Evaluation of Anti-Rheumatoid Arthritis Activity of Selected Natural Semi-Synthesized Compounds

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Abstract- Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by synovial inflammation, progressive joint destruction, and systemic complications. Despite the availability of conventional and biological disease-modifying antirheumatic drugs, their long-term use is often associated with adverse effects and high costs, necessitating the search for safer and more effective alternatives. Natural compounds and their semi-synthetic derivatives have gained significant attention due to their enhanced pharmacological activity, improved bioavailability, and reduced toxicity.

The present study aims to evaluate the anti-rheumatoid arthritis activity of selected natural semi-synthesized compounds using appropriate in vitro and in vivo experimental models. The compounds were assessed for their anti-inflammatory, immunomodulatory, and anti-arthritic potential through parameters such as inhibition of pro-inflammatory mediators, reduction of paw edema, arthritic score, and histopathological examination of joint tissues. Standard anti-arthritic drugs were used for comparison.

The findings of the study demonstrate that the selected semi-synthesized compounds exhibit significant anti-rheumatoid arthritis activity, possibly by modulating inflammatory pathways and suppressing immune-mediated joint damage. These results suggest that natural semi-synthetic compounds may serve as promising candidates for the development of novel therapeutic agents in the management of rheumatoid arthritis.

Keywords- Rheumatoid arthritis; Natural compounds; Semi-synthesized derivatives; Anti-inflammatory activity; Immunomodulatory effect; Anti-arthritic agents

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disorder primarily affecting synovial joints, resulting in persistent synovitis, cartilage degradation, bone erosion, and progressive joint deformity. The disease affects approximately 0.5–1% of the global population and is associated with significant disability and reduced quality of life. The pathophysiology of RA is complex and involves dysregulated immune responses, chronic inflammation, and aberrant activation of multiple cellular and molecular pathways.

At the molecular level, RA is characterized by activation of antigen-presenting cells, $CD4^+$ T lymphocytes, B cells, macrophages, and fibroblast-like synoviocytes (FLS). These cells produce excessive amounts of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-17 (IL-17), which play a central role in sustaining synovial inflammation and joint destruction. These cytokines activate key intracellular signaling pathways, including nuclear factor kappa-B (NF-κB), mitogen-activated protein kinases (MAPKs), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways, leading to the transcription of inflammatory genes.

The activated synovial fibroblasts in RA acquire an aggressive phenotype and contribute to pannus formation by producing matrix metalloproteinases (MMPs) such as MMP-1, MMP-3, and MMP-9, which are responsible for cartilage degradation. Additionally, pro-inflammatory cytokines stimulate osteoclast differentiation through receptor activator of nuclear factor kappa-B ligand (RANKL), resulting in increased bone resorption and erosion. Elevated levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), prostaglandins, and reactive oxygen species (ROS) further amplify inflammatory responses and oxidative stress within the joint microenvironment.

Current pharmacological treatments, including NSAIDs, corticosteroids, conventional disease-

modifying antirheumatic drugs (DMARDs), and biological agents, primarily target these inflammatory mediators and signaling pathways. However, prolonged therapy is associated with adverse effects such as gastrointestinal toxicity, immunosuppression, hepatotoxicity, and increased risk of infections, in addition to high treatment costs. These limitations highlight the need for novel therapeutic agents capable of modulating multiple inflammatory pathways with improved safety profiles.

Natural compounds have emerged as promising sources of anti-arthritic agents due to their ability to regulate inflammation, oxidative stress, and immune responses. Bioactive phytoconstituents such as flavonoids, alkaloids, terpenoids, and polyphenols have been shown to inhibit NF-κB activation, suppress pro-inflammatory cytokine production, downregulate COX-2 and iNOS expression, and scavenge reactive oxygen species. However, their clinical application is often hindered by poor bioavailability, limited stability, and suboptimal potency.

