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Comparative Study of the Effect of Diacerein and Diclofenac Sodium and Their Combination in Osteoarthritis Model Induced By Monoiodoacetate in Albino Rats.

Walla'a A Osman[1], Salwa Abd El Monaem Elmessiry[1], Olfat Gamil Shaker[2], Iman M Zaki[3], Marian Youssry Wissa[1], Mohammed Said Amer[4]*, and Ashraf Ali Shamaa[4].

[1]Department of Medical Pharmacology, Faculty of Medicine, Cairo University, Egypt.
[2]Department of Biochemistry, Faculty of Medicine, Cairo University, Egypt.
[3]Department of Pathology, Institute of Ophthalmic Research, Cairo University, Egypt.
[4]Department of Surgery, Anaesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

ABSTRACT

Osteoarthritis (OA) is the most common joint disorder. The current treatment of osteoarthritis is primarily focused on symptomatic relief by the use of rapidly acting analgesics such as NSAIDs and newer cyclooxygenase-2 (COX-2) specific inhibitors. Diacerein, an Interleukin-1β-antagonist that has been used in the last few years in the treatment of OA. This work was designed to compare the anti-inflammatory effect of Diacerein with Diclofenac Sodium and their combination on albino rats model of osteoarthritis. Ninety adult healthy female albino rats were allocated into 5 groups: normal untreated animals (negative control), the disease model group that received a single dose of monoiodoacetate (MIA) intra articularly in their right knees (positive control), and the (MIA) induced osteoarthritis treated either by Diacerein, diclofenac sodium, or their combination for 6 weeks. Level of serum cartilage oligomeric matrix protein, histopathological examination, and radiological assessment were performed. The results revealed that Diacerein has the potential to ameliorate osteoarthritic changes unlike the commonly used NSAIDs.

Keywords: Comparative, Osteoarthritis, Diacerein, Diclofenac sodium, Monoiodoacetate.

Corresponding author

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INTRODUCTION

OA has been considered as a whole organ disease because the pathological abnormalities affect many structures rather than the cartilage, such as weakness of the muscles surrounding the joint, ligaments laxity, low grade synovitis, degeneration of the menisci and alteration in the neurosensory system which are frequently present in patients [1]. Worldwide, OA is estimated to be the fourth leading cause of disability [2] and affects 12% of the total population in USA [3].

For many years, OA was considered a non-inflammatory form of arthritis (4). Nowadays, the disease is no longer considered as a passive degenerative form but as an active disease process [5]. The pathogenesis of OA appears to be the result of a cross talk between mechanical, cellular and biochemical forces [6].

Diagnosis of OA is done by the clinical findings as well as physical examination.

However identification of joint damages is indicated for both diagnostic confirmation and the extent of joint involvement [7]. Conventional plain X-ray is still used widely to detect narrowing of the joint space and osteophytes.

As OA is a disease characterized by a prolonged asymptomatic molecular phase, a pre-radiographic phase, and a later radiographic phase with evident structural joint changes, frequent pain, and loss of function, so biomarkers have the potential to provide an early warning about the initiation of matrix breakdown that could prompt earlier intervention to minimize cartilage and bone destruction that leads to disability [8].

The goals of managing osteoarthritis include controlling pain, maintaining and improving the range of movement with stability of the affected joints and limiting functional impairment [9].Current treatment is based therefore on the clinical presentation apparent at the time, age, co-morbidity, and the person's own requirements or expectations relating to physical performance. All these need to be considered on an individual basis [6]. Recommendations cover the use of pharmacological and non-pharmacological modalities [10, 11] that have been formulated for treatment of OA. However, joint replacement surgery is considered an appropriate option in patients unable to obtain meaningful functional improvement from combination of these strategies [12].

Diclofenac sodium is a non-steroidal compound with pronounced anti-rheumatic, anti-inflammatory, analgesic, and antipyretic properties. It is known that diclofenac, as other nonselective NSAIDs, is able to impair prostaglandin synthesis by the inhibition of the cyclooxygenase (COX) enzymes in the injured tissues and the central nervous system [13], however many side effects including gastrointestinal, cardiovascular and renal disorders can result from its prolonged use [14].

Diacerein, specifically its active form rhein, is an IL-1β inhibitor. It is a semi-synthetic anthraquinone derivative found in plants of the genus Cassia. Rhein is classified as a Symptomatic Slow Acting Drug in OA (SYSADOA). This class of therapy in OA is characterized by having a slow onset of efficacy and a long carry-over effect once treatment is interrupted; it has moderate anti-inflammatory and analgesic activity and weak laxative effect.

