Original Article

Anti-Inflammatory Effects of Chrysin (5,7-Dihydroxyflavone) in Freund's Complete Adjuvant-Induced Arthritis in Rats

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ABSTRACT

Background: Inflammation is a key pathological process underlying arthritis and contributes to progressive damage to the joints, cartilage, and surrounding tissues. Persistent inflammatory responses are associated with infiltration of inflammatory cells, pannus formation, cartilage degradation, and bone erosion, ultimately leading to impaired joint function. Chrysin (5,7-dihydroxyflavone) is a naturally occurring flavonoid found in various plants, honey, and propolis. It possesses several pharmacological properties, particularly anti-inflammatory and antioxidant activities, which may help attenuate inflammatory tissue damage. Therefore, the present study was designed to evaluate the anti-inflammatory effects of chrysin in an experimentally induced arthritic rat model using histopathological assessment of joint tissues.

Methodology: This study determined the healing effect of Chrysin in a FCA-induced arthritic rat model. Chrysin was injected intraperitoneally on the 8th post-administration day of FCA, and treatment continued for the next three consecutive weeks. GraphPad Prism was used to analyse the results, considering p-values ≤ 0.05 statistically significant.

Results: Anti-inflammatory effect of Chrysin upon bones and cartilage was determined by Ankle joint histopathology. Upon histopathology, Chrysin reduced the severity of arthritis in the joint, infiltration of inflammatory cells, subcutaneous inflammation, cartilage erosion, bone erosion and pannus formation. Piroxicam was used as a reference drug to compare the anti-arthritic effect of Chrysin.

Conclusion: Results showed that Chrysin possesses anti-arthritic effects and may be a potential drug for the treatment of arthritis.

Keywords: Anti-inflammatory; Immunomodulatory; Bioactive components; Chrysin; Phytotherapy; Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis is a brutally unbearable autoimmune disease due to its systemic chronic inflammation.[1] The main site of inflammation is the synovial membrane, which is a soft tissue covering over the synovial cavity of the joint except its cartilaginous parts.[1] The annual incidence is approximately 0.02 to 0.05%, and the mean age of onset of RA is the highest in the fifth decade of life.[2] The main symptoms of RA are joint pain, swelling, redness, temperature, and stiffness, particularly in the early morning or after prolonged sitting. Other symptoms of RA may include weakness, lethargy and lack of energy, loss of appetite, weight loss, fever, sweating, dry eyes, and chest pain.[3] There is a 48% increased risk of cardiovascular diseases such as atherosclerosis and MI, and a 60% increased risk of premature death in patients with RA.[4]

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclo-oxygenase enzyme type II and I both (COX II and I), which causes inhibition of prostaglandin-mediated inflammatory response.[5] Although NSAIDs were once considered front-line treatment of RA because they could relieve pain and inflammation, two major problems occurred. First, despite their analgesic effect, nonsteroidal anti-inflammatory drugs do not address the underlying disease processes that drive the pathophysiology of RA.[6] Second, continuous use of NSAIDs potentiates the risk of serious side effects of the gastrointestinal and cardiovascular systems.[7] Corticosteroids decrease inflammation in RA patients and provide pain relief, like NSAIDs. Prednisone is a known corticosteroid, which is a subclass of adrenal hormones that relieves the symptoms of arthritis.[8] Methotrexate and Leflunomide are important DMARD and immune system suppressant agents.

Chrysin is a natural flavonoid and commonly obtained from propolis plants, passion flowers, honey wax, and honey. Chrysin is 5,7-dihydroxyflavone, having a 15-carbon polyphenol skeleton.[9] Chrysin has two benzene rings (A, B)

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and a third ring (C) that contains oxygen. Chrysin has a double bond between C2 and C3, carbon 4 has a carbonyl group, and there is no hydroxyl group at carbon 3. Chrysin contains hydroxyl groups at carbons 5 and 7, as shown in Figure 1. Historically, it has been extensively used in herbal medicine for the treatment of ailments. Chrysin has the ability to protect various vital organs (Brain, Heart, Liver, Kidneys, and Lungs) against toxic agents used in industry and pesticides, but its effectiveness is linked to its bioavailability.[10] In this context, the present study was designed and conducted to evaluate the anti-inflammatory effects of Chrysin in Freund's complete adjuvant-induced arthritis in rats.

