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Original Research Article

Evaluation of the chondroprotective effect of doxycycline in monosodium-iodoacetate induced osteoarthritis in Wistar rats

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ABSTRACT

Background: Osteoarthritis is a chronic, progressive disease with drugs providing symptomatic relief but limited by side effects. Doxycycline has demonstrated protection against cartilage degradation and promoted chondrocyte proliferation in histopathological studies. However, biomarkers indicative of chondroprotection have not been thoroughly assessed. This study aimed to reconfirm the protective effects of doxycycline in osteoarthritic rats using behavioral tests, histopathology, and evaluation of Cartilage Oligomeric Matrix Protein (COMP) and Matrix Metalloproteinase-13 (MMP-13) levels.
Methods: After IAEC approval, 30 rats were divided into four groups [n=8 disease control (DC), positive control (PC), test group(TG) n=6 in sham control (SC)]. Behavioral tests were conducted at baseline, and on the 7th, 14th, 21st, and 28th days. Histopathology and biomarker analysis were performed on the 28th day. Statistical significance was set at $p<0.05$, using ANOVA for parametric data and Kruskal-Wallis tests for non-parametric data.
Results: For locomotor activity, number of squares crossed was significantly higher in TG and there was a significant decrease in the immobility time in TG when compared DC group. Number of falls on Rota-rod test was significantly lower in TG group. Hot plate test indicated higher latency time to paw licking in the TG, and grip strength significantly improved. Histopathological examination showed a 10-20% reduction in osteoarthritis severity in TG compared to 30-50% in DC. Furthermore, MMP-13 and COMP levels were significantly decreased in the TG compared to DC.
Conclusions: Doxycycline has shown antiarthritic effect by virtue of its chondroprotective action.

Keywords: Chondroprotective, COMP, Doxycycline, Grip-strength, MMP

INTRODUCTION

Osteoarthritis (OA) is most prevalent type of arthritis globally and is associated with articular cartilage abnormalities commonly affecting older people.[1] OA occurs because of an imbalance in the synthesis and degradation of the extracellular matrix, resulting in the damage and loss of articular cartilage.[2] This leads to limited joint mobility, joint instability, and discomfort, which are the primary signs of OA. The major clinical sign in radiograph is joint space narrowing due to cartilage loss.[3]

Currently available systemic drugs for osteoarthritis like NSAIDs or opioids target only symptoms however, they do not safeguard the articular cartilage. They have not shown credibility in altering the course of the disease and are associated with serious side effects. Even locally administered intraarticular medications such as steroids provide only symptomatic relief and even accelerate disease progression. Although effective and safe intra-articular hyaluronic acid is available but very costly. Inadequate predictive validity of animal models, limited understanding of pathophysiology, pain mechanism of osteoarthritis are the reasons for no major breakthrough in treatment of osteoarthritis.[4]

Chondroprotection provides a preventive approach that could slow down the progression of structural diseases and functional decline; it may also enhance pain relief by influencing inflammatory pathways.[3] Consequently, agents that preserve joint function, minimize tissue injury, and lessen the necessity for joint replacement would fulfill a significant unaddressed medical requirement.

Monosodium iodoacetate inhibits glyceraldehyde-3-phosphate dehydrogenase in chondrocytes and induce osteoarthritis. It is chemical model of indirect cartilage inhibition & is selected for study from among the various models of osteoarthritis available because it induces arthritis in 7 days, and the cartilage damage, joint dysfunction resembles with human arthritic joint pathology.[5-6] The rationale for using this model is that previous study by Matthew Thakur et al has defined the peripheral neuropathic aspect in the rat MIA model of osteoarthritis.[7]

Both the low and high dose of meloxicam were effective in improving the depth (cartilage degeneration score) and extension (total cartilage degeneration width) of the lesions in the MIA-triggered osteoarthritis. Furthermore, it is crucial that high-dose meloxicam provided protection for the deep cartilage and subchondral bone, as this functional unit could play a key role in reducing persistent inflammation and the long-term degeneration process.[8] Meloxicam is a safe and effective medication for the symptomatic treatment of OA. The evidence suggests that a daily dosage of meloxicam ranging from 7.5 to 15 mg may be effective in alleviating the pain and stiffness associated with osteoarthritis, while exhibiting gastrointestinal tolerability similar to that of a placebo.[9]

