ORIGINAL ARTICLE

MODULATORY EFFECTS OF HYALURONIC ACID AND PIROXICAM ON SYNOVIAL IL-6 AND MMP-13 IN RAT MODEL OF OSTEOARTHRITIS

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ABSTRACT

Background: Osteoarthritis, is an inflammatory joint disorder, manifested by breakdown of articular cartilage. The resultant pain and impaired joint function is due to raised inflammatory mediators comprising leukocyte derived cytokine (IL-6) and proteolytic matrix degrading metalloproteinase-13 enzyme (MMP-13). Our study compares the modulatory effects of intra-articular hyaluronic acid (HA) and piroxicam (PIRO) on IL-6 and MMP-13 levels using surgically induced osteoarthritic rat model.

Methods: Twenty-four Sprague Dawley rats were selected and randomly divided into three groups control, Hyaluronic acid (HA), and piroxicam (PIRO). Each group contained 08 animals. All animals were induced osteoarthritis by giving medial parapatellar incision. Saline water, hyaluronic acid and piroxicam were administered intra-articularly once weekly for four weeks. Synovial lavage samples were collected post-treatment and analyzed for IL-6 and MMP-13 levels. Statistical Package for Social Sciences (SPSS) version 23 was used for data analysis, considering p value <0.05 statistically significant.

Results: Comparing control group, group HA and PIRO significantly reduced synovial IL-6 and MMP-13 with p values 0.001 and 0.003 respectively. There was insignificant difference (p= >0.05) between HA and PIRO groups, showing comparable anti-inflammatory efficacy of both agents.

Conclusion: Hyaluronic acid and piroxicam were found equally effective in lowering synovial fluid samples of IL-6 and MMP-13 in osteoarthritic rats.

Keywords: Hyaluronic Acid (HA), Piroxicam (PIRO), Osteoarthritis (OA), Interleukin 6 (IL-6), Matrix metalloproteinase (MMP-13).

How to cite this article: Ishaq N, Iram Z, Aziz HMI,Tabassum S, Nazeer S, Javaid S. Modulatory Effects of Hyaluronic Acid and Piroxicam on Synovial Il-6 And Mmp-13 in Rat Model of Osteoarthritis: Pak Postgrad Med J 2025;36(4): 181-185

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Received: June19, 2025; Revised: Dec 30, 2025
Accepted: Dec 31, 2025

DOI: https://doi.org/10.51642/ppmj.v36i03.799

INTRODUCTION

Amongst musculoskeletal disorders, osteoarthritis is foremost cause of joint disability affecting elderly people worldwide. Salient features include articular cartilage erosion, synovial inflammation, and subchondral bone sclerosis. [1] It is a multifactorial disease with complex pathogenesis that is not fully elucidated. Although traditionally viewed as a wear-and-tear disease, recent evidence highlights the significant involvement of inflammatory pathways in

its progression Signaling proinflammatory protein such as interleukin-6 (IL-6) and matrix-degrading enzymes, like matrix metalloproteinase-13 (MMP-13) are recognized as pivotal mediators in the inflammatory process of OA. Elevated serum and synovial fluid interleukin 6 levels promote cartilaginous tissue catabolism and drive MMP expression, particularly MMP-13, which is primarily responsible for collagen type II degradation in articular cartilage. [3] Therefore, modulation of IL-6 and MMP-13 is of great therapeutic interest in targeting both inflammatory and degenerative aspects of OA.

A number of pharmacological and non-pharmacological modalities have been been employed in the symptomatic management of OA. [4] Among the pharmacological options, viscosupplements like Hyaluronate (HA) and Non-steroidal anti-inflammatory agents such as Piroxicam (PIRO) are widely studied. [5] Hyaluronate is a naturally occurring glycosaminoglycan predominantly present in joint fluid and cartilage, where it serves to maintain joint lubrication, viscoelasticity and mechanical integrity. Intra-articular (IA) administration of HA has demonstrated not only mechanical benefit but also biological activity. Numerous *in vivo* and *in vitro* studies have shown hyaluronic acid potential to modulate inflammatory mediators including cytokines and proteases. By reducing IL-6 production and suppressing oxidative stress-induced damage, HA helps to protect chondrocytes and maintain extracellular matrix homeostasis. Its ability to downregulate MMP-13 further supports its chondroprotective profile. [6]

