Exploring rutinoside’s impact on inflammation in a rat knee OA model induced by monosodium iodoacetate (MIA)

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INTRODUCTION

Osteoarthritis (OA) is a chronic joint condition that causes cartilage degeneration, inflammation, and pain. It is a leading cause of disability, and greatly strains afflicted individuals and healthcare systems worldwide. Current OA treatments generally focus on pain management and symptomatic alleviation. Still, there is a need for disease-modifying medications that can lower inflammation, minimize joint swelling, and maintain cartilage integrity.¹,²

Rutinoside, a natural chemical in many plants, has demonstrated potential anti-inflammatory and antioxidant capabilities in preclinical trials. Previous research indicated that rutinoside may have potential therapeutic advantages in osteoarthritis; however, its effectiveness and comparative effects with established therapies in an animal model of OA have not been thoroughly investigated.³⁻⁵

This study aimed to see how rutinoside affected inflammatory responses and joint swelling in a rat monosodium iodoacetate (MIA)-induced osteoarthritis rat model. MIA-induced OA in rats is a widely acknowledged model that substantially matches human OA regarding pathological characteristics and clinical signs. To assess rutinoside’s potential as an alternative therapeutic option, we compared its efficacy to two regularly used therapies, glucosamine sulfate and sodium diclofenac.

The outcomes assessed in this study were body weight changes, joint swelling, knee bend test scores, and concentration IL-1, IL-8, and CTX-II, key markers of inflammation, cartilage degradation, and disease severity in OA. Understanding rutinoside’s effects in this experimental model and comparing its efficacy to known therapies may give vital insights into its therapeutic potential for OA management. These findings might help design new therapy options that target both inflammatory processes and structural changes in the joint, eventually improving outcomes for osteoarthritis patients. Overall, this study aims to contribute to the existing knowledge on the therapeutic potential of rutinoside and provide a foundation for further research and clinical investigations exploring its role in the management of osteoarthritis.

MATERIAL AND METHODS

Animals

Thirty male Wistar rats weighing 161–183 grams were involved in this research. The rats were fed a regular diet and had access to mineral water. Acclimatization was performed two weeks prior to the beginning of the study to ensure that they...
were adapted to the experimental setting. The rats were assigned into five groups at random. Group I was the control group; Group II was MIA-induced, untreated osteoarthritis (OA); Group III was OA rats with rutinoside at a daily dose of 100 mg/kg (OA+Rut); Group IV was OA rats with glucosamine sulfate at a daily dose of 25 mg/kg (OA+GS); and Group V was OA rats with rutinoside with diclofenac sodium at a daily dose of 5 mg/kg (OA+SD). Administration of rutinoside, glucosamine sulfate, and sodium diclofenac began 14 days after MIA induction and was given daily for 28 days (4 weeks). The animals’ body weight, knee diameter, and knee bend test scores were measured weekly. At the end of the study, we collected rat joint synovial fluid to evaluate IL-1, IL-8, and CTX-II. All research protocols in this study obtained approval from the Ethics Committee of the Faculty of Medicine, Jember University.

**MIA-induced OA**

Three milligrams of monosodium iodoacetate (MIA) from Sigma-Aldrich in a total volume of 50 µl of saline were injected into the joint space of the left knee via the infrapatellar ligament to induce osteoarthritis (OA) in rats. The rats in the control group got the same volume of saline.

**Knee diameter**

We measured the diameter of the left knee in millimeters (mm) to determine joint swelling as osteoarthritis (OA) progressed. This measurement is carried out once a week with calibrated digital calipers. This examination tries to quantify joint edema and follow its progression during this study.

**Knee bend test**

We assessed the knee-bend test weekly to measure the pain induced by MIA during activity. We performed five alternate knee joint flexion and extension actions within the physiological range. We captured the rats’ squeaks and struggled in response to these movements on camera. The findings of the tests were as follows: 0 indicates no reaction, 0.5 indicates a struggle to maximal flexion or extension, 1 suggests an effort to moderate flexion or extension or vocalizations to maximal flexion or extension, and two shows vocalizations to maximal flexion or extension. The total of these values was the Knee-Bend score, which had a maximum score of 20 and represented the degree of movement-induced nociception in the animals.

