Trop J Nat Prod Res, June 2024; 8(6):7526-7529 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Histopathological Profile of Rats (*Rattus norvegicus*) with Collagen-Type II Rheumatoid Arthritis and treated with Purslane Tea (*Portulaca oleracea* Linn.)

Ira Asmaliani[1], Bayu Putra[2], Andi Emelda[3], Rizqi N. Azizah[4*]

[1]*Department of Pharmacology, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia*
[2]*Department of Pharmacology, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia* [3]*Department of Pharmacology, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia* [4]*Department of Pharmacology, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia*

ARTICLE INFO

Article history:
Received: 16 January 2024
Revised: 22 February 2024
Accepted: 02 June 2024
Published online 01 July 2024

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and can be treated with drugs such as methotrexate. However, the use of this drug can cause severe adverse effects. The development of herbal medicines has become an alternative therapy for treating rheumatoid arthritis. Purslane (*Portulaca oleracea* L.*)* is one of the plants that can treat RA, which contains saponins, flavonoids, omega-3 fatty acids, and phenolic acids. Purslane has been widely developed into herbal tea. This study aimed to determine the effect of purslane tea on the histopathological change of the rat's joint (*Rattus novergicus*) with rheumatoid arthritis. This test uses the collagen-induced arthritis (CIA) method. This method was carried out by inducing bovine type II collagen emulsified with Complete Freund's Adjuvant (CFA) as an immunization phase and with Incomplete Freund's Adjuvant (IFA) as a booster phase in the tail of the rats. This study consisted of 3 groups: sodium CMC (control group), methylprednisolone (control positive), and the purslane tea group. Parameters were observed in the histopathological analysis of the rats' joints. The results of the histopathological analysis showed that purslane tea influences the prevention of synovial joint damage by inhibiting joint erosion and pannus formation in the synovial joints of rheumatoid arthritis rats. The study concludes that purslane tea is an effective and alternative medicine for treating rheumatoid arthritis.

***Keywords*:** *Portulaca oleracea* L, Rheumatoid arthritis, Collagen Induced Arthritis, Purslane Tea, Rats

Introduction

The term arthritis refers to a disease with clinical manifestations such as swelling, pain, redness, and joint and connective tissue damage. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder characterized by polyarthritis, which has the potential to deform extra-articular joints.[2,3]

According to the WHO, the incidence of RA in 2019 reached 18 million of the world's population. Around 70% of patients are women, and 55% are older than 55.[1] According to Riskesdas (2018), the number of rheumatoid arthritis patients in Indonesia reached 7.30%.[4] This situation explains the lack of knowledge among Indonesian people, especially patients, to know more deeply about rheumatoid arthritis.[1,4] RA disease attacks the joints systemically and causes joint deformity if not treated. The Arthritis Foundation (AF) states that drugs that can treat arthritis are anti-inflammatory drugs, NSAIDs, corticosteroids, and DMARDs (disease-modifying anti-rheumatic drugs).[5]

*Corresponding author. E mail: rizqi.azizah@umi.ac.id
Tel: +6281241297300

Citation: Asmaliani, I, Putra, B, Emelda, A, Azizah RN. Phytochemical and Histopathological Profile of Rats (*Rattus norvegicus*) with Collagen-Type II Rheumatoid Arthritis and treated with Purslane Tea (*Portulaca oleracea* Linn.). Trop J Nat Prod Res. 2024; 8(6): 7526-7529. https://doi.org/10.26538/tjnpr/v8i6.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

NSAID-class medications are reserved for the treatment of rheumatoid arthritis symptoms. Cautious usage is advised due to the potential for severe adverse effects, including gastrointestinal bleeding.[3] Side effects of corticosteroid use include increasing blood pressure and sugar levels and can cause bone loss.[6] In addition to corticosteroids and anti-inflammatory drugs, prolonged use of DMARD drugs may result in moderate to severe adverse effects, including stomatitis, headache, rash, dyspepsia, nausea and vomiting, diarrhea, and abdominal pain. Severe adverse effects encompass vision impairments, liver function disorders, renal dysfunctions, reduced red blood cell production, and baldness.[3,6]

The extensive array of adverse effects linked to pharmaceutical interventions for rheumatoid arthritis has prompted scientists to explore botanical remedies as potential alternatives. Numerous investigations have been undertaken to identify safer and more effective options for traditional rheumatoid arthritis treatments.