Diacerein is the acetylated form of rhein. This acetylation increases the lipophilicity of the molecule, which increase absorption by the intestinal mucosa [15]. The effects of Diacerein are due to potent inhibition of the production and activity of IL-1β and other catabolic cytokines expressed in OA. This in turn prevents cytokine-induced connective tissue destruction and allow cartilage repair to take place [16]. The effects of diacerein on IL-1β concentration and activity occur at the cell membrane (pre-membrane) and also within the cell (post-membrane) effects.

This work was designed to compare the anti-inflammatory effect of Diacerein with NSAIDs diclofenac sodium and their combination on albino rats (animal) model of osteoarthritis.

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MATERIAL AND METHODS

The study protocol was in accordance with the guidelines for animal research and it was approved by the Ethical Committee of Research Institute of Ophthalmology.

Ninety adult healthy non-pregnant female *albino rats*, weighing 200-250gm matched for age and weight were bred in the animal house of Research Institute of Ophthalmology. Prior to induction of OA, 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, then by inhalation from isoflurane 1-2% for maintenance of anesthesia [17]. The left knee was used as a control, injected by the same volume of saline (50µl). X-ray on the knee joint was done by using Mobile x-ray machine (Ficher Machine, Eureka X-ray tube/ Model E-Merald-125, 1985, U.S.A).The used exposure factors were 38-40 KVP, 0.1 mAs and F.F.D (focal film distance) were 90cm. All the animals were examined in dorsal recumbency using of anterioposterior radiographic projection for the knee joint. The examination was carried out at 2, 4 and 6 weeks post osteoarthritis induction and treatment. The femoral condyle and tibial epiphysis regularity with joint space radiodensity were evaluated.

Animals were divided into two main groups, (Group I) Normal untreated animals (18 rats) served as a negative control group and (Group II) Induced OA animal model (72 rats), in which OA was induced by one single dose of 50µl of MIA solution (600mg of MIA powder in 10ml of saline) injected intra articularly in each right knee joint [18]. Animals in group (II) were further subdivided into 4 subgroups, 18 animals each as follows: Group IIA: No further treatment was applied and served as a positive control. Group IIB: Starting receiving a daily oral dose of diacerien50mg/kg for one week, then 100mg/kg/day for the following 5 weeks [19]. Group IIC: Animals received from the next day a daily oral dose of diclofenac sodium dissolved in saline orally in a dose of 2mg/kg/day for 6weeks [20]. Group IID: Animals received from the next day a combination of diacerein 50mg/kg/day and diclofenac sodium 2mg/kg/day orally for the first week then diclofenac was stopped and animals were maintained on diacerein 100mg/kg/day for the subsequent five weeks.

Six rats from each group were euthanized after the 2^{nd}, 4^{th} and 6^{th} weeks post MIA induction of OA for biochemical, histopathological, and radiological examination.

Venous blood samples were (2ml) each were obtained in the early morning at a fixed time of the day [21]. Samples were collected from all animals on day zero and then every 2 weeks, all through the study period. The serum was stored at -20^{o}C [22] for measuring the level of serum COMP using the spectrophotometer (4010-CLINCON).

ELISA kit provided by USCN LIFE, catalog No: E1197r (Wuhan EIA abScience Co, Wuhan, China) ELISA kit used for measurement of rat serum cartilage oligomeric matrix protein (COMP).

Whole right and left knee joints were fixated and were subsequently decalcified with 7.5% nitric acid [19]. Serial sections of 5 Mm thickness were stained with hematoxylin and eosin (H&E). Previously published histopathological grading scheme (20) was applied for each rat which was expressed simply by the summation of individual grade (No change= 0, Mild= 1, Moderate= 2, Severe= 3) for each of the following observations:

1. Surface irregularity and fibrillation.
2. Disorganization of chondrocytes.
3. Chondrocyte coloning and hypertrophy.
4. Chondrocyte loss.
5. Marginal osteophyte formation.
6. Subchondral bone change in form of fibrosis and cyst formation.

Diacerein (Osteocerein®, Egypt) (19). Diclofenac Sodium (Voltaren SR®, Novartis, Egypt) (20). Monoiodoacetate (MIA, Fluka, Germany). Ketamine (ketamine®, Sigma, Egypt) Xylazine HCl (Xyla-ject®, ADWIA, Egypt) and Isoflurane (Forane®, Abbott/Kahira).

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Statistical analysis

Data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows, then transferred to the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed. Data was summarized using mean and standard deviation for quantitative variables. Relative % change was calculated for COMP to detect the rate of change as follow: Relative % change :[(Post-measurement – Pre-measurement) / Pre-measurement] x100. Comparison between groups was done using one way ANOVA (with LSD post hock test).Different time measurements were compared using repeated measures ANOVA test with LSD post hoc test. P values equal to or less than 0.05 were considered statistically significant.