**Biochemical analysis**

On the last day of the study, rats were anesthetized intraperitoneally with 60 mg/kg ketamine and 5 mg/kg xylazine. The left leg of each rat was connected to and put inside a 20-ml glass vial. 100 µl of sterile saline solution was injected into the joint using a 23-G needle, and the fluid was aspirated two minutes afterward. Synovial fluid was added and dried continuously until a basic sample of 400 µl was obtained and stored in a 1.5 ml centrifuge tube. The supernatants were obtained immediately following the centrifugation of the samples. We used ELISA kits to measure numerous parameters in synovial fluid, including IL-1β [E0119Ra, BT LAB], IL-8 [E1167Ra, BT LAB], and CTX-II [E-EL-R2554 Elabscience].

**Statistical analysis**

All the data was presented as a mean and standard deviation (SD). GraphPad Prism was used for the statistical analysis. All data were tabulated and statistically analyzed to compare factors between groups using one-way ANOVA and a student t-test. p values less than 0.05 were considered significant.

**RESULTS**

**Bodyweight**

In the first and second weeks following MIA induction, rats in all treatment groups gained less weight than the control group. On the other hand, the rats in the OA+Rut, OA+GS, and OA+SD groups began to gain weight on day 21. The body weight of rats in the OA+Rut group was equivalent to that of the OA+GS and OA+SD groups on day 42 (p > 0.05). This finding demonstrated that rutinoside may restore OA-affected body weight and metabolism and that glucosamine sulfate and sodium diclofenac can have a similar effect (Figure 1a).

**Knee diameter**

All treated groups significantly increased the diameter of the left knee compared to the control group (p < 0.05). We recorded the largest diameter measurement on the first day after the MIA injection. When comparing the OA+Rut group to the OA group on day 21, there was a significant decrease in the diameter of the left knee. Knee diameters did not differ significantly amongst the treated groups (OA+Rut,
OA+GS, and OA+SD). This study reports that rutinoside can reduce joint swelling in knee OA, and the result is the same as glucosamine sulfate or sodium diclofenac (Figure 1b).

**Knee bends score**
Prior to the MIA injection, there was no sign of spontaneous nociceptive behavior or discomfort. However, all treated groups significantly improved knee bend scores (p < 0.05) compared to the control group. After day 21, all treatment groups’ knee bend test scores began to improve. The study found that either rutinoside, glucosamine sulfate, or sodium diclofenac reduced osteoarthritic knee discomfort (Figure 1c).

**Biochemical markers**
At the end of the study, we took synovial fluid and evaluated IL-1, IL-8, and CTX-II levels. The IL-1 level in the synovial fluid of rats in the OA group was significantly higher than in the control group (p < 0.05). Meanwhile, IL-1 levels decreased significantly in the OA+Rut group compared to the OA group (p < 0.05). The groups that received glucosamine sulfate and diclofenac sodium had similar IL-1β levels to those that received rutinoside (p > 0.05) (Figure 2a). IL-8 levels in the OA group increased significantly compared to the OA group (p < 0.05), and rutinoside successfully prevented this increase, resulting in a substantial drop in IL-8 (p < 0.05). IL-8 levels in the diclofenac sodium and glucosamine sulfate groups were comparable to those in the rutinoside group (p > 0.05) (Figure 2b).

CTX-II levels were also higher in the OA group than controls (p < 0.05). Rutinoside decreased CTX-II levels significantly (p < 0.05) compared to the OA group. CTX-II levels were comparable in the diclofenac sodium and glucosamine sulfate groups to those in the rutinoside group (p > 0.05) (Figure 2c). The results of this study show that rutinoside reduces inflammatory cytokines (IL-1 and IL-8) and improves cartilage degradation in OA conditions, as well as glucosamine sulfate and sodium diclofenac.

**DISCUSSION**
The current study compared the effectiveness of rutinoside with two commonly prescribed therapies, glucosamine sulfate and sodium diclofenac, in a rat osteoarthritis (OA) rat model caused by monosodium iodoacetate (MIA). We evaluated joint swelling, knee bend test scores, body weight changes, IL-1, IL-8, and CTX-II levels.