As demonstrated by histopathological change, jackfruit leaf extract can treat rheumatoid arthritis by repairing collagen-induced injuries to the leg joints of rats.[7] An additional viable botanical specimen is purslane, composed of chemical compounds that impart biological activity, including flavonoids, phenolic acids, and carotenoids.[8] A chemical constituent, specifically flavonoids, has been recognized for its anti-inflammatory properties through the inhibition of the cyclooxygenase enzyme, which consequently impedes prostaglandin formation.[9] Purslane has been documented to possess pharmacological properties, including antithrombotic[10], anti-inflammatory[11,12], antioxidant, and antidiabetic effects.[13] An additional study demonstrated that purslane ethanol extract inhibits the progression of rheumatoid arthritis.[14]

Due to the numerous health advantages of the purslane plant, micro, small, and medium enterprise traders have begun cultivating it as an

herbal tea. Utilizing purslane tea is simpler and more effective than using it in extract form. People brew tea with heated water to extract the active compounds from the purslane plant. This study aimed to explore the potential anti-rheumatoid properties of purslane tea in collagen-induced arthritis in rats.

Materials and Methods

Materials

The purslane sample was collected during field research in Makassar, South Sulawesi, Indonesia. The herb was identified at the Faculty of Pharmacy, Natural Product Laboratory, Universitas Muslim Indonesia (UMI), Makassar, Indonesia. Sodium carboxymethyl cellulose (Sodium CMC), buffered Formalin, Chloroform, EDTA, and Sodium Chloride 0.9% were acquired from CV. Sumber Rejeki, Makassar, Indonesia.

Ethical approval was obtained from the Universitas Muslim Indonesia Ethics Committee (UMI) with reference number UMI012208488.

Methods

Preparation of plant samples

Purslane tea (Portulaca oleracea L.) was prepared using 50 g of purslane herbs that were chopped into small pieces. Subsequently, the sample was dried in an oven until the desired state of dryness was achieved, resulting in the formation of dried herbs. The dried herbs were pulverized into a fine powder. The powder sample (1.5 g) was then immersed in 200 mL of water. The recommended dose of purslane tea is 7.5 g/L.[15]

Preparation of collagen emulsion

The source of the Type II collagen was Chondrex, Inc. The collagen emulsion was created by combining bovine type II collagen with complete Freund's adjuvant (CFA) in a 1:1 ratio. This mixture was then homogenized and used for immunization. Type II collagen was emulsified with incomplete Freund's adjuvant (IFA) in a 1:1 ratio for the booster.[16]

Anti-rheumatoid arthritis effect by collagen-induced arthritis (CIA)

Wistar female rats were separated into three groups: As the control group, sodium carboxymethyl cellulose (0.3%) was administered to Group 1. Group 2 received methylprednisolone at a dose of 0.41 mg/kg BW, and Group 3 was administered purslane tea at a dose of 7.5 g/L. A collagen-induced arthritis (CIA) model was utilized to generate an animal arthritis model. As part of the immunization phase, collagen emulsion was administered to the base of rats' tails on day 1. Additionally, on day 7, rats were administered emulsified collagen as a booster phase at an area 1-2 cm below the injection site of immunization, specifically at the base of the tail. The experiment indicates that the most severe stage of arthritis occurs on day 21, and treatment can be initiated from that point until day 25.

Histopathological assay of rheumatoid arthritis rats

All of the rats were sacrificed under chloroform anesthesia on day 26 post-induction. The left legs of the experimental animals were surgically removed, immobilized, and washed with 0.9% sodium chloride to remove blood following surgery. After placing the limb joints of the rats in a pot, they were soaked for 24 hours in a 10% buffered formalin solution before being labeled.[23] Fixation serves to preserve cell and matrix structure, prevent tissue decomposition, and intensify subsequent staining.[17] Afterward, EDTA liquid was used to decalcify the bones for two weeks or until they became pliable. This process softens the organ, thereby facilitating the cutting process. Dehydration and clarification were subsequently performed, followed by infiltration and embedding, and tissue blocks were then cut.[18]

The cutting results were then transferred using a brush into warm water at 38-40^{o}C to unfold the creases and smooth out delicate wrinkles. The ensuing flawlessly taut incision was captured using a glass object. The chosen pieces were dehydrated and positioned on a heated surface at a temperature of 38-40^{o}C until they became completely dry. Subsequently, the preparations were stored in an incubator at a temperature range of 38-40^{o}C, after which they were prepared for staining using Hematoxylin-Eosin.[18] Subsequently, a histological examination was conducted on the rat's leg joint tissue using a light microscope set at a magnification of 400x to observe any alterations in the joint tissue.[17,18.]

