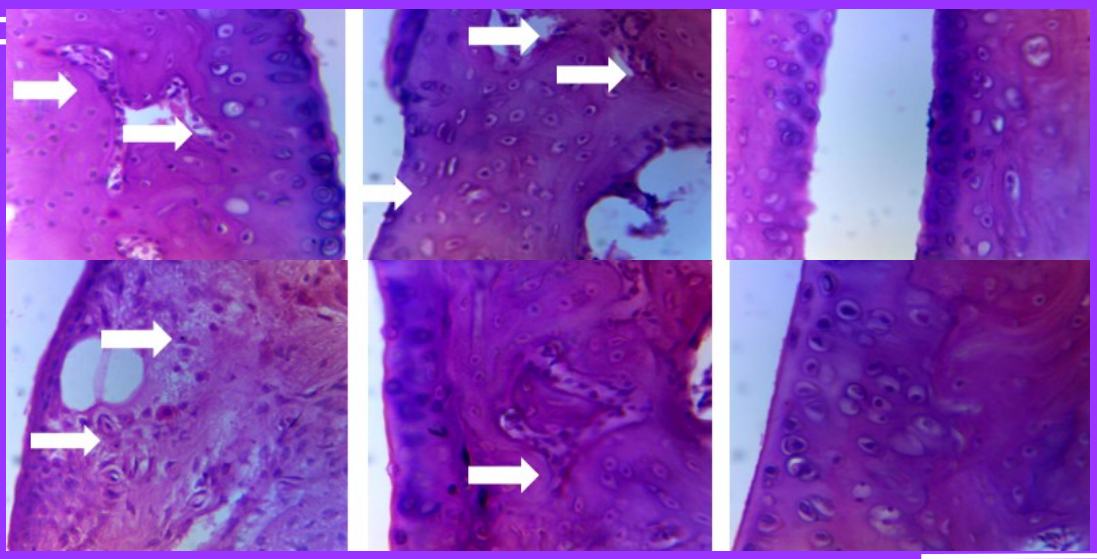

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Anti-arthritic activity of *Alternanthera paronychioides* through suppression of inflammatory cytokines and NF-κB signaling

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Abstract

Alternanthera paronychioides is traditionally used for managing inflammatory disorders; however, its anti-arthritic efficacy and underlying mechanisms remain insufficiently characterized. The present study evaluated the anti-arthritic potential of *A. paronychioides* in acute and chronic animal models of arthritis. Previously, sequential extracts were assessed for inhibition of protein denaturation, proteinase activity, and erythrocyte membrane destabilization. The most active *n*-hexane extract was further investigated in carrageenan -induced inflammation and complete Freund's adjuvant-induced arthritis in rats. *n*-Hexane extract treatment significantly reduced paw edema, arthritic severity, hyperalgesia, and allodynia, while improving histopathological, and radiographic outcomes. Molecular analysis revealed marked suppression of pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, NF-κB, and COX-2, alongside up-regulation of IL-4. These findings demonstrate that *A. paronychioides* exerts potent anti-arthritic effects through multi-target immunomodulation.

Introduction

Rheumatoid arthritis is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, progressive cartilage destruction, and irreversible joint damage, ultimately resulting in pain, functional impairment, and reduced quality of life.

At the molecular level, rheumatoid arthritis pathogenesis involves dysregulated immune responses marked by excessive production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), together with aberrant activation of intracellular signaling pathways such as NF-κB, MAPK, and PI3K/AKT. These mechanisms collectively drive synovial hyperplasia, pannus formation, and bone erosion (McInnes and Schett, 2017).

Current therapeutic strategies primarily involve nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs). Although these agents provide symptomtic relief and delay disease progression, their long-term use is frequently associated with adverse effects, including gastrointestinal and cardiovascular toxicity, hepatotoxicity, immunosuppression, and increased susceptibility to infections. In addition, a significant proportion of patients exhibit suboptimal responses or develop treatment resistance, highlighting the need for safer and more effective multi-target therapeutic approaches capable of modulating the complex inflammatory networks underlying rheumatoid arthritis (Smolen et al., 2007).

Medicinal plants constitute an important source of

structurally diverse bioactive compounds with inherent polypharmacological properties, making them attractive candidates for the management of multifactorial inflammatory disorders such as rheumatoid arthritis. *Alternanthera paronychioides* A. St.-Hil. (Amaranthaceae) has been traditionally used for the treatment of rheumatoid arthritis, gout, hyperuricemia, and nephritis (Mandal et al., 2023; Wu et al., 2013). Phytochemical investigations of *Alternanthera* species have revealed the presence of phenolics, terpenoids, sterols, and triterpenes, compound classes widely recognized for their anti-inflammatory, antioxidant, and immunomodulatory activities (Chen et al., 2022). Despite its ethnomedicinal importance, the anti-arthritic efficacy of *A. paronychioides* and its underlying molecular mechanisms remain insufficiently characterized.