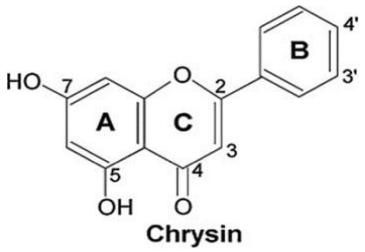

Figure 1: Chemical structures of Chrysin (Mohos *et al.,* 2018)[11]

METHODOLOGY

Male Wistar rats of 6-8 weeks old, weighing 180-200 grams, were used in this study. Standard rat pellet diet and water in a feeding bottle were provided to animals *ad libitum*. They were kept in the animal house under standard laboratory conditions of 12-hour day/night cycles and a temperature of 24°C with 60-70% humidity. The Institutional Ethics Committee approved the study.

The sample size was determined using the resource equation method, which is suitable for experimental animal studies where a reliable estimate of effect size is not available. The resource equation was calculated as E = N – T, where *N* represents the total number of experimental animals, and T represents the number of treatment groups. Based on the experimental design and the required degrees of freedom for statistical analysis, a total of 60 Wistar rats were selected, providing 10 rats per group across six experimental groups. Ten rats were included in each group:

- Group I: Vehicle control and i.p. 0.1% DMSO.
- Group II: Arthritic control and i.p. 0.1% DMSO.
- Group III: Arthritic and Chrysin treated (50mg/kg) i.p., dissolved in 0.1% DMSO.
- Group IV: Arthritic and Chrysin treated (100mg/kg) i.p. dissolved in 0.1% DMSO.
- Group V: Arthritic and Piroxicam treated (10mg/kg) i.p. dissolved in 0.1% DMSO.
- Group VI: Acute toxicity Study.
- 0.2 ml of Freund's complete adjuvant was injected in the subplantar region of the left hind paw in all groups except the vehicle control group on day 0.

Treatment was initiated on the 8th day of arthritis induction. All animals were sacrificed on day 29.[12]

The degree of arthritis was determined by modified arthritic scoring methods of Mali *et al.,* 2011 and Zhu *et al.,* 2011. The features of arthritis include swelling, redness, and erythema that were determined by visual criteria on days 4, 8, 12, 16, 20, 24, and 28. Score 0 was given to the normal paw, while scores 1 to 4 were given according to the level of severity of swelling and redness.

All rats were sacrificed, and ankle joints were preserved in 10% formalin solution for fixation. Subsequently, bones were decalcified by using 10% boric acid in 10% formalin solution. Slides were made by hematoxylin and eosin (H&E) staining.[13] Slides were examined for infiltration of inflammatory cells, synovial inflammation, subcutaneous inflammation, cartilage erosion, bone erosion, and pannus formation. Normal, minimal, mild, moderate, and severe changes were scored as 0, 1, 2, 3, and 4, respectively, and the results were compiled by GraphPad Prism 13.

Data were analysed by GraphPad Prism version 13 and presented as mean ± SD. One-way ANOVA followed by post-hoc Tukey's test was applied to measure the significant mean difference among groups. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