Piroxicam, belonging to oxicam class, possess pain and inflammation reducing properties due to its non-selective inhibition of cyclooxygenase (COX) enzymes, hence reducing prostaglandin synthesis. [7] Although its systemic use is associated with gastrointestinal and renal side effects, localized IA administration is being explored as a safer alternative to deliver therapeutic effects directly to the affected joint. [8] Recent preclinical studies suggest that piroxicam also exert modulatory effects on cytokine release and matrix-degrading enzymes. Some evidence indicates that piroxicam can attenuate IL-6 expression and inhibit MMP activity, thus extending its role beyond mere symptomatic relief. [9]

Our previous study evaluated and compared the histological and radiographic outcomes of HA and PIRO in a surgically induced OA rat model. [10,11] Building upon those findings, the rationale of current project aims to analyze the modifying influence of intra-articular hyaluronic acid and piroxicam on IL-6 and MMP-13 levels. By targeting these specific biomarkers, the study seeks to provide insight into the inflammation suppressing and chondroprotective potential of these therapeutic agents, thus contributing to the evolving understanding of disease-modifying interventions in osteoarthritis.

METHOD

This experimental study was carried out at the Department of Pharmacology and Therapeutics, Army Medical College (AMC), Rawalpindi, in collaboration with the Animal Research Facility at the National Institute of Health (NIH), Islamabad. Ethical approval was granted by AMC's Ethical Review Committee (dated 3rd May 2019). The study spanned two months (April–June 2019).

A total of 24 Sprague Dawley rats (adult males or non-pregnant females), aged 8–10 weeks and weighing approximately 500 g, were selected via non-probability convenience sampling. Lottery method was used to divide animals into three group. Each group comprising 8 animals and labeled as I (control), II (HA) , and III (piroxicam) respectively. Animals were housed under standard conditions (25±5°C, adequate humidity, 12-hour light-dark cycle) with free access to food and water.

Table 1: Nucleotide sequences of qRT-PCR primers.

Gene	Primar sequence
IL-6	Forward 5′-GCTGGAGTCACAGAAGGAGTGGC-3′ Reverse 5′-GGCATAAC GCACTAGGTTTGCCG-3′
MMP-13	Forward 5′-CCCTGGAGCCCTGATGTTT-3′ Reverse 5′-CTCTGGTGTTTTGGGGTGCT-3′

Surgical induction of osteoarthritis was made using anesthesia (5% xylazine + 1% ketamine, intraperitoneally). A medial para patellar incision was given. After anterior cruciate ligament was transected, medial meniscus was removed. [12] Postoperatively, animals were allowed unrestricted movement in cages.

One week post-induction, synovial lavage was done with 0.3 ml sterile saline was fluid was collected for IL-6 and MMP-13 analysis. [13]. Polymerase chain reaction was used to determine IL6 and MMP expression while TRIZOL reagent was used to extract RNA subsequently quantified by Nano drop spectrophotometer. Complementary DNA (cDNA) was synthesized using a commercial kit, and primers for IL-6 and MMP-13 were designed using Primer-3 software Sequence as described in Table 01.

Afterwards, intra-articular treatments were administered once weekly for four weeks: 100 µL saline (control), 300 µL of 0.9 % HA, and 500 µL of (10 mg/ml) PIRO, respectively. [14-16] One week after the final injection, joints were flushed with sterile saline (0.3 ml) and lavage samples collected for final cytokine assessment. Due to limited synovial volume, direct aspiration was not feasible.

Difference between groups' mean was tested by one-way ANOVA followed by *Tukey*'s *post hoc* test. A p-value ≤0.05 was considered statistically significant.

RESULTS

Table 02 presents the mean ± standard deviation (SD) values of IL-6 and MMP-13 levels measured after induction and following intervention in three groups: Control, HA, and PIRO, each consisting of eight rats. After induction, IL-6 levels were comparable across the groups, with the control group at 226.5±17.1, HA group at 232±28.6, and PIRO group at 246±27.6. Following the intervention, a negligible change was observed in the control group (226.12±22), whereas both HA and PIRO groups exhibited a noticeable decreased IL-6 levels, reaching 166.5±19.6 and 177±16.6, respectively. Similarly, MMP-13 levels after induction were relatively similar among the groups: 199±16.7 in control, 203.2±25.9 in HA, and 210±25.1 in PIRO. After the intervention, MMP-13 levels remained nearly unchanged in the control group (199.88±18.2), while significant decreases were noted in the HA (164.3±18.5) and PIRO (158±20.1) groups. These findings suggest a potential anti-inflammatory effect of the HA and PIRO interventions on IL-6 and MMP-13 expression.