Accordingly, the present study was designed to systematically evaluate the anti-arthritic potential of *A. paronychioides* using acute and chronic experimental models of arthritis. These findings scientifically validate the traditional use of *A. paronychioides* in inflammatory and rheumatic disorders and highlight its potential as a promising plant-derived candidate for safer rheumatoid arthritis management.

Materials and Methods

Plant material collection and authentication

The aerial parts (stems, leaves, and flowers) of *A. paronychioides* were collected between March and April 2023 from the greenbelt area near the hostel block at the University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan (31.4775° N, 74.3275°E).

The plant was identified and authenticated by Prof. Zaheer-Ud-Din Khan, Department of Botany, Government College University, Lahore, Pakistan. A voucher specimen was deposited under reference number GC.Herb.Bot.3937.

Preparation of plant extracts

Shade-dried aerial parts were pulverized, and 350 g of powdered material was subjected to sequential soxhlet extraction using solvents of increasing polarity: n-hexane, chloroform, and methanol. The extraction procedure was conducted as previously described (Azwanida, 2015), with minor modifications to optimize solvent efficiency. Defatting with n-hexane was performed at 50–55°C for approximately 72 hours until the siphon solvent appeared clear. The remaining plant residue was subjected to similar extraction procedures using chloroform and then methanol, ensuring thorough extraction of intermediate and polar phytochemicals, respectively. Extracts were filtered, concentrated under reduced pressure using a rotary evaporator (Heidolph, Germany), and dried at 40°C to constant weight. Percentage yields were calculated, and dried extracts were stored at 4–5°C until further use.

Carrageenan-induced acute inflammation

Female Wistar rats (9–10 weeks old) were obtained from the Punjab University College of Pharmacy animal facility. Carrageenan-induced paw edema was performed as described previously (Vinegar et al., 1969). The *n*-hexane extract doses (100, 200, and 400 mg/kg) were selected based on prior studies on related *Alternanthera* species (Pelisoli Formagio et al., 2012; Biella et al., 2008). Thirty non-pregnant female Wistar rats weighing 160 ± 20 g were randomly allocated into six groups (n=5). One hour after oral administration of the respective treatments (Table I), acute paw edema was induced by subplantar injection of 0.1 mL of freshly prepared carrageenan solution (1% w/v in distilled water) into the left hind paw. Baseline paw diameters were measured prior to injection using the vernier calipers, and subsequent

Table I

Experimental design and treatment groups

Description	Regimen for carrageenan-induced acute inflammatory assay	Regimen for complete Freund's adjuvant-induced chronic inflammatory assay
Control	1% Carboxymethyl cellulose orally + 0.1 mL saline	1% Carboxymethyl cellulose orally + 0.1 mL saline
Disease control	1% Carboxymethyl cellulose orally + 0.1 mL carrageenan	1% Carboxymethyl cellulose orally + 0.1 mL complete Freund's adjuvant
Standard (10 mg/kg)	Diclofenac sodium orally + 0.1 mL carrageenan	Diclofenac sodium orally + 0.1 mL complete Freund's adjuvant
n-Hexane extract (100 mg/kg)	Extract orally + 0.1 mL carrageenan	Extract orally + 0.1 mL complete Freund's adjuvant
n-Hexane extract (200 mg/kg)	Extract orally + 0.1 mL carrageenan	Extract orally + 0.1 mL complete Freund's adjuvant
n-Hexane extract (400 mg/kg)	Extract orally + 0.1 mL carrageenan	Extract orally + 0.1 mL complete Freund's adjuvant

Box 1: Complete Freund's adjuvant–induced arthritis model

Principle

Chronic arthritis was induced by subplantar injection of complete Freund's adjuvant

Requirements

Complete Freund's adjuvant; Diclofenac sodium (as standard); Carboxymethyl cellulose; Hot plate; Rat (Wistar; Non-pregnant female; 9–10 weeks old; 170 ± 50 g); Vernier calipers

Preparation

Extract: The n-hexane extract of *A. paronychioides* was freshly prepared each day in carboxymethyl cellulose (1%)

Procedure

Step 1: Animals were randomly divided into six groups (n=5 per group): normal control, disease control, standard drug–treated, and three extract-treated groups (100, 200, and 400 mg/kg) (Table I).