The vehicle control group (Group I), which received 0.1% DMSO intraperitoneally (i.p.), did not develop arthritis. In contrast, the FCA-induced arthritic control group (Group II), which received 0.1% DMSO i.p., developed progressive arthritis, with the mean arthritis score reaching 3.94 ± 0.30 at 4 weeks. Treatment with Chrysin 50 mg/kg i.p. (Group III), Chrysin 100 mg/kg i.p. (Group IV), and Piroxicam 10 mg/kg i.p. (Group V) significantly reduced the arthritis score compared with the arthritic control group. The mean arthritis scores were 1.19 ± 0.20 in the Chrysin 50 mg/kg group, 0.63 ± 0.30 in the Chrysin 100 mg/kg group, and 2.19 ± 0.50 in the Piroxicam 10 mg/kg group. The greatest reduction in arthritis score was observed with Chrysin 100 mg/kg, followed by Chrysin 50 mg/kg and Piroxicam 10 mg/kg, respectively. These findings suggest a dose-dependent anti-arthritic effect of Chrysin in FCA-induced arthritis, as shown in Figure 2.

Histopathological examination of the ankle joint of the control group showed normal joint architecture with no evidence of inflammatory cell infiltration, cartilage erosion, bone erosion, or pannus formation. In contrast, the FCA-induced arthritis control group demonstrated severe inflammatory changes, characterised by marked infiltration of inflammatory cells, subcutaneous inflammation, cartilage erosion, bone erosion, and pannus formation.

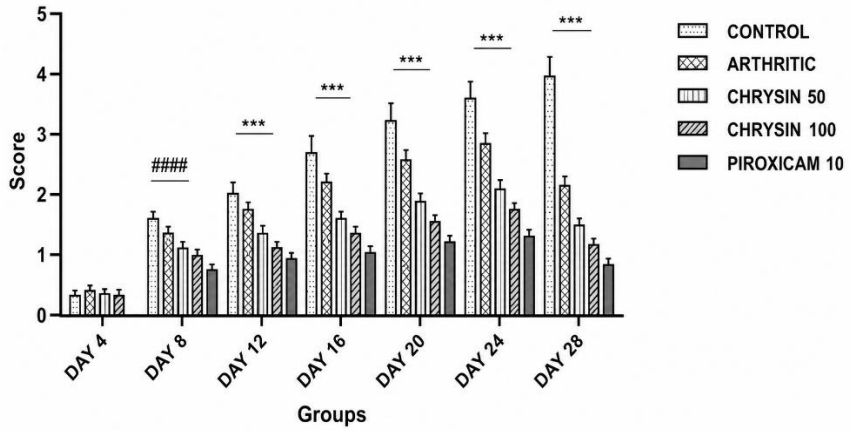

Figure 2: Showing the comparison of arthritic score, where ### shows a significant difference from the control and * shows a significant difference from the arthritic group.**