Table 02: mean ± standard deviation (SD) values of IL-6 and MMP-13 levels

Groups	IL-6 before induction of OA	IL-6 after induction of OA	MMP-13 before induction of OA	MMP-13 after induction of OA
Control group (n=08)	226.5±17.1	226.12±22	199±16.7	199.88±18.2
HA group (n=08)	232±28.6	166.5±19.6	203.2±25.9	164.3±18.5
PIRO group (n=08)	246±27.6	177±16.6	210±25.1	158±20.1

Table 03 presents post intervention groups' comparison of IL1 and MMP-13 levels by using one-way ANOVA. Both parameters in pretreatment groups show statistically insignificant p-values IL-6 (p = 0.295) and MMP-13 (p = 0.633), confirming that the groups were comparable before the intervention. However, post intervention, statistically significant differences were observed in IL-6 (p = 0.001) and MMP-13 (p = 0.006) levels, indicating that the treatments had a differential impact on reducing these inflammatory markers.

Table 04 presents the results of the *post hoc Tukey's* test, showing pairwise comparisons between the study groups for IL-6 and MMP-13 levels after induction and intervention. No statistically significant differences were observed between any groups after induction for both IL-6 and MMP-13, confirming baseline homogeneity (p > 0.05). After the intervention, however, significant reductions in IL-6 and MMP-13 levels were found in both the HA and PIRO groups when compared to the control group, with p-values of 0.001 and 0.003 for HA, and 0.001 and 0.001 for PIRO, respectively. In contrast, the differences between the HA and PIRO groups were statistically insignificant for either marker (p > 0.05), suggesting that both agents were comparably effective in reducing IL-6 and MMP-13 levels.

Table 3: Anova-based intergroup comparison of IL-6 and MMP-13 levels before and after oa induction

Groups	IL-6 before induction	IL-6 after induction	MMP-13 before induction	MMP-13 after induction
Inter group comparison	0.295	0.001	0.633	0.006

*p<0.05 is statistically significant

Table 4: Post Hoc Tukey Test: Intergroup Comparison of IL-6 and MMP-13 Levels after Induction and Intervention (p-values)

Inter group comparison	IL-6 after induction	IL-6 after intervention	MMP-13 after induction	MMP-13 after intervention
Control & HA	0.899	0.001	0.927	0.003
Control & PIRO	0.285	0.001	0.610	0.001
HA & PIRO	0.513	0.541	0.828	0.798

*p<0.05 Statistically significant

DISCUSSION

Osteoarthritis is a multifactorial joint disorder accompanied by structural changes including articular cartilage damage, synovial inflammation, and bone remodeling along with sclerosis and resorption. Although classically associated with mechanical degradation, current research emphasizes the central role of inflammatory signaling molecule and matrix-degrading enzymes in OA pathogenesis. IL-6, a, and matrix MMP-13, a primary collagenase targeting type II collagen, are critical contributors to cartilage catabolism and joint destruction in OA . [3,17] This study compared the effects of intra-articular hyaluronic acid and piroxicam on IL-6 and MMP-13 levels in OA-induced rats. Both treatments significantly reduced these markers versus control (IL-6: p=0.001; MMP-13: p=0.006, ANOVA), without significant difference between HA and PIRO (p>0.05). Likewise, the research project conducted by Lixia Jin reported that the administration of HA significantly decreased the levels of pro-inflammatory cytokines IL-6 and IL-8 (P<0.001) in patients diagnosed with knee osteoarthritis. [18] These findings provide additional support and validation for our observed outcomes regarding the anti-inflammatory effects of hyaluronic acid in osteoarthritic conditions. Furthermore, the research conducted by Sree Samanvitha Kuppa also demonstrated that HA possesses significant immunomodulatory properties. It was shown to reduce the expression of pro-inflammatory cytokine IL-6 (p<0.01) and the catabolic enzyme MMP-13 (p<0.05) by suppressing the mitogen-activated protein kinase (MAPK) signaling pathway in chondrocytes. [19]

Piroxicam, a non-selective cyclooxygenase inhibitor, block prostaglandin synthesis thereby alleviate inflammation. However, recent research has highlighted its broader anti-inflammatory potential beyond COX inhibition. In line with the objectives of our study, Eshwa Dar conducted an experimental study using a rat model of rheumatoid arthritis. The results of her research demonstrated that piroxicam administration led to a statistically significant reduction in pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), IL-6, and MMP-3, with a p-value of less than 0.001. These findings strongly support the outcomes observed in our project, further validating the anti-inflammatory efficacy of piroxicam in managing inflammatory joint disorders.[20] Meanwhile, the research project conducted by Sara Sattar also demonstrated that piroxicam has significant effect in reducing the levels of IL-6, MMP-2 and MMP-3 in OA rat model, with a high level of statistical significance (p<0.001). [21] These findings are consistent with and lend further support to the results observed in our own study regarding the anti-inflammatory effects of piroxicam.