Step 2: On day 0, chronic arthritis was induced in all groups except the normal control by a single subplantar injection of 0.1 mL complete Freund's adjuvant into the left hind paw. Normal control animals received an equal volume of saline.

Step 3: Twenty-four hours after complete Freund's adjuvant administration (day 1), oral treatments of n-hexane extract, diclofenac sodium (10 mg/kg), or carboxymethyl cellulose (1%) at a volume of 10 mL were administered and continued once daily (approximately the same time each morning) for 28 consecutive days.

Step 4: Arthritis progression was monitored on day 0, 7, 14, 21, and 28 by measurement of paw diameter using vernier calipers, assessment of arthritic score, and recording of body weight. Behavioral assessments, including thermal hyperalgesia, mechanical allodynia, and anxiety-like behavior, were performed during the treatment period.

Step 5: On day 28, rats were euthanized for histopathological, radiographic, and molecular analyses to evaluate joint damage and inflammatory mediators.

Reference

Manan et al., 2020

measurements were taken at hourly intervals for up to 6 hours, with a final measurement at 24 hours. The percentage inhibition of paw edema for each treatment group was calculated at 6 and 24 hours using the following formula:

$$\%\text{Inhibition of edema} = \frac{(\text{PeC} - \text{PeT})}{\text{PeC}} \times 100$$

Where PeC represents paw edema (mean paw thickness (mm) in the control group, and PeT indicates paw edema (mean paw thickness (mm) in the treated groups

Behavioral assessments

Thermal hyperalgesia was evaluated using the hot plate method, mechanical allodynia using the von Frey up–down method, and anxiety-like behavior using the open-field test following standard protocols (Gunn et al., 2011; Deuis et al., 2017; Parent et al., 2012). Thermal hyperalgesia was assessed on days 0, 7, 14, 21, and 28 using the hot plate method. Rats were placed on a metallic surface maintained at 52.5 ± 0.5°C, and the latency period until hind paw licking or hopping was recorded as an index of pain threshold. The plate was enclosed with plexiglas barriers to prevent escape. Rats were removed immediately after a hind paw response or if no reaction occurred within 60 sec, to avoid injury. The percentage change in pain sensitivity was calculated as follows:

$$\%\text{Latency reduction} = \frac{(\text{Control latency-Treatment latency})}{\text{Control latency}} \times 100$$

Mechanical allodynia was evaluated on days 0, 7, 14, 21, and 28 by manually applying a constant force of 50 g to the inflamed ankle joint. The withdrawal latency following the nociceptive response was recorded with a stopwatch. To minimize stress, rats were tested in a calm environment free of external stimuli. A cut-off time of 10 sec was imposed to prevent tissue injury.

Anxiety-like behavior was assessed using the open field test. The open field apparatus consisted of a wood square arena (72 cm × 36 cm) placed in a normally lit room. Rats were acclimatized to the experimental conditions but not to the arena. For testing, each rat was placed in one corner of the field, and exploratory behavior was recorded for 5 min. The arena was virtually divided into 16 equal squares. All sessions were recorded with a ceiling-mounted digital video camera positioned directly above the arena. Behavioral scoring was performed from the video files by manual counts. Behavioral parameters included the time period spent in the center, the frequency of entries into the central four squares, the duration spent in the periphery, rearing events, and the total distance traveled. An entry was recorded when the midpoint of the animal's body crossed into a square.

Histopathological and radiographic evaluation

Joint tissues were processed for histopathological examination using hematoxylin and eosin staining. Following deep anesthesia, animals were humanely euthanized on day 28, and hind paw tissues were excised for histological analysis. Samples were fixed in 10% formalin, decalcified, dehydrated in graded alco-hols, and embedded in molten paraffin. Tissue sections of 5 µm thickness were prepared, mounted on slides, air-dried, and stained with hematoxylin and eosin. Histopathological changes such as pannus formation, bone erosion, and joint swelling were evaluated under a light microscope.

Hind limbs were dissected at the ankle joint and subjec-

ted to computerized radiographic imaging to assess structural changes in the joints. Radiographic analysis was performed to assess joint integrity (Atkinson et al., 2012; Esser et al., 1995).