Various drugs aimed at reducing the tissue damage associated with osteoarthritis are currently being tested. One notable agent is the antibiotic doxycycline, which has been reported to restrict the destruction of cartilage and bone while significantly improving the degenerative alterations observed in osteoarthritic joints in both animal models and human subjects.[10]

Current research aims to evaluate the chondroprotective effect of doxycycline in monosodium iodoacetate induced osteoarthritis in Wistar rats.

METHODS

Approval from the Institutional Animal Ethics Committee (IAEC/GSMC/03/2020) was obtained before the initiation of the study. Animals randomly bred in the Centre for animal Studies of the Institute were used. The research was carried out in compliance with the guidelines established by CPCSEA. Animals were accommodated in polypropylene cages equipped with a stainless steel top grill, which included provisions for dispensing food in pellet form and filtered water. Paddy husk served as the bedding material. Controlled conditions were upheld with a temperature range of 18°C to 29°C, humidity levels between 30% and 70%, and a light-dark cycle lasting 12 hours. Animals were housed under standard laboratory conditions with access to filtered water and animal feed.

30 Swiss albino Wistar rats of either sex ageing 2-3 months and weighing 150-250 gm were used in this study. Study was conducted for 28 days in month of October 2021. 4 groups of Wistar rats with 6 rats in Normal control group and 8 rats in the rest of the groups were used respectively. Animals from group 1 received normal saline on left knee on day 1 and animals from group 2 to 4 received MIA. MIA was purchased from Sigma Aldrich Chemical Pvt Ltd.

For the induction of OA, rats were anaesthetized with ketamine 0.2ml (50mg/kg) and xylazine (10 mg/kg) intraperitoneal and MIA was injected into the left knee joint cavity using Hamilton syringe a 30 -gauge needle by inserting through the intra patellar ligament.

The variables of disease activity were assessed on baseline day 0, day 7, 14, 21 and 28. Meloxicam and MIA drugs were procured from Sigma Aldrich Company and doxycycline from Zydus Cadila Health care Limited. Meloxicam 1mg/kg (positive control group) and doxycycline 3mg/kg were administered from day 0 till day 28.[8,11] The details of study groups are as given below:

Group 1: Sham (Normal) control (SC): 25 µl. of saline via 26.5-G needle in left knee on day 0 + SC 1ml 0.9% saline from day 0 to day 28;

Group 2: Disease control (DC): 2mg of MIA I/A in 25 µl. of saline in left knee on day 0 +SC 1ml 0.9% saline from day 0 to day 28;

Group 3: Positive Control (PC): 2mg of MIA in 25 µl. of saline via 26.5-G needle in left knee on day 0 + Oral Meloxicam 1mg/kg from day 0 to day 28 daily;

Group 4: Test group (TG): 2mg of MIA in 25 µl. of saline via 26.5-G needle in left knee on day 0 + Oral Doxycycline 3mg/1000 mg body weight daily from day 0 to day 28.

Following behavioural parameters were assessed on day 0,7,14,21 and 28. Retro-orbital blood was collected for ELISA for testing biomarkers (COMP and MMP-13) on day 28 and following that animals were subjected to sacrifice, and a sample comprising the left femur along with the tibia and fibula was dispatched for histopathological examination of the knee joint.

Behavioural variables

Hot plate analgesiometer: Time of licking hind paw in seconds was noted.

Rota rod test: The latency to fall from the Rota rod, along with the percentage of animals that fell from the Rota rod during the one-minute test period, was determined.