Semi-synthetic modification of natural compounds represents a strategic approach to overcome these limitations by enhancing molecular stability, target selectivity, and pharmacokinetic properties. Semi-synthesized derivatives can be designed to improve interaction with key molecular targets involved in RA pathogenesis, such as cytokine receptors, signaling kinases, and transcription factors. In this context, the present study aims to evaluate the anti-rheumatoid arthritis activity of selected natural semi-synthesized compounds using appropriate experimental models. The study focuses on elucidating their anti-inflammatory, antioxidant, and immunomodulatory mechanisms and comparing their efficacy with standard anti-arthritic drugs, thereby exploring their potential as safer and more effective therapeutic agents for the management of rheumatoid arthritis.

II. MATERIALS AND METHODS

Materials

The natural compounds selected for the present study were obtained from authenticated natural sources and subjected to semi-synthetic modification using standard chemical procedures. All chemicals and reagents used for synthesis and pharmacological evaluation were of analytical grade and procured from reputed suppliers. Complete Freund's Adjuvant (CFA), standard anti-rheumatoid arthritis drug methotrexate and biochemical assay kits were obtained from certified manufacturers.

Experimental animals, male Wistar albino rats (150–200 g), were procured from the institutional animal house. Animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum.

Preparation of Semi-Synthesized Compounds

The selected natural compounds were subjected to semi-synthetic modification using appropriate chemical reactions to enhance their pharmacological properties. The synthesized derivatives were purified by standard techniques such as recrystallization or column chromatography. Structural characterization was carried out using spectroscopic methods including UV, IR, NMR, and mass spectrometry. The purity of the compounds was confirmed prior to biological evaluation.

Acute Toxicity Study

An acute oral toxicity study of the semi-synthesized compounds was conducted according to OECD guideline 423. Animals were observed continuously for the first 4 hours and periodically for 14 days for signs of toxicity, behavioral changes, and mortality. Based on the results, safe doses were selected for the anti-arthritic activity study.

Induction of Rheumatoid Arthritis

Rheumatoid arthritis was induced in rats by a single intradermal injection of 0.1 mL of Complete Freund's Adjuvant (CFA) into the left hind paw. The development of arthritis was confirmed by progressive paw edema and increased arthritic score.

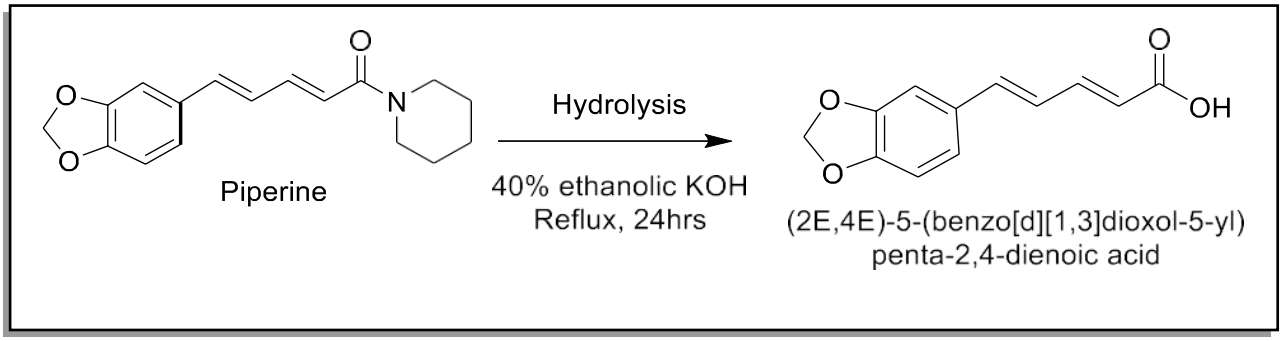
Experimental Design

Animals were randomly divided into the following groups (n = 6):