RT-qPCR analysis

Expression levels of key inflammatory mediators, including NF-κB, TNF-α, PGE_2, IL-4, IL-6, and IL-1β, were quantified using real-time qPCR. Total RNA was extracted from blood samples collected in EDTA tubes using the GeneJET RNA purification kit (Thermo Scientific, Cat. No. K0732). RNA quality and concentration were confirmed with Nanodrop spectrophotometry. Reverse transcription was performed using the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific, Cat. No. K1622) with a minimum input of 10 ng/µL RNA. qPCR was carried out using SYBR Green Master Mix (Zokeyo, China) in a Quant3 Real-Time PCR System (Thermo Fisher Scientific). Each reaction contained 2 µL cDNA, 1 µL of each primer, 5 µL nuclease-free water, and 10 µL SYBR mix. Cycling conditions were as follows: initial denaturation at 95°C, annealing at 60°C, and extension at 72°C for 40 cycles. Relative expression was calculated by comparing cycle threshold (Ct) values of target genes with those of the housekeeping gene. Primer sequences were designed using Primer3, PrimerQuest, and validated against the NCBI GenBank database. Gene expression levels of NF-κB, TNF-α, IL-1β, IL-6, IL-4, and PTGS2 (COX-2) were normalized to GAPDH and calculated using the 2^ΔΔCt method.

Statistical analysis

All data were expressed as mean ± SD (n=5). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism (Version 8.0), with $p<0.05$ considered statistically significant.

Results

Extraction yield

Sequential soxhlet extraction of the aerial parts of *A. paronychioides* yielded *n*-hexane (1.1%), chloroform (1.9%), and methanol (9.0%) extracts, indicating a higher abundance of polar constituents. Despite the lower yield, the *n*-hexane extract exhibited superior *in vitro* anti-arthritic activity (data not shown) and was, therefore, prioritized for further analysis.

Carrageenan-induced acute inflammation

In the carrageenan-induced paw edema model, disease control animals exhibited a time-dependent increase in paw thickness, peaking at 6 hours post-induction (Figure 1). Oral administration of the *n*-hexane (100–400 mg/kg) produced significant, dose-dependent inhibition of paw edema at all measured time points. The highest dose (400 mg/kg) showed inhibitory effects comparable to diclofenac sodium, indicating potent acute anti-inflammatory activity.

Complete Freund's adjuvant-induced chronic arthritis

Complete Freund's adjuvant administration resulted in progressive paw swelling, increased arthritic scores, and body weight loss in disease control animals (Table II). Treatment with the *n*-hexane extract significantly attenuated paw edema and arthritic scores in a dose-dependent manner, with the 400 mg/kg dose producing the most pronounced effects by day 28. Extract-treated animals also exhibited protection against complete Freund's adjuvant-induced body weight loss.

Behavioral assessments

CFA-induced arthritis significantly reduced thermal withdrawal latency and mechanical withdrawal thresholds, indicating hyperalgesia and allodynia (Figure 2). Treatment with the *n*-hexane extract improved both

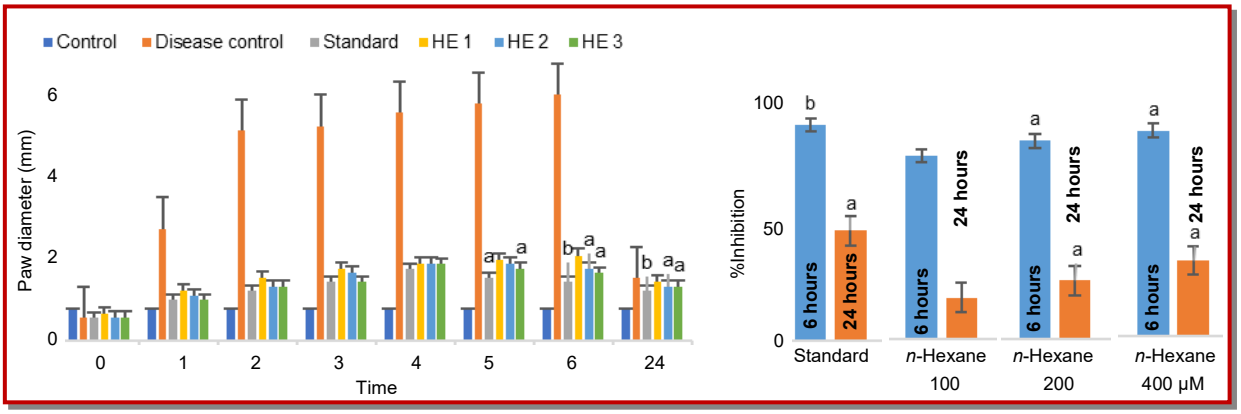

Figure 1: Effect of *n*-hexane extract on paw diameter (mm) (A) and %inhibition of paw edema (B) in carrageenan-induced acute inflammatory assay. Values are mean ± SD (n=5); [a]p<0.05, [b]p<0.01 vs disease control, HE: *n*-Hexane extract; HE1: 100 mg/kg; HE2: 200 mg/kg; HE3: 400 mg/kg)

Table II

Effect of *n*-hexane extract on paw diameter and arthritic score of CFA-induced chronic arthritic rat model