Result and Discussion

Purslane is an herbaceous plant that contains numerous compounds. It contains high levels of potassium, magnesium, calcium, and omega-3 fatty acids.[19] It also contains secondary metabolites such as flavonoids, phenolic acids, and carotenoids. Purslane has anti-inflammatory,[11,12] anti-hyperlipidemic,[26] immunomodulatory[25], anti-cancer, and antioxidant properties.[20] It is also effective in treating obesity. [8,20,24]

This study examined the histological changes in the leg joints of rats with rheumatoid arthritis who were administered purslane tea. Observation of histopathological images is one of the measures used to determine the extent of damage caused by Collagen-induced arthritis (CIA) in rats.

The CIA method has advantages over other methods. It is more potent in demonstrating the success of modeling RA disease in rats. This method produces polyarthritis, characterized by cartilage damage associated with immune complex deposits on the articular surface, inflammation of inflammatory cells, proliferation of synovial membranes, and even erosion of joints.[16] These methods present an arthritic state that is more like the arthritic state in humans. The histopathological change of the leg joints of rheumatoid arthritis rats induced by CIA and after being treated with purslane tea was observed using the hematoxylin-eosin (HE) staining technique, as shown in Figures 1–3. Figure 1 depicts the histopathological changes observed in the leg joints of rheumatoid arthritis rats administered 0.3% Na CMC orally (group 1). In this group, several synovial joint damages occurred, including inflammatory infiltration (red arrow), joint surface erosion (green arrow), and pannus development (yellow arrow). The presence of pannus and joint erosion indicates efficacy in modeling rheumatoid arthritis illness in rats using the CIA approach.[16] This is consistent with previous studies indicating that the CIA technique generated inflammatory cell infiltration, pannus development on the synovial membrane, and cartilage erosion.[7] Figure 2 depicts the histopathological changes in the leg joints of rheumatoid arthritis rats that received methylprednisolone orally. In Figure 2, there was no joint erosion or inflammatory cell infiltration, and the synovium cells were still healthy. This demonstrated that the rats' joints improved after being treated with methylprednisolone. These findings suggest that methylprednisolone effectively improves the histopathological alteration in the rat's joints. This medication is intended to treat rheumatoid arthritis.[22] Methylprednisolone is an anti-inflammatory corticosteroid that inhibits the synthesis of arachidonic acid by phospholipids, preventing prostaglandins and leukotrienes from forming and releasing inflammatory mediators. It also reduces vascular permeability in areas of inflammation.[2,3,22] Also, figure 3 depicts the histopathological changes in the leg joints of rheumatoid arthritis rats that were administered purslane tea. The figure shows multiple damages, including inflammatory cell infiltration (red arrow) and synovial membrane hypertrophy (blue arrow), but no pannus development. Chronic inflammation induces hypertrophy and proliferation of the synovial membrane, resulting in pannus. Pannus is caused by the thickening of the synovium, which is bordered by granular tissue.[2,16,22] Although it did not completely cure the damage to the rats' joints, purslane tea therapy effectively prevented pannus development. This finding is consistent with previous research, demonstrating that purslane ethanol extract can effectively treat rheumatoid arthritis in rats.[14] Purslane samples with higher concentrations of active substances exhibit enhanced anti-inflammatory activity and efficacy in treating rheumatoid arthritis in rats. This indicates that the active substance at the administered dose can rectify the histopathological alterations in the joints of the rats. It is hypothesized that the anti-rheumatoid arthritis properties of purslane ethanol extract are attributable to its phytochemical constituents, including saponins, flavonoids, omega-3 fatty acids, and phenolic

acids.[8,21] The anti-inflammatory properties of flavonoids are attributed to their ability to inhibit the cyclooxygenase enzyme, which prevents the synthesis of prostaglandins.[9,14] While purslane tea does not entirely restore joint injury in rats with rheumatoid arthritis, it can prevent the development of pannus and may be utilized as an alternative treatment for rheumatoid arthritis.