- Group I: Normal control (vehicle only)
- Group II: Arthritic control (CFA-induced)
- Group III: Standard drug-treated group
- Group IV: Semi-synthesized compound – low dose
- Group V: Semi-synthesized compound – high dose

III. RESULTS AND DISCUSSION

Synthesis methods and steps for pyrroleeste
minutes to ensure thorough dissolution. To get a yellow precipitate of piperic acid a, add dilute hydrochloric acid drop by drop, making up 30% of the solution. To extract yellow powder of piperic acid, the precipitate should be filtered and dried in a hot air oven at 50°C. The molecule 2, which is (2E,4E)-5-(benzo[d][1,3]dioxol- 5-yl) penta-2,4-dienoicacid, is obtained with a high level of purity. Therefore, it can be directly employed in the subsequent procedure without any further purification.



Synthesis of Pipericestermethyl
Take 500mg of piperic acid (compound2) in a round-bottomed flask. Add15mlof methanol and a few drops of concentrated H2SO4 and maintain a temperature 80- 90^{O}cforabout3hours.Verify if the reaction as reached completion by using TLC. Once the reaction is finished, the solvent is left to evaporate naturally, and the resulting product is obtained (2E,4E). Compound 3, also known as -5- (benzo[d] [1,3] dioxol-5-yl) penta-2,4-dienoate, is in the form of a brown powder purity, such as brown powder, is isolated by column chromatography (50% ethyl acetateinhexane) (260mg). The compound is Pyrrole ester, abbreviated as PY and coded as AU-5.

Isolation of clerodane diterpene lactone
The 25kg fresh seeds of *Polyalthia longifolia* var Pendula were dried under shade.
The powder was then soaked in a mixture of 10% ethyl acetate and methanol at room temperature for 7 days. Afterwards, the mixture was filtered to separate the liquid from the solid.
The collected filtrates were concentrated to a syrupy mass under reduced pressure (yield:1.2kg).50gmoffreshseedsextractfrom*Polyalthia longifolia*varPendula were separated into different fractions using a silica gel column chromatography with a mesh size of 60-120. This was done by passing 2.5L of n-hexane, ethyl acetate, and methanol through the column.
From main column we have collected 10% ethyl acetate: n-hexane fraction (50g), which has the maximum clerodane diterpenes level, was run into column chromatographyusingn-hexane, graded with ethylacetate (n-hexane: ethylacetate (100:0,99:1,98:2upto0:100). Fromfraction1,5% ethylacetate: n-hexane (5g) of Lactonec lerodane diterpene was isolated. The isolated compound was subjected.

Two compounds SP- 3 and Pyrrole ester (PY/AU-5) were synthesized from isolated compounds Piperine and Lactone from *Piper nigrum* (black pepper) and *Polyalthia longifolia* (Ashoka) respectively.
The initial analysis of semi-synthetic compounds Pyrroleester (PY/AU-5) and sP- 38 revealed the existence of anti-rheumatoid activity at different levels.
An in vivo investigation was conducted to determine parameters such as arthritis, arthritic index, the volume of paw edema and other inflammatory responses.
The results of an in vivo investigation indicated that, the investigated compounds had a substantial impact on reducing inflammation and preventing joint damage.
The biochemical analysis was evaluated by estimating the serum values like rheumatoid factors and C-creative protein, which yielded beneficial benefits.
An in vivo investigation demonstrated the impact of the semi-synthetic compounds AU-5 and SP-38 on the

degree of inhibition of inflammatory markers including rheumatoid factor and some pro-inflammatory cytokines, resulting in significant reactions.

Additional techniques, such as histology and radiographic X-ray examination, yielded favourable outcomes in the studied groups by providing support to the initial result of anti-rheumatoid activity of the investigated compounds.