Description	Day 0	Day 7	Day 14	Day 21	Day 28
Paw diameter (mm)					
Control	2.6 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	2.1 ± 0.6	1.9 ± 0.5
Disease control	2.3 ± 0.5	2.7 ± 0.6	2.8 ± 0.4	3.4 ± 0.2	3.1 ± 0.8
Standard (10 mg/kg)	2.6 ± 0.3	2.6 ± 0.1	3.1 ± 0.2	2.6 ± 0.3[a]	2.3 ± 0.2[b]
n-Hexane extract (100 mg/kg)	2.0 ± 0.4	2.7 ± 0.6	2.7 ± 0.6	3.4 ± 0.4	3.3 ± 0.5
n-Hexane extract (200 mg/kg)	2.1 ± 0.5	3.5 ± 0.5	3.6 ± 0.3	3.4 ± 0.7	3.4 ± 0.3[a]
n-Hexane extract (400 mg/kg)	2.4 ± 0.5	2.9 ± 0.5	2.7 ± 0.5	2.6 ± 0.5[a]	2.5 ± 0.3[b]
Arthritic score					
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Disease control	0.0 ± 0.0	2.3 ± 0.6	3.0 ± 0.2	3.3 ± 0.4	3.5 ± 0.2
Standard (10 mg/kg)	0.0 ± 0.0	1.7 ± 0.5	2.6 ± 0.3	1.5 ± 0.6[a]	0.7 ± 0.4[b]
n-Hexane extract (100 mg/kg)	0.0 ± 0.0	2.4 ± 0.3	2.7 ± 0.5	1.4 ± 0.2	1.1 ± 0.1
n-Hexane extract (200 mg/kg)	0.0 ± 0.0	1.8 ± 0.2	1.8 ± 0.7	1.8 ± 0.3[a]	1.8 ± 0.5[a]
n-Hexane extract (400 mg/kg)	0.0 ± 0.0	1.8 ± 0.6	2.5 ± 0.4	1.9 ± 0.3[b]	1.3 ± 0.5[b]

Values are mean ± SD (n=5); [a]p<0.05, [b]p<0.01 vs disease control; CFA means complete Freund's adjuvant

parameters in a dose-dependent manner, with the highest dose restoring responses toward baseline values.

In the open-field test, arthritic animals displayed reduced locomotor activity and exploratory behavior. Extract treatment significantly improved total distance