RESULTS

Biochemical findings

Comparing the serum cartilage oligomeric matrix protein (COMP) level (pg/ml) which expressed as mean±SD and percentage difference showed that, in group I (-ve control) an insignificant change among the mean levels of serum (COMP) all through the study period (P=0.991), as it was 744.7±83.1, 749.7±85.1 and 746.8±60.5 at 2nd, 4th and 6th weeks respectively. While in the disease model group (IIA) there was a progressive significant rise in serum COMP level at 2nd, 4th and 6th weeks (P<0.001) as it attained a level of 1052.5±65.1 (41%; P<0.001), 1505.3±129.8 (101%; P<0.001) and 1784.2±135.4 (139%; P<0.001) respectively, in comparison with the normal untreated group. In group (IIB), the level of COMP showed a significant progressive drop all through the observation period (P=0.02) as the mean serum levels were 1024.5±67.4, 956.5±37.2 and 939.7±39.8 at 2, 4 and 6 weeks respectively and the percentage drop in COMP level by the end of the 6th week after diacerein was (-8.3%). In diclofenac treated group (IIC) the mean level of serum COMP showed a progressive slow rise all through the study period (P=0.003), the mean values in this group were 1036.3±57.4, 1057.7±62.0 and 1083.2±61.3 at 2, 4 and 6 weeks respectively, while the overall percentage change in COMP level by the end of the study in diclofenac treated group was (+4.5%). Passing to group (IID) in which animals received combination of diacerin with diclofenac, an insignificant drop in COMP level was reported all through the study period at 2nd, 4th and 6th weeks (P=0.724) with mean levels of 1027.5±99.1, 991.8±20.4 and 968.5±61.7 respectively and the percentage change in COMP at the end of the study was (-5.7%). Data are summarized and represented in Table (1) and Fig. (1).

Table 1: Changes in mean serum COMP level (pg/ml) in each group measured at 2nd, 4th and 6th weeks (repeated measures ANOVA)

	Normal Control group (I)	MIA induced OA group (IIA) 50µl/right knee joint	Diacerin treated OA group (IIB)	Diclofenac treated OA group (IIC)	Diacerin +Diclofenac treated OA group (IID)
COMP at 2 weeks	744.7±83.1	1052.5±65.1	1024.5±67.4	1036.3±57.4	1027.5±99.1
	A	A	C	A	A
COMP at 4weeks	749.7±85.1	1505.3±129.8	956.5±37.2	1057.7±62.0	991.8±20.4
	A	B	B	B	A
COMP at 6 weeks	746.8±60.5	1784.2±135.4	939.7±39.8	1083.2±61.3	968.5±61.7
	A	C	A	C	A
P value	0.991 NS	**<0.001 HS**	**0.02 S**	**0.003 HS**	0.724 NS

- Groups having different letters or color labels are statistically significantly different at P value of 0.05 (post hoc LSD test).
- Data were expressed as mean± SD.
- Dose of diacerein in group (IIB) is 50mg/kg/day in the 1st week then doubled to 100mg/kg/day in the subsequent 5 weeks.
- Dose of diclofenac in group (IIC) is 2mg/kg/day.
- Dose in group (IID) is: diacerein 50mg/kg /day and diclofenac sodium 2mg/kg/day orally for the first week then diclofenac was stopped and the animals were maintained on diacerein 100mg/kg/day orally for the subsequent five weeks.

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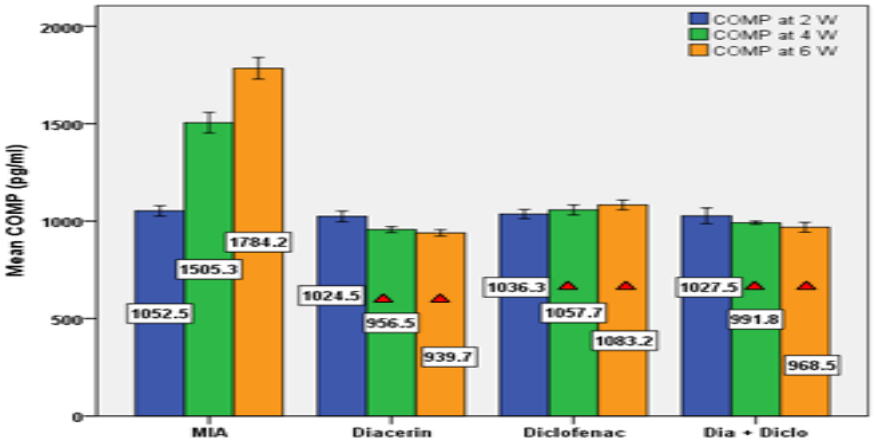
Figure 1: Changes in serum COMP level (pg/ml) in osteoarthritis untreated model group (IIA) versus the disease model groups receiving either diacerein (IIB), diclofenac (IIC) or their combination (IID).