The study’s outcomes showed that rutinoside significantly decreased joint swelling and inflammation in the MIA-produced OA rat model. The observed decrease in joint swelling, which reduced the inflammatory process, implies that rutinoside as an anti-inflammatory medication may benefit OA—these findings back with previous studies that emphasized rutinoside’s anti-inflammatory properties in various illness types. The study also sheds light on the effect of rutinoside on body weight and knee bend scores in the setting of osteoarthritis (OA). Observing decreased body weight and improved knee bend scores in Rutinoside-treated rats suggests that rutinoside may benefit OA patients’ overall mobility and physical function. These results indicate that rutinoside has the potential to relieve OA-related pain and improve the quality of life for people who suffer from it. Rutinoside showed equivalent effectiveness in reducing body weight loss, joint swelling, and knee bend test results compared to glucosamine sulfate and sodium diclofenac. These results are crucial gauges of the development of OA and functional impairment. Given the same results shown between it and the two main therapies, Rutinoside may be a potential alternative therapy option for treating OA-related symptoms.8–9

Additionally, it was shown through a study of inflammatory biomarkers, including IL-1 and IL-8, that rutinoside therapy significantly reduced their levels. IL-1 and IL-8 are essential for inducing inflammation and cartilage degeneration in OA. The decrease in these cytokines suggests that rutinoside may be able to control the inflammatory environment in the joint, preserving cartilage integrity and improving function.10–12

Following rutinoside treatment, a decrease in CTX-II levels—a marker of cartilage degeneration—was also seen. This demonstrates that type II collagen in articular cartilage is resistant to degradation, suggesting that rutinoside may have chondroprotective qualities. Maintaining the cartilage’s structural integrity is essential for keeping joints functioning and preventing the onset of OA.13–15

Compared to sodium diclofenac, a commonly used nonsteroidal anti-inflammatory medicine (NSAID), rutinoside demonstrated comparable anti-inflammatory activity without the gastrointestinal side effects often associated with NSAIDs. This shows that rutinoside may be a safer long-term option for OA treatment. Furthermore, the study discovered that rutinoside performed similarly to glucosamine sulfate, a common supplement for joint health. This suggests that rutinoside might be a natural alternative to standard OA treatments, with potential advantages for patients seeking complementary

![Figure 2.](image-url) The synovial concentration of (a) IL-1, (b) IL-8, and (c) CTX-II in all groups. OA: osteoarthritis; OA/Rut: Osteoarthritis and Rutinoside treated group; OA/GS: Osteoarthritis and glucosamine sulfate treated group; OA/SD: Osteoarthritis and sodium diclofenac treated group.
therapy. It outperformed two well-known OA treatments: sodium diclofenac and glucosamine sulfate. Rutinoside was also shown to be more effective in lowering IL-1 and IL-8 levels, implying that it might be a helpful anti-inflammatory therapy for OA.

However, there are some limitations in the study that should be resolved. An OA animal model may not accurately represent the complex nature of human OA pathogenesis. Furthermore, the study focused on short-term results while neglecting long-term consequences and safety concerns associated with rutinoside intervention. More research is needed to understand the underlying mechanisms and confirm these findings in human clinical trials, which would open the way for future research into new OA treatment alternatives.

CONCLUSION

In conclusion, this study reveals that rutinoside offers potential as a therapeutic intervention for osteoarthritis. Its results on body weight, knee bend scores, knee diameter, and the modulation of key inflammatory markers and CTX-II hint at its promise in boosting mobility, lowering inflammation, and protecting cartilage in osteoarthritis.

CONFLICT OF INTEREST

The authors declare that no competing financial, professional, or personal interests might have affected the performance or presentation of the work described in this manuscript.

ETHICAL STATEMENT

The study was conducted following the Declaration of Helsinki and approved by the Research Ethics Committee of the Faculty of Medicine, Jember University, with number 1554/H25.1.11/KE/2022.

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AUTHOR CONTRIBUTION

RN responsible for concept of the study, design of the study, definition of intellectual content literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. BP responsible for concept of the study, design of the study, definition of intellectual content literature search, experimental studies, manuscript editing, manuscript review, and guarantor of the study. DT responsible for concept of the study, design of the study, definition of intellectual content literature search, experimental studies, manuscript editing, manuscript review, and guarantor of the study.

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