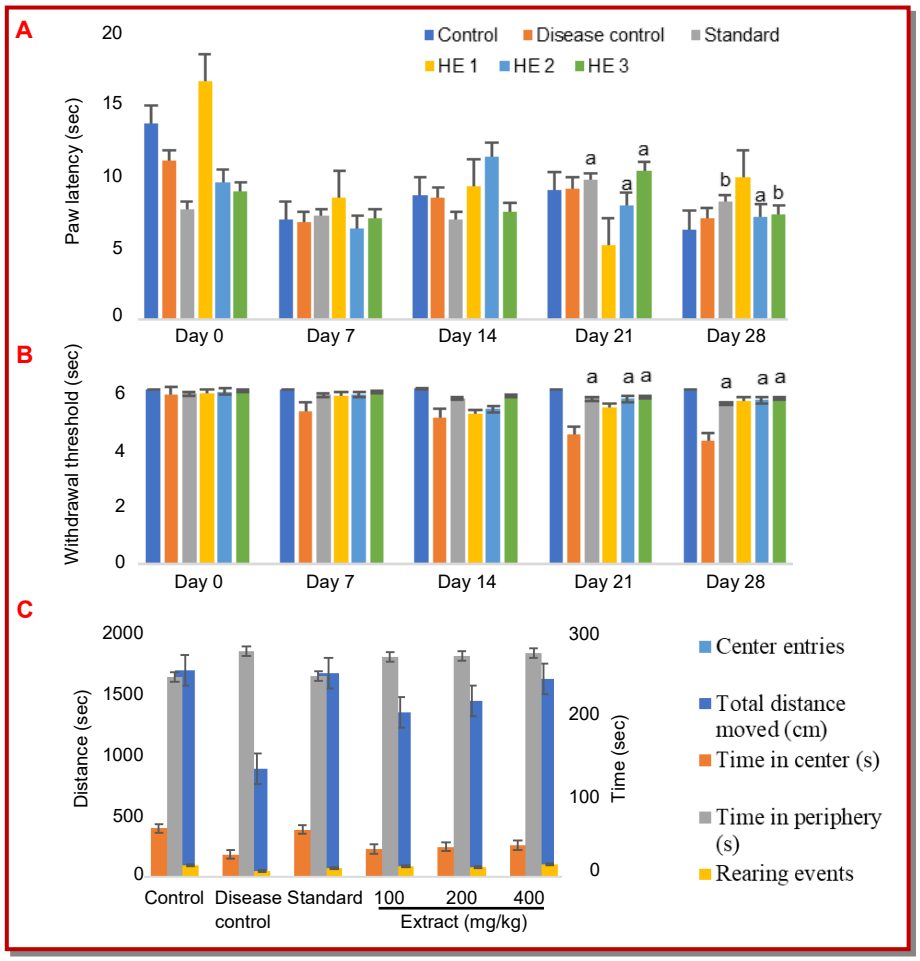

Figure 2: Effect of *n*-hexane extract of *A. paronychioides* on thermal hyperalgesia (A), mechanical allodynia (B), and open field test (C) in CFA-induced chronic arthritic rat model (Values are expressed as mean ± SD (n=5). [a]p<0.05; [b]p<0.01 vs disease control; HE1: 100 mg/kg; HE2: 200 mg/kg; HE3: 400 mg/kg); CFA means complete Freund's adjuvant

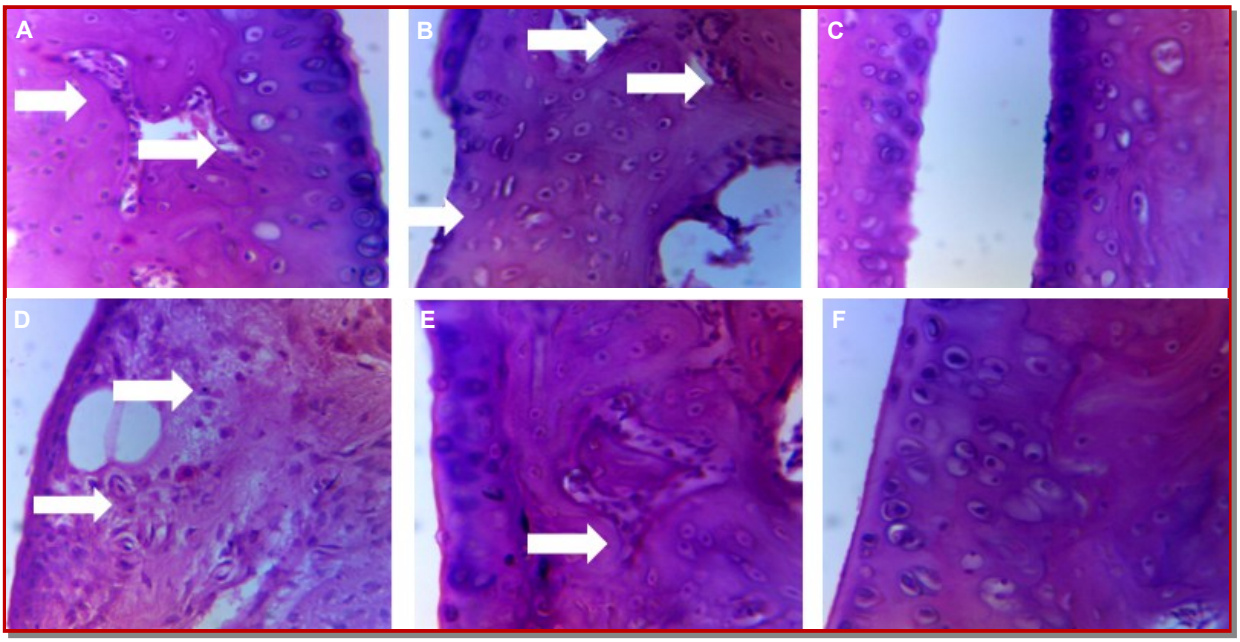

Figure 3: Representative histopathological sections of ankle joints from CFA-induced arthritic rats (H&E, 40x). (A) Control showing intact hyaline cartilage with well-organized chondrocytes and normal subchondral bone.; (B) Disease control displaying severe cartilage erosion, chondrocyte loss, pannus formation, and dense inflammatory cell infiltration.; (C) Standard-treated rats showing mild cartilage degeneration, moderate synovial hyperplasia, and reduced inflammatory infiltration; (D) *n*-Hexane extract-treated 100 mg/kg rats showing moderate cartilage disruption, mild synovial thickening, and scattered inflammatory cells; (E) *n*-Hexane extract-treated 200 mg/kg rats showing moderate chondroprotection with persistent synovial hyperplasia and inflammatory infiltration; (F) *n*-Hexane extract-treated 400 mg/kg rats showing near-normal cartilage morphology, minimal synovial hyperplasia, and markedly reduced inflammatory infiltration