Histopathological and Radiological findings

Microscopic examination of serial sections prepared from right knee joints of group I (negative control group) revealed smooth articular cartilage surface overlying a uniformly distributed layer of normal flattened chondrocytes in the tangential zone. Chondrocytes were distributed in parallel rows in the transitional and radial zones of the non-calcified part of the articular cartilage. The intercellular matrix was homogenous and uniformly stained with H&E stain. Normal radiological picture was seen (Fig.4). A single purple wavy line (tidemark) was occasionally seen separating the three layers of articular cartilage (non-calcified part) from a narrow zone of calcified cartilage that lies just above the subchondral bone. The subchondral bone exhibited normally distributed bone trabecullae composed of osteocytes and canaliculi surrounding bone marrow (Fig.2; Plates 2, 3&4). The total average pathology score was 0.0±0.0 (Table 2).There was gradual deterioration in pathological scoring as well as the radiological finding which were detected predominantly after the 4th and 6th weeks of OA induction in group (IIA), as the histopathological examination revealed irregularity and fibrillation in cartilage surface, chondrocyte disorganization, cellular degeneration and loss, osteophyte formation and subchondral fibrosis and cyst formation (Fig. 2; Plates 5, 6&7). The score of histopathology show significant increase from 0.0±0.0 in –ve control group to 2.17±0.8 (P<0.001), 7.67±1.4 (P<0.001) and 13.33±1.3 (p<0.001) at the 2nd, 4th and 6th weeks respectively (Fig. 3). While the radiology showed increased joint opacity, decreasing joint space, dystrophic changes in the articular surface, osteophyte expressed as new bone formation and finally complete loss of joint space (Fig. 4; Plate 19).

At the 2nd weeks of the study, histopathological findings in diacerein treated group (IIB) as well as other model group treated by either diclofenac (IIC) or diacerein/diclofenac drug combination (IID) didn't show any significant change from model untreated group, while at the 4th and 6th weeks of treatment by diacerein, a significant decrease in pathological score was detected which represent a marked improvement in the histopathological finding that were previously described in the untreated model group (Table 2, Fig.3). Multiple tidemarks were seen indicating continuous trial of cartilage regeneration (Fig. 2; Plates 8, 9&10). The radiological finding improved at the 4th weeks with diacerein treatment in form of presence of minimal changes in articular surface and clear intercondyloid space, furthermore marked improvement in joint appearance was detected at the 6th week where the joint space appeared almost normal (Fig. 4; Plates 20). On the other hand, the histopathological examination of group (IIC) which treated by diclofenac at 4th and 6th weeks were closely resembling to the untreated OA model (IIA) group, with progressive continuous rise in pathological score (Table 2, Fig.3), the histopathology sections showed progressive irregularity in the articular surface and fibrillation with chondrocytes loss and subchondral bone change in the form of cyst and osteophyte formation that increased in severity all through the study period (Fig. 4; Plates 11,12&13), however the score was significantly lower than that of the untreated model group at 6th week. Moreover the radiological finding showed that the osteoarthritic changes in diclofenac group (IIC) did not prove a significant improvement from those detected in the disease model (IIA) (Fig.21). In group (IID), the total average pathology score showed insignificant change in comparison to the disease model at 2nd week, while at the 4 and 6 weeks a significant drop in the pathological score was noticed (Table 2, Fig.3), these results was matched with that detected by x-ray (Fig.2, 4; Plats14, 15, 16&22).