Notably, Suppression of IL-6 and MMP-13 was similar between HA and PIRO groups, showing insignificant difference. (p=0.541 and p=0.798, respectively). Our research work is novel because previously no study has directly compared hyaluronic acid and piroxicam in osteoarthritis (OA) models. However our earlier study where radiographic and histological scores revealed comparable cartilage regeneration and osteophyte inhibition between both treatments. [10,11]

These results are particularly relevant as OA management continues to shift toward disease-modifying approaches rather than symptomatic relief alone. Targeting IL-6 and MMP-13 offers a promising strategy to mitigate cartilage loss and joint degeneration.

CONCLUSION:

In this experimental osteoarthritis model, intra-articular hyaluronic acid and piroxicam significantly reduced synovial IL-6 and MMP-13 levels Both agents demonstrated comparable efficacy, with no significant difference between them. These findings support chondroprotective roles of hyaluronic acid and piroxicam in osteoarthritis induced rat models.

STUDY LIMITATIONS:

Study limitation include that it was conducted on small sample size with a relative short duration of intervention. Additionally, being an animal-based model, the results may not fully translate to clinical outcomes in human osteoarthritis.

ETHICAL APPROVAL

Ethical approval of article was granted by the Institutional Ethical Review Board of Army Medical College, Rawalpindi, dated 03 May, 2019.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

FUNDING SOURCE: None

AUTHOR'S CONTRIBUTIONS

NI: Study design, manuscript writing, data collection
ZI: Study design, biostatistics
HMIA: Result compilation
ST: Study design, manuscript writing, biostatistics
SN: Result compilation, data analysis
SJ: Biostatistics, Literature review
All Authors: Approval of the final version of the manuscript to be published