Grip strength test using Grip strength meter (Orchid scientific TM innovative India Pvt. Ltd): The Rat was lowered over the angled grid mesh facing away from the meter keeping the torso parallel with the grid allowing both its hind paw attach to mesh. The rat was gently pulled back by its tail ensuring the torso remains parallel with the grid and maximal grip strength value that was displayed on the screen was recorded.[12] Same Procedure was repeated twice. Before repeating the same procedure, grid was cleaned with spirit and allowed to dry and the experiment was repeated till the last number of animals are completed . Three readings were recorded for each rat and average of the 3 readings was used as the final force expressed in Newton Force (N). The maximum tension was recorded as the force exerted on the grid or the bar immediately prior to its loss of grip.

Open field test: Open field apparatus with dimension, floor: 60 × 60 cm and wall height: 25 cm with floor divided in nine equal squares was used for the study. We examined number of squares crossed and immobility time in 5 minutes using open filed apparatus connected to video tracking system. Decrease in number of squares crossed with increased immobility time accounts for decreased locomotor activity which indicates inflammatory pain.

Biochemical variables

On day 28, retro orbital blood (2ml) collected from rats and centrifuged to get clear serum for measurement of Cartilage oligomeric matrix protein (COMP) Matrix metalloproteinase-13 (MMP13). ELISA kits for estimation of COMP, MMP13 were purchased from Krishgen Biosystems, Mumbai, Maharashtra-400018.

Histopathology of knee joint

All the rats were euthanized after 28 days using high doses of thiopentone (100 mg/kg), and their knee joints were then examined histopathologically. For the histology of subchondral bone and cartilage, tissue samples from knee joints were taken and stored in a 40% formalin solution. Samples were embedded in paraffin after being decalcified in 10% EDTA. Once the paraffin was dissolved, sections (5 µm) were cut. The sections were stained with hematoxylin and eosin stain for the evaluation of the histological arthritis score, and an independent blinded pathologist assessed them. The Brenner et al.[13] scoring system parameters were used. The relevant parameters used in the histologic scoring of the knee joint in our study are given below: 1) Osteophyte score = 0-4; 2) Cartilage degeneration score = 0-4; 3) Synovial membrane inflammation score = 0-4; 4) Calcified cartilage and subchondral bone damage score = 0-4; 5) Histopathology analysis total score = 16.

Statistical analysis

Results were expressed as Mean ± Standard Deviation (SD). Normality of data was checked by Kolmogrov-Smirnoff test. For parametric data, ANOVA with post hoc Tukey's test was done, whereas for non-parametric data, Kruskal Wallis test with post hoc Dunn's test was applied. GraphPad Instat software was used for statistical analysis.

RESULTS

During the study, 3 animals died due to MIA complications. The presented data is only of 27 animals.

Behavioural variables

Hot plate analgesiometer

In hot plate analgesiometer test, we measured latency to lick hind paw. On day 0, all the groups were comparable. The latency to lick hind paw was found to be significant ($p<0.0001$) when compared to disease control on day 28. Depicted in Figure 1.

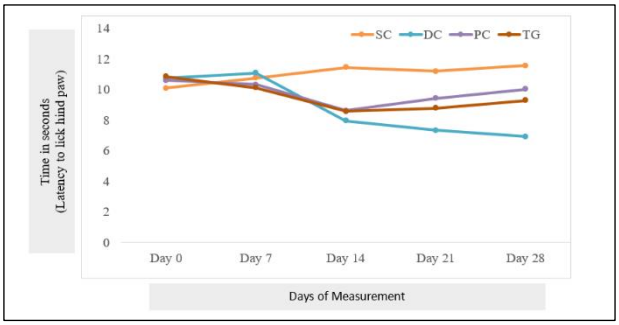

Figure 1: Results of hot plate analgesiometer test.

Grip strength

In grip strength test we measured the force applied by hind paw in newton before it leaves the grid. On day 0, all the groups were comparable and the force applied by hind paw was significantly higher in treatment group ($p< 0.05$) when compared to disease control on day 28. Depicted in Figure 2.

