α and those of the fibrinolytic system including plasminogen, tissue and urokinase plasminogen activators. This coincides with the observations of⁽²¹⁾, who noted a rise in pro-inflammatory cytokines levels in synovial fluid and blood in osteoarthritic rats induced by MIA intraarticular injection, when compared with control negative group. It had been suggested⁽²²⁾ the concept that IL-1 β and perhaps TNF- α are the major catabolic systems involved in the destruction of joint tissues, and may constitute the in situ source of articular tissue damage and degradation. The current findings showed that osteoarthritic rats treated with sodium hyaluronate (0.036 ml once weekly/ month I/A), after 2 weeks from MIA injection showed a significant decrease in the levels of serum pro-inflammatory cytokines when compared with osteoarthritic (non-treated) group. Our results were in accordance with⁽²³⁾ who used high fat diet for induction of OA and when treated with I/P injection of Hyalubrix For 10 days ,there were a significant decrease in the serum level of IL-6 , IL-1 β and TNF- α when compared with osteoarthritic (non-treated) group. It have been demonstrated⁽²⁴⁾ that the long-term using of intra-articular injection of HA in to osteoarthritic rat knee joint induced by adjuvant-induced arthritis, decreased TNF α level to become similar to those of non-arthritis animals. It have been found⁽²⁵⁾ that the production of reactive oxygen species (ROS), such as nitric oxide (NO), resulted in degeneration of cartilage through increased chondrocyte apoptosis and intra-articular HA treatment demonstrated a reduction in IL-1 β -induced oxidative stress, through inhibition of NO production within the synovium. Our data revealed that osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt / twice weekly / month), after 2 weeks from MIA injection revealed a significant increase in serum pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) concentration when compared with osteoarthritic (non-treated) group. These results were in accordance with those reported by⁽²⁶⁾ who examined the effect of diclofenac sodium on the production of clinically validated pro inflammatory cytokines; they found that diclofenac sodium induced production of TNF- α from human peripheral blood monocytes and rheumatoid

synovial membrane cultures. These findings contrasts studies in the acute heterologous (bovine) type II collagen-induced mouse arthritis model, which have shown beneficial effects of NSAIDs on joint pathology and proinflammatory cytokine levels⁽²⁶⁾. On the contrary, Gallelli et al., (2013) reported that, Shortterm NSAID treatment improved the patient disease-specific quality of life with a parallel decrease in pro-inflammatory synovial fluid cytokine (IL-6 and TNF- α) levels in knee OA. In our study, treatment of osteoarthritic rats with I/A injection of sodium hyaluronate (0.036 ml once weekly/ month), after 2 weeks from MIA injection showed a significant decrease in MDA serum level and significant increase in the serum level of antioxidant enzymes as GPx and SOD when compared with osteoarthritic (non-treated) group. These findings endorsed those obtained by⁽²⁸⁾ who examined synovial fluid (SF) samples aspirated from OA patients before the commencement of the treatment and 6 weeks after treatment with HA products. Synovial fluid nitric oxide (SF NO) levels were significantly higher in patients with OA before the commencement of the treatment compared with post-treated and control groups. The synovial fluid SOD activity of patients before the commencement of the treatment was lower than the values in the control and post-treated groups. It well documented that oxidative stress is one of the reasons for apoptotic cell death, and SOD can play a pivotal role in preventing the apoptosis. The use of SOD mimetics has been shown to reduce the severity of inflammation in collageninduced arthritis⁽²⁹⁾. Although low levels of hydrogen peroxide have been shown to stimulate synthesis and may help to repair the matrix after mechanical injury with inadequate extracellular SOD, the balance may shift to an oxidized/inflammatory state with the formation of the more toxic radicals⁽³⁰⁾. It have been reported⁽³¹⁾ a significant decrease in erythrocyte SOD activity in rheumatoid arthritis patients, whereas have observed a significant increase in SOD activity in the synovial fluid (SF) after injection of HA⁽³²⁾. It was demonstrated that sodium hyaluronic acid (Na-HA) had a suppressive effect on MDA and NO production in the meniscus and synovium during the development of OA in the animal models⁽³³⁾. It have been reported⁽³⁴⁾ that in avian embryonic fibroblasts, HA reduced cell damage induced by hydroxyl radicals in a molecular weight (MW) and dose-dependent manner. In this study, and concerning to osteoarthritic rats treated with diclofenac sodium I/M injection of 0.054 ml (6.75 mg/kg b.wt/twice weekly/month), 2 weeks post MIA injection revealed a significant increase in serum MDA concentration and significant decrease in serum GPX and SOD levels when compared with osteoarthritic (non-treated) group. These findings may be due to the damage of hepatic and kidney tissue caused by long term using of diclofenac