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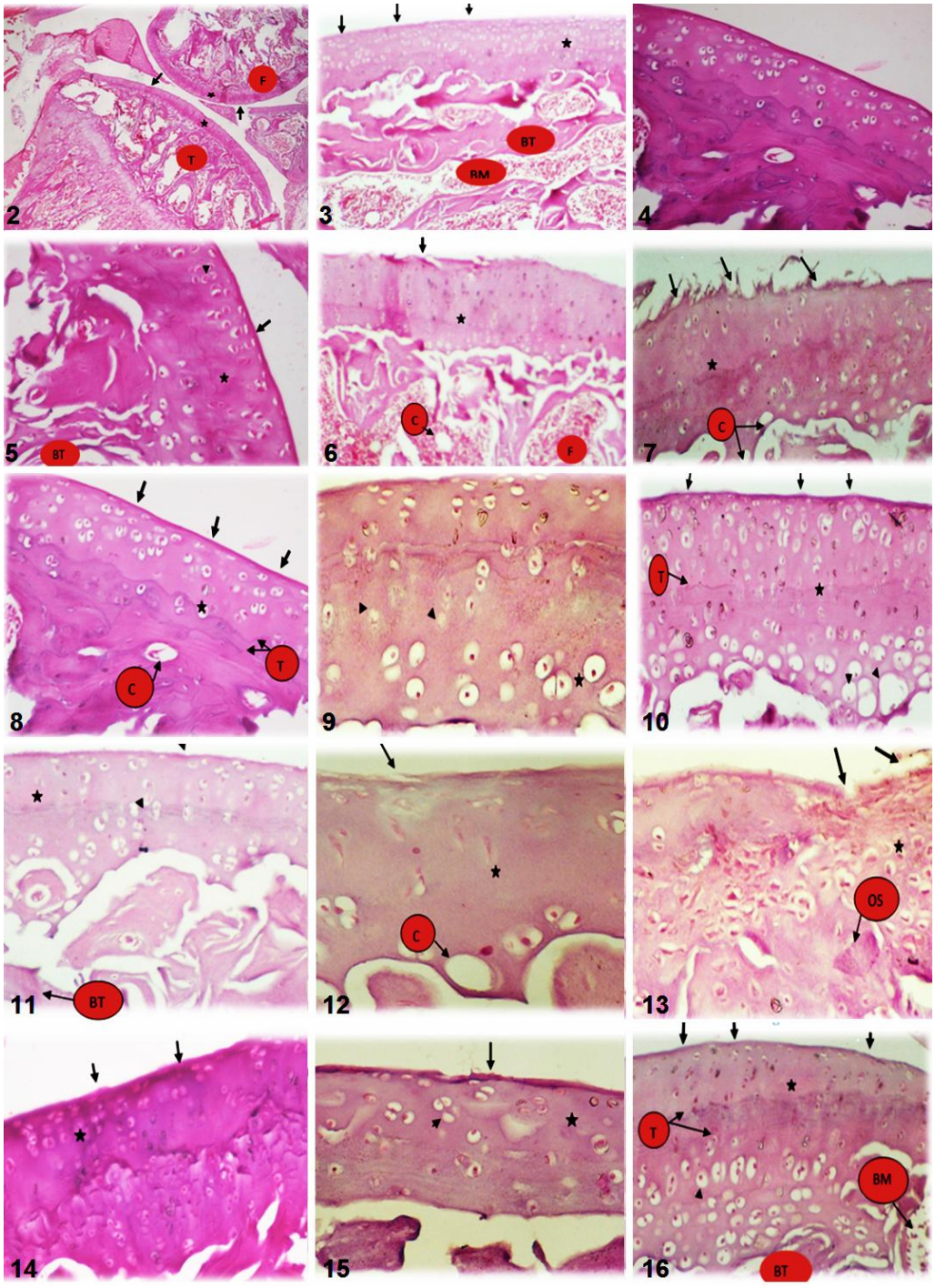
Figure 2: [Plat 2, 3 &4] Light photomicrographs of rat's right knee joint from group I (negative control group) showing (2): Normal structure of the articular cartilage and subchondral bone of both the tibial plateau (T) and the femoral condyle (F). The articular surfaces are smooth (arrows). The chondrocytes are normally distributed (star) (H&E×50).(3): Smooth articular surface (arrow) and uniformly distributed chondrocytes (star).There are normally distributed bone trabeculae (BT) surrounding the bone marrow (BM) (H&E×125(4): A single prominent tidemark (arrow) (H&E×250).



[Plat 5, 6&7]Light photomicrograph of rat's right knee joint from group IIA (MIA induced OA model) at 2,4 and 6 weeks respectively, showing(5): smooth articular surface (arrow), mild chondrocytes loss (star), mild disorganization of chondrocytes (arrow head) and normal bone trabeculae (BT) (H&E×250).(6):surface irregularity with fibrillation and cracking (arrow). There is chondrocytes loss (star). The subchondral bone shows cyst formation(c) and early fibrous tissue formation in the bone marrow space (F) (H&E×125). (7): Marked surface fibrillation (arrow), chondrocytes loss (star) and cyst formation (C) (H&E×250).

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[Plat 8, 9&10]Light photomicrograph of rat's right knee joint from group IIB (diacerein treated OA group) at2, 4 and 6 weeks respectively, showing(8):Fairly smooth articular surface (arrow). The chondrocytes exhibit mild disorganization and hypertrophy (star). Multiple tidemarks are seen (T) with cyst formation in the subchondral bone (C) (H&E×250). (9): prominent hypertrophied chondrocytes (star) and shadow of degenerated chondrocytes (arrow head) (H&E×500). (10): Mild irregularity of articular surface with mild fibrillation (arrows). There is a mild degree of chondrocytes depletion and disorganization (star) with hypertrophy (arrow head). Faint multiple tidemarks are seen (T) (H&E×250).