REFERENCES

1. Allen KD, Thoma LM, Golightly YM. Epidemiology of osteoarthritis. Osteoarthritis cartilage. 2022 Feb 1;30(2):184-95. doi.org/10.1016/j.joca.2021.04.020
2. Liu CJ. Beyond wear and tear at the joint. Science. 2025 Apr 4;388(6742):30-1. doi: 10.1126/science.adw4656
3. Nambi G. Does low level laser therapy has effects on inflammatory biomarkers IL-1β, IL-6, TNF-α, and MMP-13 in osteoarthritis of rat models—a systemic review and meta-analysis. Lasers Med Sci. 2021 Apr;36(3):475-84. https://doi.org/10.1007/s10103-020-03124-w
4. Magni A, Agostoni P, Bonezzi C, Massazza G, Menè P, Savarino V, et al. Management of osteoarthritis: expert opinion on NSAIDs. Pain ther. 2021 Dec;10(2):783-808. https://doi.org/10.6084/m9.figshare.14308292.
5. Elakkad YE, Younis MK, Allam RM, Mohsen AF, Khalil IA. Tenoxicam loaded hyalcubosomes for osteoarthritis. Int J pharm. 2021 May 15; 601:120483-96. https://doi.org/10.1016/j.ijpharm.2021.120483
6. Zhang ZW, Zhao JY, Feng Y, Yin K, Li PC, Wei XC. Study on the mechanism of cross-linked hyaluronic acid-dexamethasone hydrogelin post-traumatic osteoarthritis. Zhonghua yi xue za zhi. 2024 Mar 1;104(9):695-703. doi.org/10.3760/cma.j.cn112137-20231008-00672
7. Gaber DA, Radwan MA, Alzughaibi DA, Alail JA, Aljumah RS, Aloqla RM, et al. ormulation and evaluation of Piroxicam nanosponge for improved internal solubility and analgesic activity. Drug delivery. 2023 Dec 31;30(1):2174208. doi.org/10.1080/10717544.2023.2174208-17.
8. Gupta A, Ali I, Zeeshan M, Singh S, Kumar A, Adil A. Role of intra-articular piroxicam in the temporomandibular joint after arthrocentesis for anterior disc displacement without reduction. Cureus. 2023 Feb 3;15(2) 1-11. https://doi 10.7759/cureus.34580
9. Li J, Wang L, Zeng G, Li H, Luo J, Tian Q, Zhang Z. Chymotrypsin attenuates adjuvant- -induced arthritis by downregulating TLR4, NF-κB, MMP-1, TNF-α, IL-1β, and IL-6 expression in Sprague–Dawley rats. Immunopharmacol Immunotoxicol. 2022 Nov 2;44(6):959-69. https://doi.org/10.1080/08923973.2022.2093743
10. Ishaq N, Batool S, Qammar M, Aziz MI, Khan MW, Javaid S. Radiographic comparative regenerative effects of Hyaluronate and Piroxicam in Osteoarthritis Models Of Rats. Pak J Physiol. 2022 Sep 30;18(3):19-22. https://doi org/10.69656/pjp.v18i3.1462
11. Ishaq N, Saqib M, Salahuddin Z, Gul S, Ata N, Rahman Z. Comparison of Chondroprotective Efficacy of Hyaluronic Acid and Piroxicam in Murine Model of Osteoarthritis. Esculapio – JSIMS. 2022 Sep 1;18(3) 282-6. https://doi.org/10.51273/esc22.251838
12. Chapman JH, Ghosh D, Attari S, Ude CC, Laurencin CT. Animal Models of Osteoarthritis: Updated Models and Outcome Measures 2016–2023. Regen eng transl med. 2024 Jun;10(2):127-46. doi.org/10.1007/s40883-023-00309-x
13. Julovi SM, Dao A, Trinh K, O'Donohue AK, Shu C, Smith S, et al. Disease-modifying interactions between chronic kidney disease and osteoarthritis: a new comorbid mouse model. RMD open. 2023 Aug 1;9(3):e003109-19. doi 10.1136/ rmdopen-2023-003109
14. Yang J, Wang L, Zhang Z, Sun Q, Zhang Y. Downregulation of HAS-2 regulates the chondrocyte cytoskeleton and induces cartilage degeneration by activating the RhoA/ROCK signaling pathway. Int J Mol Med. 2023 May 25;51(1):57-71. https://doi.org/10.3892/ijmm.2023.5300
15. Filho MC, dos Santos Haupenthal DP, Zaccaron RP, de Bem Silveira G, de Roch Casagrande L, Lupselo FS, et al. Intra-articular treatment with hyaluronic acid associated with gold nanoparticles in a mechanical osteoarthritis model in Wistar rats. J Orthop Res. 2021 Dec;39(12):2546-55. https://doi.org/10.1002/jor.25008
16. Faheem MA, Akhtar T, Naseem N, Aftab U, Zafar MS, Hussain S,et al. Chrysin is immunomodulatory and anti-inflammatory against complete Freund's adjuvant-induced arthritis in a pre-clinical rodent model. Pharmaceutics. 2023 Apr 12;15(4):1225-38. https://doi.org/10.3390/pharmaceutics15041225
17. Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2021;17(1):33–44. https://doi:10.1038/s41584-020-00522-8
18. Jin L, Xu K, Liang Y, Du P, Wan S, Jiang C. Effect of hyaluronic acid on cytokines and immune cells change in patients of knee osteoarthritis. BMC musculoskelet disord. 2022 Aug 25;23(1):812-20. https://doi.org/10.1186/s12891-022-05767-y
19. Kuppa SS, Kang JY, Yang HY, Lee SC, Sankaranarayanan J, Kim HK, et al Hyaluronic Acid Viscosupplement Modulates Inflammatory Mediators in Chondrocyte and Macrophage Coculture via MAPK and NF-κB Signaling Pathways. ACS omega. 2024 May 1;9(19):21467-83. doi.org/10.1021/acsomega.4c01911
20. Dar E, Mobashar A, Shabbir A, Sharif A, Saleem A, Mushtaq MN, et al. Therapeutic potential of d-limonene in rheumatoid arthritis: Modulation of inflammatory, anti-inflammatory cytokines, and prostaglandin E2. Archiv der Pharmazie. 2024 Dec;357(12):2400388. https://doi.org/10.1002/ardp.202400388
21. Sattar S, Shabbir A, Shahzad M, Akhtar T, Ahmad A, Alnasser SM, et al. Eichhornia crassipes Ameliorated Rheumatoid Arthritis by Modulating Inflammatory Cytokines and Metalloproteinase Enzymes in a Rat Model. Medicina. 2023 Sep 4;59(9):1594-609. https://doi.org/10.3390/medicina59091594