sodium. One of the products of lipid damage is malondialdehyde MDA⁽³⁵⁾. Measurement of MDA is widely used as an indicator of lipid peroxidation⁽³⁶⁾ and elevated levels of MDA have been reported in the serum and synovial fluid of patients with rheumatoid arthritis⁽³⁷⁾. These results were in accordance with those reported by⁽³⁸⁾ who found that administration of rats with diclofenac sodium at dose of 13.5 mg/kg b.wt. for 2 successive weeks, induced a marked increase in lipid peroxidation product, malondialdehyde (MDA) content in liver and kidney tissues and a significant decrease in GSH value in both tissues at the 2 weeks of the experiment when compared to control group. During diclofenac sodium metabolism, the number of reactive oxygen species increased. These products induced prooxidative damage in renal tissue. The increase in MDA activity in renal tissue may indicate per oxidative damage and cause cell damage in kidney tissue^{(39), (40)}. Our results also were supported by those of⁽⁴¹⁾ who suggested that diclofenac induced nephrotoxicity may involve production of reactive oxygen species leading to oxidative stress and massive genomic DNA fragmentation and these two free radical mediated events may ultimately be translated into apoptotic cell death of kidney cells in vivo and revealed a DNA-active role for diclofenac. Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are very effective parameters for diagnosis of liver function⁽⁴²⁾. In this study, osteoarthritic (non-treated) rats and sodium hyaluronate treated rats revealed non-significant changes in measured serum levels of ALT and AST when compared with control group. One of the most important advantages of intra articular drug administration is that it could achieve a targeted delivery of the drug to affected tissues allowing local treatment and minimizing side effects typical of systemically administered drugs⁽⁴³⁾. In our study, osteoarthritic rats treated with diclofenac sodium showed a significant increase in serum ALT and AST levels when compared with the control group. Activities of AST and ALT are most commonly used as biochemical markers of liver damage⁽⁴⁴⁾. The current results were in accordance with⁽⁴⁵⁾ who reported that diclofenac sodium caused an elevation in the levels of liver function enzymes and was found to initiate hepatitis and hepatotoxicity. The mechanisms of diclofenac induced hepatic idiosyncratic adverse drug reactions remain largely unknown⁽⁴⁶⁾. Hepatocellular cytotoxicity from diclofenac or its metabolites is only observed in vitro with large concentrations of diclofenac that are not achieved in vivo^{(47), (48)}. Inhibition of prostaglandins is the most important cause of the adverse effects of diclofenac on liver and kidney tissues in sensitive persons or animal species and potentially during longterm use^{(49), (50)}. The obtained biochemical results used for evaluation of liver function were confirmed by histopathological studies of liver of diclofenac sodium

treated rats which showed prehepatitis, thickening of hepatic capsule by fibrous tissue and extensive leukocytic infiltration. Our results agreed with those of⁽⁵¹⁾ who observed that histopathological changes in liver of rats treated with different doses of diclofenac sodium were hepatitis and cloudy swelling and hydropic degeneration of the liver cells. The present study data revealed that osteoarthritic (nontreated) rats and sodium hyaluronate treated animal's provoked non-significant changes in measured serum levels of urea and creatinine when compared with the control group. Meanwhile, treatment with diclofenac sodium elicited a significant increase in serum levels of urea and creatinine. The kidney is another organ affected by the toxic effect of NSAIDs⁽⁵²⁾. Our data were in accordance with those obtained by⁽⁵³⁾ who noticed that serum creatinine and urea levels were elevated in 4 avian species treated with diclofenac sodium. On the same line,⁽⁵⁴⁾ observed a marked increase in urinary uric acid level in rats treated with diclofenac. Similarly,⁽⁵⁵⁾ supported the evidence of renal function impairment by diclofenac sodium and diclofenac potassium, as these drugs increased serum urea and creatinine in rabbits. The biochemical findings of kidney function obtained in the present work were confirmed by histopathological studies of kidney of diclofenac sodium treated rats as kidney showed acute renal failure, severe hydropic degeneration with individual coagulative necrosis of the tubular epithelium and peritubular congestion. Similar outcomes were reported by⁽⁵⁶⁾ who observed cloudy swelling and hydropic degeneration in the tubular epithelial cells of the kidney tissue of all diclofenac sodium treated groups. Our histopathological and X-ray findings in the right stifle joint go side by side with the obtained biochemical alterations. Identification of joint damages is indicated for both diagnostic confirmation and the extent of joint involvement⁽⁵⁷⁾. Conventional plain X-ray is still used widely to detect narrowing of the joint space and osteophytes⁽⁵⁸⁾. In the current study, radiography on osteoarthritic (non-treated) rats injected with Single dose I/A injection of 50µl of MIA solution (40mg of MIA powder in 1ml of saline) to the right stifle joint showed complete joint space disappearance with osteophyte formation and irregularity in articular surface. These results were supported with our histopathological findings which revealed severe degeneration in the bone matrix and infiltration of bone marrow with large numbers of mononuclear cells. Our data were in accordance with those recorded by⁽⁵⁸⁾ who administered a single dose of 50µl of MIA solution intra- articularly in right knee joint of rats. The authors observed a progressive irregularity in articular surface and fibrillation, with chondrocytes loss, and subchondral bone change in the form of cyst and osteophytes formation after for 4 weeks from MIA