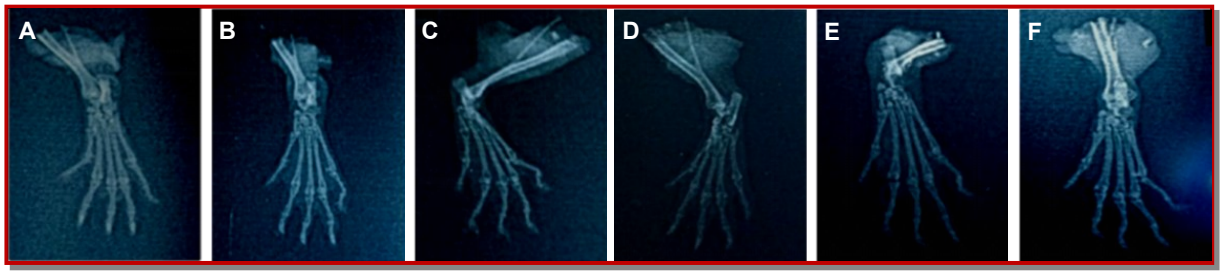

Figure 4: Radiographic images of joints and limbs of CFA-induced arthritic rats (A) Control; (B) Disease control; (C) Standard-treated; *n*-Hexane extract-treated 100 mg/kg (D); 200 mg/kg (E); and 400 mg/kg (F)

traveled, rearing frequency, and time spent in the center zone, indicating amelioration of arthritis-associated anxiety-like behavior and functional impairment. Open-field behavioral outcomes are summarized in Figure 2C.

Histopathological and radiographic evaluation

Histopathological examination of ankle joints from arthritic control animals revealed severe synovial hyperplasia, inflammatory cell infiltration, pannus formation, and cartilage erosion. In contrast, extract-treated groups exhibited dose-dependent preservation of joint architecture, reduced inflammatory infiltration, and protection against cartilage destruction. The highest dose group showed near-normal joint morphology. Representative histological sections are shown in Figure 3.

Radiographic analysis supported histological findings, with extract-treated animals displaying reduced periarticular soft tissue swelling and improved joint space preservation compared with arthritic controls (Figure 4).

Molecular validation by RT-qPCR

RT-qPCR analysis demonstrated significant upregulation of TNF-α, IL-1β, IL-6, NF-κB, and PTGS2 (COX-2) in arthritic control animals as compared with the standard and control groups. Treatment with *n*-hexane extract resulted in a reduced expression of pro-inflammatory genes, with the magnitude of reduction varying among treatment doses. In particular, *n*-hexane extract-treated (200 and 400 mg/kg) showed lower expression levels of IL-1β, TNF-α, and NF-κB relative to the disease control group, whereas more modest chan-

Table III

qPCR analysis of gene expression in CFA-induced arthritic rats

	Relative expression ($2^{-}\Delta\Delta Ct$)					
	IL-1β	IL-6	IL-4	TNF-α	NF-κB	**PTGS2**
Control	2.0 ± 0.0	0.6 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	0.5 ± 0.1
Disease control	4.3 ± 0. 6	1.4 ± 0. 4	1.0 ± 0.4	3.4 ± 0.5	12.3 ± 1.9	1.1 ± 0.7
Standard (10 mg/kg)	1.1 ± 0.5	0.7 ± 0.5	1.9 ± 0.2	0.7 ± 0.2	1.6 ± 0.7	0.5 ± 0.1
n-Hexane extract (100 mg/kg)	2.2 ± 0.8	0.9 ± 0. 3	1.5 ± 0.7	1.3 ± 0.4	1.9 ± 0.2	0.6 ± 0.3
n-Hexane extract (200 mg/kg)	0.9 ± 0.7	0.8 ± 0.7	1.7 ± 0.9	1. 1 ± 0.1	1.0 ± 0.6	0.9 ± 0.2
n-Hexane extract (400 mg/kg)	0.6 ± 0.9	0.7 ± 0.2	1.9 ± 0.6	1. 0 ± 0.4	1.2 ± 0.9	0.9 ± 0.4

Relative gene expression was determined by quantitative real-time PCR using the $2^{-}\Delta\Delta Ct$ method and values are expressed as mean ± SD

ges were observed for IL-6 and $PTGS2_2$. In contrast, expression of the anti-inflammatory cytokine IL-4 was comparatively higher in the standard and n-hexane-treated groups than in the disease control group. Overall, these findings indicate that *n*-hexane extract treatment modulated the inflammatory response by suppressing pro-inflammatory gene expression while maintaining or enhancing IL-4 expression (Table III).