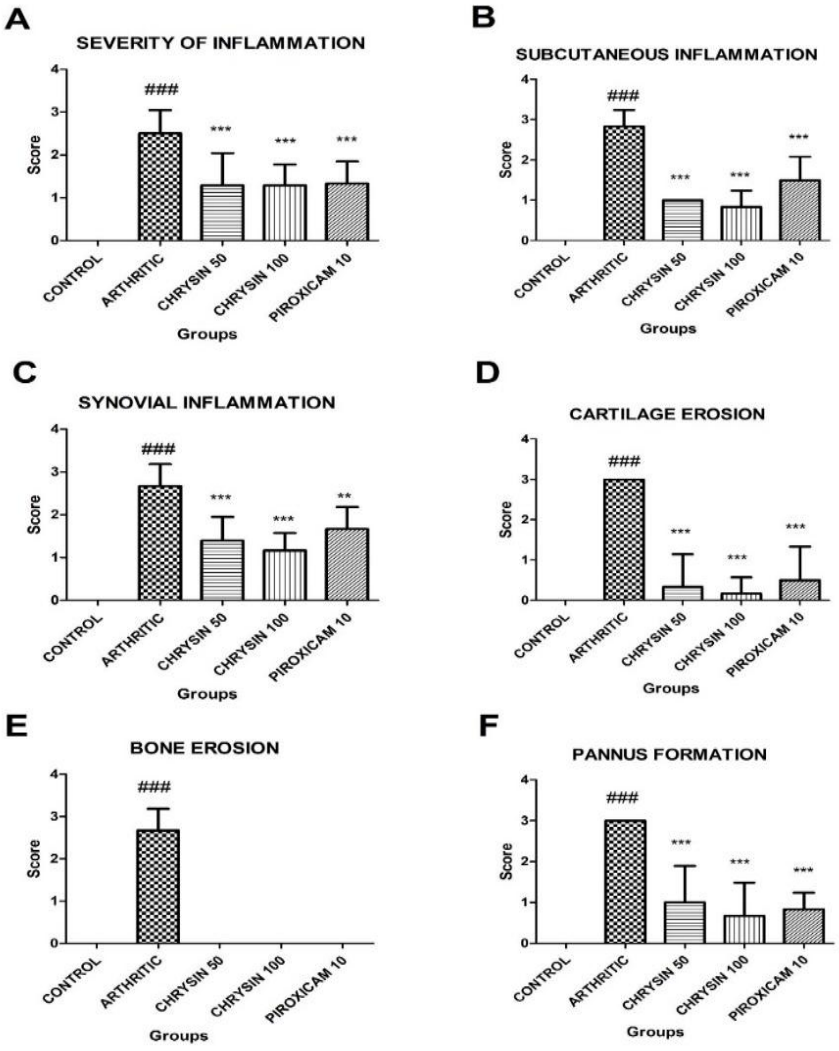

Figure 3: Showing comparison of histological features of arthritic ankle joints of rats of different groups.

Treatment with Chrysin 50 mg/kg i.p. resulted in a noticeable reduction in inflammatory changes compared with the arthritic control group. The Chrysin 100 mg/kg i.p. group showed a greater reduction in inflammatory cell infiltration and tissue damage, with comparatively preserved cartilage and bone architecture. The Piroxicam 10 mg/kg i.p. group also demonstrated improvement in histopathological changes. Overall, the 100 mg/kg Chrysin-treated group showed the most pronounced improvement among the treatment groups, followed by the 50 mg/kg Chrysin and Piroxicam groups, as shown in Figure 3.

DISCUSSION

Experimental inflammatory arthritis is commonly stimulated in rodents using Freund's complete adjuvant (FCA), which is known for its capacity to generate reproducible clinical and histopathological characteristics of joint inflammation. The FCA-induced arthritis model is frequently employed to evaluate possible anti-inflammatory and anti-arthritic drugs, and has some pathological similarities with rheumatoid arthritis, such as swollen joints, inflammatory cell infiltration, cartilage damage, and pannus formation.[14] FCA-induced arthritis is an experimental model that does not fully reflect the complex immunopathology of human rheumatoid arthritis (RA). Injection of FCA results in significant joint inflammation, swelling, pain and impaired mobility, and typically, paw swelling is evident within the first few days after injection.[15,16]

The present study began treatment on the 8th day after FCA, and the treatment period was 3 weeks to assess the therapeutic effect of Chrysin on the established inflammatory changes. A distinct rise in arthritis score was noted in the arthritis control group with FCA, with the joints showing obvious morphological inflammatory changes. The results showed that experimental arthritis had been successfully induced. Chrysin at both doses (50 mg/kg and 100mg/kg) showed a reduction in the arthritis score when compared to the untreated arthritic control group. The decrease seen with Chrysin 100 mg/kg is larger, which indicates a possible dose-related anti-inflammatory effect. The arthritis score was also lowered in the case of the anti-inflammatory drug (Piroxicam), used as a reference drug. The decrease achieved with high-dose Chrysin was, however, greater (numerically) than that achieved with Piroxicam in the current experimental conditions. The results indicate that Chrysin might have anti-inflammatory properties in FCA-induced arthritis. Macroscopic examination also revealed significant inflammatory changes in the FCA-induced arthritis group, such as redness and swelling of the joints. Treatment with Chrysin was less effective in inducing these changes, especially at 100 mg/kg. The decrease in the visible inflammatory changes is in line with the previous results of Chrysin anti-inflammatory activity and indicates that Chrysin could suppress the inflammatory response in FCA-induced arthritis.[17]