[Plat 11, 12& 13]Light photomicrograph of rat's right knee joint from group IIC (diclofenac treated OA group) at 2, 4 and 6 weeks respectively showing (11): mild surface irregularity with mild fibrillation (arrow). Moderate chondrocytes depletion (star) and disorganization are seen (arrow head). The bone trabeculae are of average thickness (BT) (H&E×125). (12): Surface irregularity (arrow). Moderate chondrocytes depletion (star) with cyst formation(C) and scattered shadows of degenerated cells (H&E×500). (13): Evident and marked surface irregularity with cracking (arrow). Chondrocytes loss and disorganization (star). There is osteophyte formation in the subchondral bone (OS) (H&E×500).

[Plat 14, 15& 16]Light photomicrograph of rat's right knee joint from group IID (combination of diacerein and diclofenac OA treated group) at 2, 4 and 6 weeks respectively, showing (14): mild surface irregularity and fibrillation (arrow). Chondrocytes show mild hypertrophy and disorganization (star) (H&E×250). (15): surface irregularity with fibrillation (arrow). Chondrocytes depletion (star) with necrosis and empty lacunae is seen (arrow head) (H&E×500). (16): Mild surface irregularity (arrows). Chondrocytes show moderate depletion (star) with hypertrophy of the remaining population (arrow head). Multiple faint tidemarks are seen (T). The subchondral bone show thinned out bone trabeculae (BT) with focal depletion of blood elements (BM) (H&E×250).

Figure 3: Differences between average histopathological scoring of animal groups (normal control, MIA induced OA, diacerein treated OA, diclofenac treated OA and combination of diacerein with diclofenac treated OA groups) in the three consecutive durations (2nd, 4th and 6th weeks).

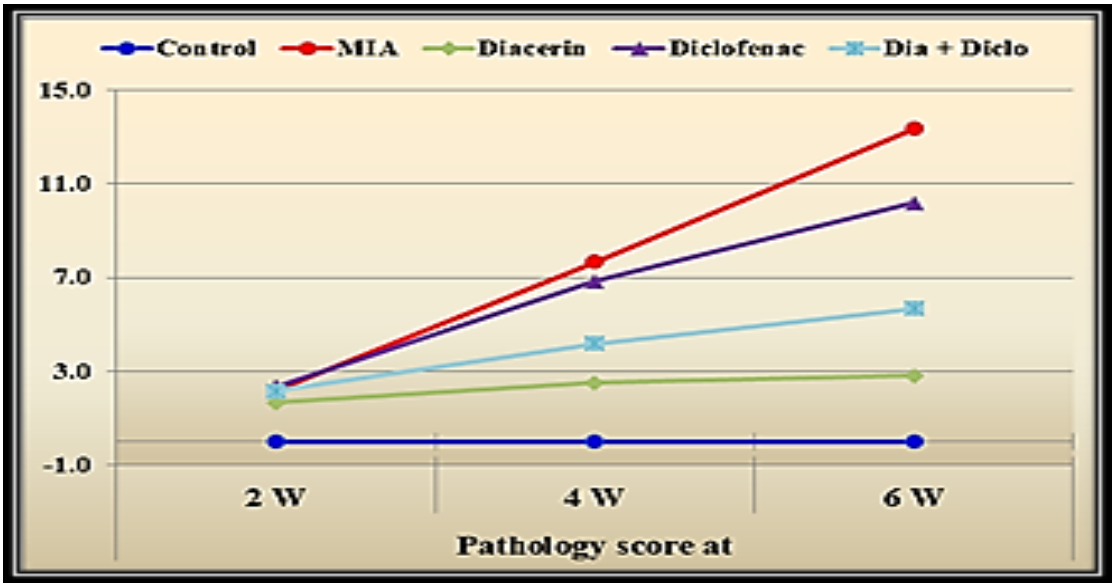
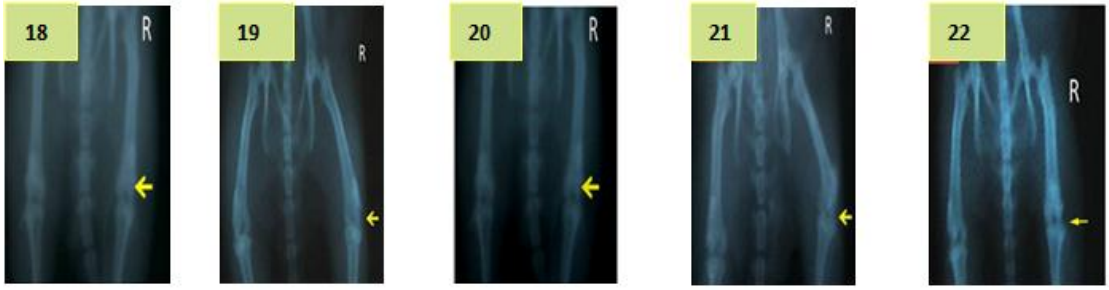


Figure (4): Plat 18) Radiograph of normal rat's knee joint showing normal opacities, smooth articular surfaces with clear radiolucent microfilm of synovial fluid.