Discussion

This study presents a comprehensive, multi-level evaluation of the anti-arthritic potential of *A. paronychioides* using in *vivo* acute and chronic models of inflammation and arthritis, providing evidence that the plant exerts broad anti-inflammatory, analgesic, immunomodulatory, and joint-protective effects through modulation of signaling pathways involved in inflammatory joint disorders. The combined use of carrageenan-induced acute inflammation and complete Freund's adjuvant-induced chronic arthritis enabled assessment of both short-term anti-inflammatory effects and long-term disease-modifying potential, thereby strengthening the translational relevance of the findings.

The results suggest that in *A. paronychioides*, critical anti -inflammatory compounds are concentrated in the non-polar extract, as reflected by its low extraction yield yet high bioactivity, supporting reports on the role of triterpenoids and sterol derivatives in immunomodulation (Loza-Mejía and Salazar, 2015). This contrasts with studies on *A. sessilis* and *A. tenella*, where polar (methanolic or ethanolic) fractions were prioritized based on yield and moderate bioactivity (Sharma et al., 2025).

Previous studies on *Alternanthera* species have largely focused on edema suppression and biochemical markers, with limited assessment of pain behavior or neuroimmune involvement (Pelisoli Formagio et al., 2012; Marchete et al., 2021). In this context, the present findings extend the pharmacological profile of the genus to include the modulation of the sensory and affective dimensions of inflammatory pain.

In the carrageenan-induced paw edema model, dose-dependent suppression of inflammation, particularly during the late inflammatory phase suggests modulation of prostaglandins, cytokines, nitric oxide, and oxidative stress, which are central to chronic synovial inflammation and arthritis progression (Necas and Bartosikova, 2013). Similar late-phase inhibition has been reported for *A. sessilis* and *A. brasiliana*, however, these studies did not extend their evaluation to chronic autoimmune arthritis or molecular cytokine profiling.

The complete Freund's adjuvant-induced arthritis model provided strong evidence of the disease-modifying potential. The extract dose-dependently reduced paw edema, arthritic scores, hyperalgesia, allodynia, and anxiety-like behavior closely reflecting therapeutic endpoints relevant to human rheumatoid arthritis. The highest dose (400 mg/kg) produced maximal suppression of chronic inflammation and joint pathology, whereas 200 mg/kg yielded the strongest antinociceptive effects during the later disease stages. This divergence may reflect the differential dose-dependent engagement of peripheral anti-inflammatory mechanisms versus central or neuroimmune pathways involved in pain modulation, a phenomenon reported for other plant-derived anti-arthritic agents but rarely discussed in *Alternanthera* studies (Manan et al., 2022; Mohd Razali et al., 2022).

Histopathological and radiographic analyses corroborated these findings, demonstrating the preservation of cartilage architecture, reduced synovial hyperplasia, diminished pannus formation, and protection against bone erosion. While previous studies on *Alternanthera* have reported reductions in paw swelling and inflammatory markers, few have provided radiographic or histological evidence of structural joint preservation (Brand et al., 2007), highlighting the added translational relevance of the present study.

At the molecular level, n-hexane extract downregulated TNF-α, IL-1β, IL-6, NF-κB, and PTGS2 while restoring IL-4 expression, indicating a shift from a pro-inflammatory Th1-dominant response toward anti-inflammatory immune regulation (Liao et al., 2025). Unlike NSAIDs,

which primarily inhibit prostaglandin synthesis, the extract appears to act by suppressing NF-κB signaling, thereby simultaneously influencing multiple inflammatory mediators and distinguishes the present study from previous reports that lack gene-level validation.

Comparison of acute and chronic models indicated that the n-hexane extract was effective in both inflammatory phases; however, the chronic complete Freund's adjuvant model demonstrated more pronounced disease-modifying and joint-protective effects, highlighting its superiority for evaluating therapeutic relevance in rheumatoid arthritis, while the acute model confirmed rapid and potent suppression of anti-inflammatory responses.

This study has limitations like the identification of specific bioactive constituents, gene expression analysis without complementary protein-level validation, and the role of the preventive efficacy of arthritis by the extract.

Conclusion

A. paronychioides demonstrated significant anti-arthritic activity in experimental models through suppression of pro-inflammatory cytokines and inhibition of NF-κB signaling. These findings support its traditional use and highlight its potential as a multi-target therapeutic candidate for inflammatory arthritis.

Financial Support

Self-funded

Ethical Issue

All experimental procedures were approved by the Punjab University Institutional Ethics Review Board (Approval No. D/140/FIMS, dated 24-09-2025) and conducted after approval in accordance with NIH guidelines

Conflict of Interest

Authors declare no conflict of interest

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