The anti-inflammatory effect of Chrysin was also confirmed by histopathological analysis. The inflammatory cell infiltration, subcutaneous and synovial inflammation, cartilage and bone erosion and pannus formation were marked in the FCA-induced arthritis group. These pathological changes are hallmark features of inflammatory arthritis and are linked with advancing deterioration of joint tissues.[18]

Both Chrysin-treated groups showed improvement in histopathological abnormalities compared with the FCA-induced arthritis control group. The 100 mg/kg Chrysin group demonstrated greater preservation of joint architecture and reduction in inflammatory changes than the 50 mg/kg group. Cartilage erosion, bone erosion, and pannus formation were prominent in the arthritic control group but were reduced in the treated groups. No evidence of bone erosion was observed in the Chrysin- or Piroxicam-treated groups, suggesting attenuation of inflammation-associated structural damage. However, these findings do not establish prevention of bone erosion and should be interpreted within the limitations of the animal model and short treatment duration. The greater improvement with 100 mg/kg Chrysin suggests a possible dose-related effect, although a definitive dose-response relationship cannot be established using only two doses. These findings are consistent with previous experimental studies reporting anti-inflammatory and anti-rheumatic effects of Chrysin, including evidence from arthritis scoring, radiographic, and histopathological assessments.[19]

The present study further supports this by showing that clinical arthritis scores and histopathological abnormalities of joint inflammation were improved. The present results suggest a beneficial effect of Chrysin, but no attempt was made in the study to examine the molecular mechanisms underlying this effect. The observed effects must be considered as evidence of anti-inflammatory and anti-arthritic effects and are not sufficient to conclude that the effects are immunosuppressive or disease-modifying. Likewise, the superior numerical effect of high-dose Chrysin with Piroxicam should not be interpreted as a superior clinical effect unless statistical comparison of these treatment groups has been conducted. In general, the present results demonstrate that Chrysin reduced the clinical and histopathological features of FCA-induced arthritis in rats and that 100 mg/kg was the most effective dose. The results are similar to the previous investigations regarding anti-inflammatory and anti-rheumatic effects of Chry-

sin.[17,19] Further experimental studies using quantitative inflammatory biomarkers, molecular investigation, multiple doses, extended follow-up and comparison with other well-established disease-modifying therapies are warranted to assess the therapeutic potential and safety of Chrysin.

CONCLUSION

The results of this experimental study indicate that Chrysin has anti-inflammatory and anti-arthritic activity in FCA-induced arthritis in rats. Chrysin treatment lowered the arthritis score and diminished histopathological parameters of joint inflammation: infiltration of inflammatory cells, erosion of cartilage, erosion of bone and pannus formation. The 100 mg/kg dose of Chrysin yielded greater benefits than the 50 mg/kg dose, and both Chrysin doses led to improvement when compared to the arthritic control group. Piroxicam was also seen to have a beneficial effect on the inflammatory changes, while the high dose group of Chrysin was comparatively more histopathological. These results show that Chrysin could be used in the therapy of inflammatory joint diseases.

Author Contributions

MAF: Conception and design, analysis and interpretation of data, drafting the article, critical revision for important intellectual content, final approval.
MI: Conception and design, analysis and interpretation of data.
WS: Analysis and interpretation of data, drafting the article.
HM: Acquisition of data, conception and design, analysis and interpretation.
MS: Analysis and interpretation of data, proofreading.

Artificial Intelligence (AI) Use Disclosure

ChatGPT (OpenAI) was used during the preparation of this manuscript for language editing and improvement of manuscript clarity. The AI tool was not used to generate or manipulate original research data, perform statistical analysis, or independently develop scientific conclusions or interpretations. All AI-assisted text and suggestions were critically reviewed, edited, and approved by the authors. The authors remain fully responsible for the accuracy, originality, integrity, scientific content, data interpretation, and conclusions of the manuscript.

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