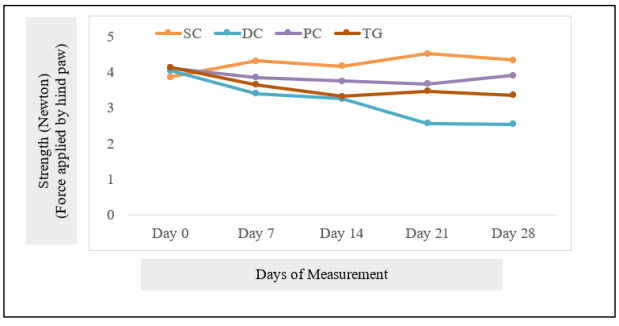

Figure 2: Results of grip strength test.

Open field test

In open field test, we measured number of squares crossed and the immobility time. On Day 0, all the groups were

comparable. The number of squares crossed was significantly higher ($p<0.0001$) in treatment group when compared to disease control on day 28. Also the immobility time was significantly lower ($p<0.0001$) in treatment group as compared to disease control group. Depicted in Figure 3 and Figure 4.

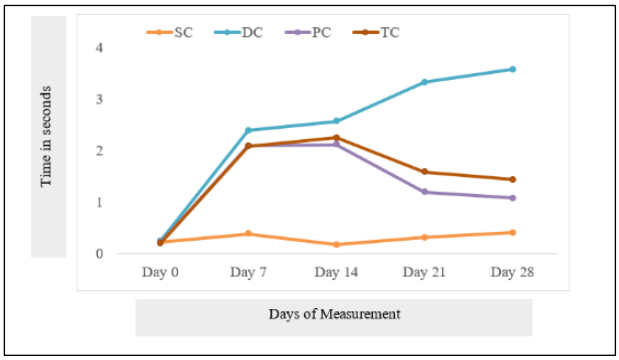

Figure 3: Results of open field test (immobility time).

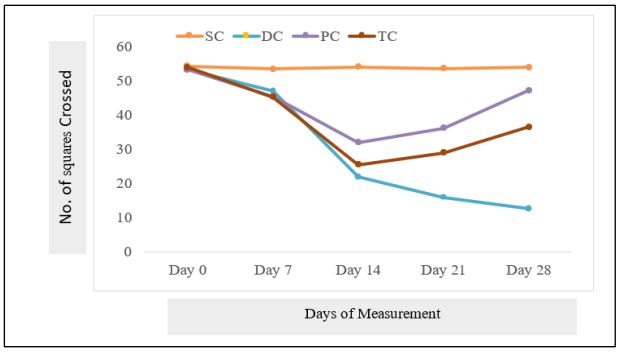

Figure 4: Results of open field test (Number of squares crossed).

Rota rod test

In Rota rod test we measured number of falls per minute. On day 0, all the groups were comparable. The number of falls was significantly lower ($p<0.05$) in treatment group when compared to disease control on day 28. Depicted in Figure 5.

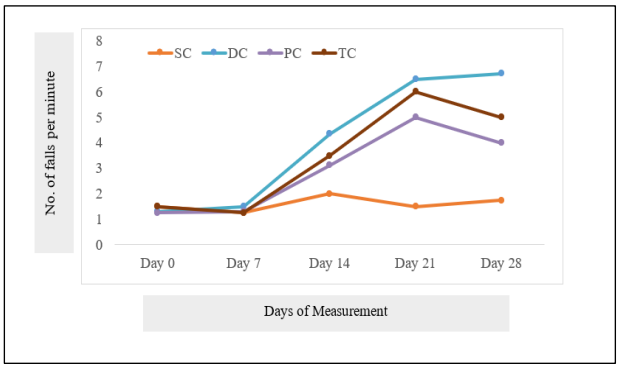

Figure 5: Results of rota rod test.