treatment. An early study recommended intra-articular hyaluronan (HA) therapy as not only a symptom-modifying therapy but also a treatment, which may significantly decreased the rate of deterioration of joint structure⁽⁶⁾. On a similar ground, ⁽⁵⁹⁾ noted that rabbits treated with 0.3 ml cross-linked hyaluronic acid (33 mg/ml) into the right knee joint showed a significant improvement in knee articular cartilage degeneration in a rabbit model of collagenase-induced osteoarthritis. Intra-articular hyaluronic acid administration produced well-documented structure-modifying effects, as observed in several animal species. For instance, ⁽⁶⁰⁾ reported that administration of a single injection of Healon, immediately after anterior cruciate ligament transaction in rabbits, resulted in significantly decreased inflammation, increased collagen synthesis and angiogenesis and enhanced tissue repair if compared to a single injection of saline solution. In our study, radiographic changes in right rat's stifle joint of diclofenac sodium treated osteoarthritic rats showed increased joint space radiopacity, osteophyte formation with irregular articular surface and erosions at the femoral condyles. In addition the histopathological examination revealed signs of progressive OA degeneration (Shrinkage of chondrocytes with increased eosinophilia, nuclear pyknosis in the tangential and transitional zones with cellular loss in the calcified zone, perivascular edema, mild leukocytic infiltration in the joint capsule, irregular joint surface and loss of chondrocytes). These results matched with those previously obtained by ⁽⁶¹⁾ from studies on NSAIDs. The authors concluded that NSAIDs suppressed proteoglycan synthesis in chondrocytes. Similar observations recorded by ⁽⁶²⁾ who found that the chronic use of diclofenac, but not ibuprofen, naproxen, or piroxicam, accelerated the progression of knee and hip OA in patients over 55 years old. It have been explained⁽⁶³⁾ the destructive effect of NSAIDs on articular cartilage from data obtained from in vitro and in vivo experimental and human studies by inhibition of chondrocyte proliferation, synthesis of cellular matrix components, glycosaminoglycan synthesis, collagen synthesis and proteoglycan synthesis. The net effect of all

or some of the above is an acceleration of articular cartilage breakdown. Our study revealed that there were no significant differences in combination treated rats (sodium hyaluronate and diclofenac sodium) in all biochemical parameters measures when compared with diclofenac treated group. Since prostaglandins (PGs), as well as, other inflammatory mediators act as messenger molecules in the process of inflammation. It was hoped that the use of NSAIDs would decrease the catabolic process in OA, so resulting in disease modifying effect. Unfortunately, research has shown that PGs, especially PGE2 has an important role in differentiation of chondrocytes, and is an important contributor to cartilage formation and promotes DNA and matrix synthesis in chondrocytes⁽⁶⁴⁾. Sodium hyaluronate act through different mechanism, which is the inhibition for production and activity of IL-1 β both at the pre and post membrane effect. Therefore, it can alter the pathogenesis of OA, since IL-1 β was found to play an important role in this disease⁽⁶⁵⁾.

CONCLUSION

On conclusive note, osteoarthritis is the most common type of arthritis. The best method for treatment of osteoarthritis not only depends on removing the inflammatory cause but also trying to regenerate the cartilaginous tissue in the joint. For control of osteoarthritis, a number of medications have been used lately. Diclofenac sodium is the most commonly used non -steroidal anti-inflammatory drug nowadays. Unfortunately, long-term use of diclofenac sodium alone or with sodium hyaluronate resulted in an undesirable effect including hepatotoxicity, nephrotoxicity, gastritis and accelerating the progression of osteoarthritis. Another alternative therapy for treating osteoarthritis is intra-articular injection of Sodium hyaluronate Intra-articular (HA) therapy proved its efficacy in treating OA. It is not only a symptom-modifying therapy but also a treatment, which may significantly decrease the rate of deterioration of joint structure. Sodium hyaluronate administration can be used for treatment and regeneration of the cartilaginous tissue in rats affected with OA without side effect.

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