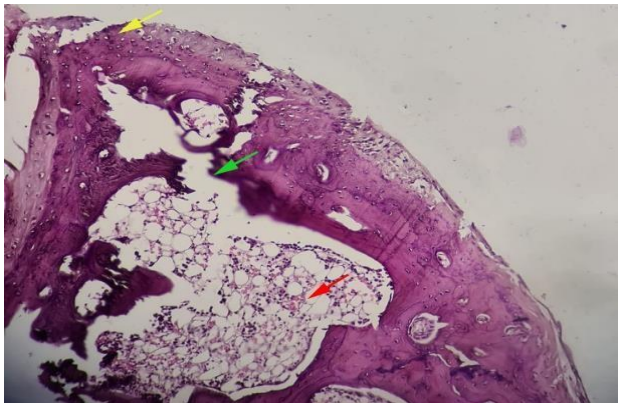

Figure 1: Histopathological changes were observed in the leg joints of rheumatoid arthritis rats given 0.3% Sodium CMC orally (group 1). H&E at x400 magnification. In this group, multiple synovial joint damages occurred. The red arrow showed inflammatory infiltration, the green arrow indicated joint surface erosion, and the yellow arrow showed pannus development.

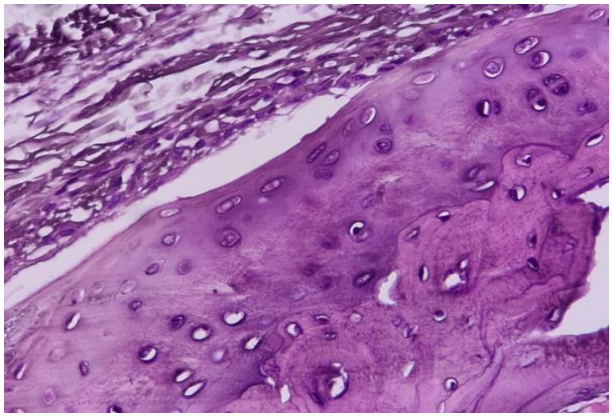

Figure 2: Histopathological changes were observed in the leg joints of rheumatoid arthritis rats given methylprednisolone (group 2). H&E at x400 magnification. In Figure 2, there was no joint erosion or inflammatory cell infiltration, and the synovium cells remained healthy.

Conclusion

Based on the histopathological analysis, it can be inferred that purslane tea has a preventive impact on synovial joint damage in rheumatoid arthritis rats by reducing joint erosion and pannus development in the synovial joints.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

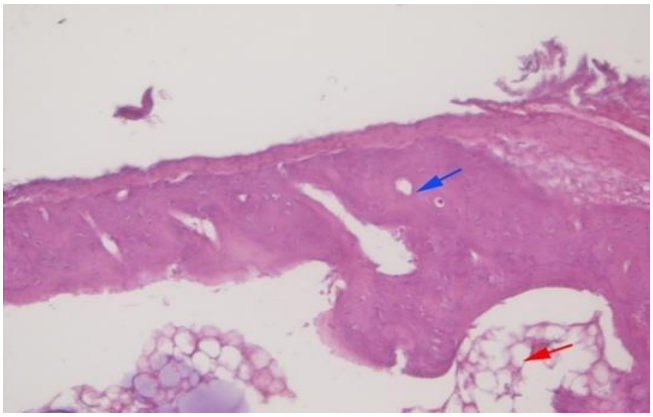

Figure 3: Histopathological changes were observed in the leg joints of rheumatoid arthritis rats given purslane tea (group 3). H&E at x400 magnification. Figure 3 shows multiple damages, including the red arrow indicating inflammatory cell infiltration and the blue arrow indicating synovial membrane hypertrophy, but there was no pannus development.

Acknowledgments

The Research Team would also like to thank the Rector of Universitas Muslim Indonesia (UMI) and the Resource Research and Development Department of UMI (LP2S) for their research funding support and significant contributions to this research.

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