Plat 19) Radiographic changes in rat's right knee joint in untreated OA model group (IIA) at 6 weeks showing complete joint space disappearance with intercondyloid osteophyte formation.

Plat 20) Radiographic changes in rat's right knee joints in (diacerein treated OA group (IIB) at 6weeks showing improvement in the form of minimal changes in the articular surface and clear intercondyloid space and the joint space appears almost normal.

Plat 21) Radiographic changes in right rat's knee joints in diclofenac treated OA group (IIC) at 6 weeks showing increased joint space radiodensity, osteophytic reaction with irregular articular surface and erosions at the femoral condyles are detected.

Plat 22) Radiographic changes in rat's right knee joint in combination of diacerein and diclofenac treated OA group (IID) at 6 weeks showing that the joint and intercondyloid space appeared nearly normal in radiograph.

Table 2: Changes in average pathological scoring at 2nd, 4th and 6th weeks in each group (repeated measures ANOVA).

	Normal Control group (I)	MIA induced OA group (IIA) 50µl/right knee joint	Diacerin treated OA group (IIB)	Diclofenac treated OA group (IIC)	Diacerin +Diclofenac treated OA group (IID)
Pathology Score at 2weeks	0.0±0.0	2.17±0.8	1.66 ±0.8	2.33±0.9	2.17±0.8
	A	A	A	A	A
Pathology Score at 4weeks	0.0±0.0	7.67±1.4	2.5±0.9	6.84±1.1	4.17±1.0
	A	B	A	B	B
Pathology Score at 6 weeks	0.0±0.0	13.33±1.3	2.8±0.9	10.17±1.3	5.68±1.3
	A	C	A	C	B

- Groups having different letters or color labels are statistically significantly different at P value of 0.05 (post hoc LSD test)
- Data were expressed as mean ± SD
- Dose of diacerein in group (IIB) is 50mg/kg/day in the 1st week then doubled to 100mg/kg/day in the subsequent 5 weeks.
- Dose of diclofenac in group (IIC) is 2mg/kg/day.
- Dose in group (IID) is: diacerein 50mg/kg /day and diclofenac sodium 2mg/kg/day orally for the first week then diclofenac was stopped and the animals were maintained on diacerein 100mg/kg/day orally for the subsequent five weeks.

DISCUSSION

This study was carried out to compare the anti-inflammatory effect of diacerein, a selective inhibitor for production and activity of IL-1β 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 monoiodoacetate (MIA).

Selection of MIA was based on its metabolic inhibitory property on chondrocytes, it induce disruption of glycolysis through inhibition of glyceraldehhyde–3 phosphate dehydrogenase enzyme in the chondrocyte with subsequent cell death. Since articular cartilage integrity requires the balance between anabolic and catabolic processes that are under the control of chondrocytes, MIA was selected from different methods of OA induction as it induces histopathological changes in the knee joint resembling to those of human OA in a short period of time [18].

The result of the present work has shown that, in the control groups of animals, when comparing normal animals in group (I), with model untreated (IIA) group, the biochemical marker serum COMP level increased progressively and significantly after induction of OA in the disease model group, when estimated at the three consecutive fixed timings of the study period (2^{nd}, 4^{th}, and 6^{th} weeks). This coincides with the observation of Larsson et al. [24] that correlated the degree of rise in COMP level with the severity and duration of osteoarthritis.

Deterioration in pathological scoring, as well as, the radiological findings has been detected predominantly after the 4^{th} and the 6^{th} weeks of OA induction in the right knee joint in group (IIA). These finding were in accordance with the findings described in previous studies done by Al-saffar et al. [25, 26] who used MIA to induce OA model in rats.