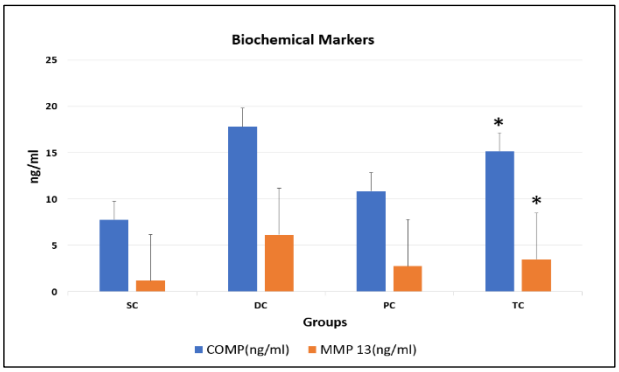

Figure 6: Biochemical markers.
***P<0.0001 Test group vs Disease Control**

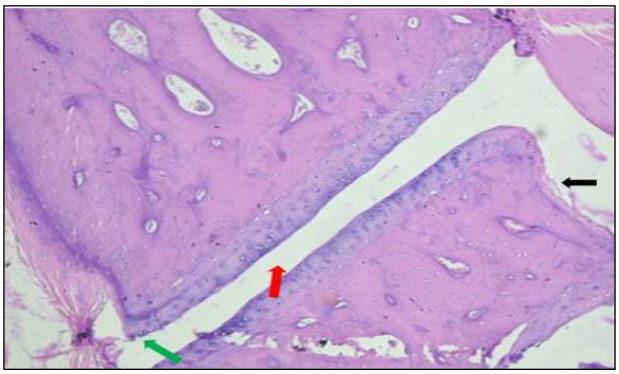

Figure 7: Sham control group. Black arrow: Synovial membrane, Red arrow: Synovial space, Green arrow: Cartilage surface, Magnification - 100X.

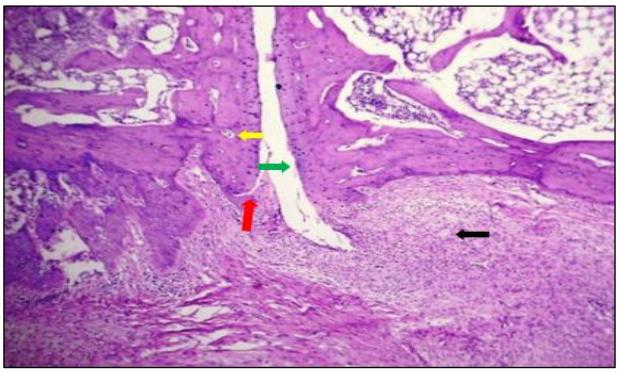

Figure 8: Positive control. Black arrow: Fibroplasia and mixed leucocytic infiltration, Red arrow: Synovial hyperplasia, Green arrow: Uneven/ irregular cartilage surface, Yellow arrow: Bone erosion, Magnification-100X.

Biochemical variables

There was significant increase in COMP & MMP13 levels in the disease control compared to the sham control group. The level of COMP and MMP13 was significantly low ($P<0.0001$) in test group when compared to disease control group. Depicted in Figure 6.

Histopathology: knee joint

Histopathological scoring of DC group [Median= 3, IQR (1,3)] compared to the test group [Median= 2, IQR (1,2)] was significantly reduced ($p<0.05$). It indicates that there was decreased inflammation and damage of the joint. Depicted in Figure 7-10.

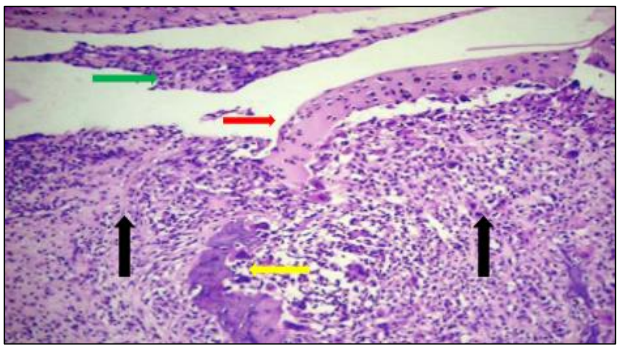

Figure 9: Disease control. Black arrows: Severe mixed leucocytic infiltration, Red arrow: Cartilage erosion, Green arrow: Synovial hyperplasia, pannus formation, Magnification-100X.