The current study outlined that oral administration of diacerein alone or in combination with diclofenac reduced significantly the level of serum COMP, and lowering significantly the global pathological

score at the 4th and 6th weeks, these findings also supported by the X. ray. These results were in accordance with in vivo studies done on different OA animal models, as well as, in vitro studies done on human articular cartilage, using different indices of cartilage degradation. They found that, diacerein decreased the collagenase level in the cartilage of dogs and rabbits [27], while Moore et al. (28)stated that diacerein also reduced the loss of hydroxyproline and proteoglycans in mouse cartilage, and decreased the inducible NO synthase (iNOS) in dog cartilage, Pelletier et al. [29] and Tamura et al. [30] found that rhein which is the active metabolite of diacerein, down regulates the gene expression and production of matrix metalloproteinases and up-regulates the production of tissue inhibitors of metalloproteinase-1 in rabbit articular chondrocytes. Hwa et al. [31] described a reduction in subchondral bone remodeling in sheep with diacerein treatment. Another study done by Rezende et al. [19] also found that diacerein lowered the degree of articular stiffness in experimentally induced osteoarthritis model in rats with reduction in the histopathological score. While Legendre et al. [32] found that, rhein inhibits proliferation of synoviocytes, suggesting that the drug may decrease the development of the inflammatory synovial tissue that accompanies joint pathologies. Its action as anti-catabolic on chondrocytes and anti-proliferative on synoviocytes may explain its beneficial effect in the treatment of joint diseases.

On the contrary, an older study was carried out as one-year, double-blind, placebo-controlled trial concluded that diacerein (100 mg/kg per day) failed to improve symptoms (as measured by pain, VAS, Lequesne's index, patient's global assessment of disease activity, and percentage of painful days), or to delay radio- logically determined joint space narrowing in patients with knee OA [33].

Concerning diclofenac treated OA group (IIC), an insignificant change in COMP level was found at the 2nd week, while a significant drop at the 4th and 6th weeks was detected when comparing this group with the untreated disease model. However, a significant progressive rise in serum COMP level measured at 2nd, 4thand 6th weeks was detected.

The histopathological examination revealed a progressive irregularity in articular surface and fibrillation, with chondrocytes loss, and subchondral bone change in the form of cyst and osteophytes formation that increased in severity all through the study period. According to the pathology scoring system such changes were insignificant from those of the model untreated control group at both the 2nd and 4th weeks. But at the 6th week such changes were extensive, although its score was significantly lower than that of the untreated model group. Moreover, the radiological findings showed that the osteoarthritic changes in the diclofenac treated group (IIC) did not prove a remarkable improvement from those detected in the disease model.

These results matched with the previous results of Palmoski and Brandt [34] who published several research papers showing that NSAIDs suppress proteoglycan synthesis by chondrocytes. Reijman et al. [35] made the observation that the chronic use of diclofenac, but not ibuprofen, naproxen, or piroxicam, accelerated the progression of knee and hip OA in subjects over 55 years old. Hauser [36] explained 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.

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 [37].The articular chondrocytes produce PGE2 in response to injury to stimulate healing. Osteoarthritic cartilage spontaneously releases PGE2 in levels at least 50 fold higher than normal cartilage. NSAIDs will block this response, although pain may be improved, but the repair mechanism for the affected joint is inhibited. The long-term consequences can be an acceleration of the degenerative osteoarthritic process [38] through inhibition of cyclooxygenase enzyme (COX) which is responsible for PGE2 release in chondrocytes (39). Accordingly the net result of their blockade is the acceleration of articular cartilage degeneration. Long-term NSAIDs treatment not only blocks PGE2 production by direct inhibition of COX-2 activity but also by down-regulating COX-2 synthesis [40].

This could explain the failure of diclofenac to improve the picture of OA induced by MIA, while diacerein act through different mechanism which is the inhibition for production and activity of IL-1β both at the pre and post membrane [41] effect so it can alter the pathogenesis of OA, since IL-1β was found to play an important role in this disease [42].

In addition of the ability of diacerein to inhibit the activity and synthesis of IL-1β, it can also increase the release of PGE2 [43] which promote the healing process in the cartilage and this could explain why the use of diacerein alone give better result than its use in combination with diclofenac, as the amount of PGE2 that produced by diacerein could be altered or decreased to some degree by the combination of the two drugs.

In conclusion, the results of the present study revealed that diacerein has the potential to ameliorate the inflammatory response caused by MIA in the knee joint, and so provides a good alternative treatment of OA. Furthermore diacerein slowed down the progression of OA when compared to diclofenac alone. Taking into consideration its devoid of known adverse effects, this may allow diacerein to be used alone in mild cases that can tolerate the pain or in conjunction with reduced doses of analgesics in those who can't wait until its effect appear, to decrease the numerous adverse effect which occur with chronic consumption of analgesics.

Furthermore studies of diacerein on different OA disease models, for longer durations are recommended. Also its therapeutics benefits in other diseases in which interleukin-1 β play an important role is needed.

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