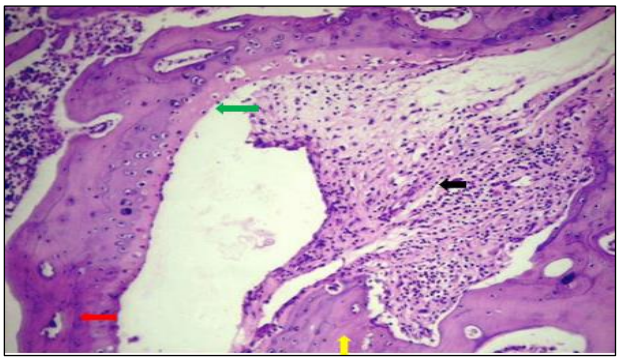

Figure 10: Test group. Black arrow: Mixed leucocytic infiltration, Red arrow: Irregular/ uneven cartilage surface, Green arrow: Synovial hyperplasia, Yellow arrow: Bone erosion, Magnification-100X.

DISCUSSION

We used behavioural testing, histological examinations, and biomarker studies to ascertain if doxycycline has an analgesic, anti-inflammatory, and chondroprotective effect in an animal model of MIA-induced osteoarthritis. Behavioural tests simulate human mobility testing so they were included.

As a variable in the hot plate test, we measured the latency to lick the hind paw, or pain threshold. The TG group showed a higher pain threshold than the DC group. In the grip strength test, which measured the force applied by the hind paw, or muscular strength as a variable, the TG group outperformed the DC group, suggesting a decrease in osteoarthritis damage. Similar results were reported by Pardy et al study.[10]

In open field test, we measured number of squares crossed and the immobility time and it was found that the number of squares crossed was increased in TG group as compared to DC group and immobility time decreased in TG group as compared to DC group. Using the Rota Rod, we measured the number of falls per minute as a measure of joint damage in the rats. Compared to the DC group, the TG group showed increased motor coordination and conserved muscle strength, indicating less joint damage.

The numerous behavioural tests, including the hot plate, rota rod, and open field tests that were used in our study were comparable to those proposed by Mcllwain et al in the arthritis model of pain. These tests are equivalent to those rheumatologists use to evaluate individuals with OA.[14]

Doxycycline significantly reduced the level of biomarkers in the ELISA for COMP and MMP-13 analyses when compared to disease control. Therefore, based on our research, we can conclude that doxycycline has chondroprotective potential and can slow the progression of OA.

Comparing the doxycycline group to the disease control group, MMP-13 levels were considerably lower in the doxycycline group. In osteoarthritis, MMP-13 is important for the degradation of articular cartilage. Patients with osteoarthritis have elevated MMP-13 levels, which are normally undetectable. A new area of interest in the treatment of osteoarthritis involves inhibiting MMP-13.[15] MMP 13 levels have decreased due to doxycycline, which is consistent with histopathological findings. By suppressing MMP-13, doxyxycline may have a chondroprotective impact, as evidenced by the decrease in MMP 13 levels.

MMP -1, MMP -13, and ADAMTS play a significant role in the elevation of COMP levels in OA. COMP levels are found in cartilage, synovial fluid, and the serum in osteoarthritis.[16] The decrease in levels in the doxycycline group as compared to the disease control group indicates chondroprotective effect and anti-arthritic potential of doxycycline.

In histopathology analysis we found out that there was significant reduction in histopathological scoring test group. Therefore, our results show that doxycycline has antiarthritic properties and decreases the progression of osteoarthritis. The efficacy of doxycycline in treating knee OA was demonstrated in a prior clinical trial by Aydin et al and Cylwik et al.[17,11] The mechanism of action of this medication is synergistically acting, making it an effective therapy option for chronic OA.

Doxycycline can therefore be used as a new line of treatment for OA in order to increase compliance, decrease the number of pills needed, and be more economical because it can delay joint surgery for an extended amount

of time, which can also have a significant positive impact on quality of life (QOL) and financial benefits.

This study has few limitations. There was no sample size calculation. Due to the lack of randomization and blinding, investigator bias cannot be ruled out. The study results will be generalizable to the same species as the construct validity may differ between species.

CONCLUSION

Doxycycline has demonstrated an antiarthritic effect due to its chondroprotective properties, in addition to its analgesic and anti-inflammatory effects.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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