Overall, this study has confirmed that the semi-synthesized compounds Pyrrole ester (PY/AU-5) and SP-38 from selected plants effectively reduced joint inflammation and damage in rats with adjuvant arthritis. Experiments in vivo indicated that compounds exhibited an ameliorating effect on the arthritic rats. Pathological analysis and immune histochemical effects also demostrated that it presented an ameliorating effect of Pyrrole ester (PY/AU-5) and SP-38 on subcutaneous edema of the rats plan tar and draw back the expression of TNF-α and IL-6 inflammatory cytokines. The study ascertain that data produced offer mechanistic proof for the anti-arthritic application of the semi-synthesized compounds as candidates for a new therapeutic agent for Rheumatoid arthritis

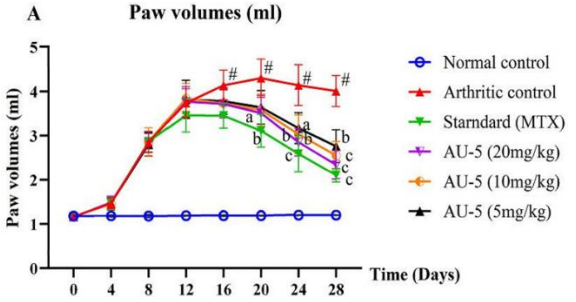


Figure :PY/AU-5 oral administration effects on paw volume. (A) Paw volume from day 0 and (B) % Paw volume changes from day

All values were reported as Mean±Standard Error Mean(SEM), with a sample size of oral administration effects on inflammatory biomarkers Table-

PY/AU-5 oral administration effects on inflammatory markers.

	CRP(mg/dl)	RF (IU/mL)	ESR(mm)
NegativeControl	4.18±0.49	8.84±1.26	3.46±0.27
DiseaseControl	26.01±3.43$^{\#}$	29.18±3.08$^{\#}$	8.71±0.83$^{\#}$
Standard (MTX)	7.68±0.68^{c}	14.18±1.53^{c}	5.09±0.22^{c}
PY/AU-5(20mg/kgbw)	14.84±1.48^{b}	16.84±1.73^{b}	5.76±0.38^{c}
PY/AU-5(10mg/kgbw)	17.34±1.62^{a}	19.51±1.68^{a}	6.33±0.41^{b}
PY/AU-5(5mg/kgbw)	19.51±2.06^{ns}	22.34± 1.26^{ns}	6.69±0.28^{a}

Anti-inflammatory medication was anticipated to regulate inflammation in adjuvant-induced arthritis by reducing the arthritis index, which indicates the intensity of inflammation (31). The disease control group obtained the maximum arthritis index rating on the 28th day (8.18 ± 0.13), which differs remarkably from the drug-administered groups. Conversely, both the standard group and PY/AU-5 administered groups exhibited a significant reduction in the disease score from the 20thto the 28th day, in comparison to a disease control group. The decline was sustained on the 28th day in all PY/AU-5 dosed group.

IV. CONCLUSION

The present study demonstrates that the semi-synthesized compounds pyrrole ester (PY/AU-5) and SP-38, derived from natural sources, possess significant anti-rheumatoid arthritis activity in an adjuvant-induced arthritic rat model. Treatment with these compounds resulted in a marked reduction in paw edema, arthritic index, and overall inflammatory response, indicating their effectiveness in suppressing disease progression.

Biochemical evaluation revealed a significant decrease in serum inflammatory markers, including rheumatoid factor and C-reactive protein, suggesting modulation of systemic inflammation. Furthermore, the observed inhibition of pro-inflammatory cytokines such as TNF-α and IL-6 supports the immunomodulatory potential of the investigated compounds. Histopathological and radiographic analyses further confirmed the protective effects of

PY/AU-5 and SP-38 against joint inflammation, synovial hyperplasia, and tissue damage.

Overall, the findings of this study provide mechanistic and experimental evidence supporting the anti-arthritic potential of the semi-synthesized compounds PY/AU-5 and SP-38. These compounds may serve as promising lead candidates for the development of safer and more effective therapeutic agents for the management of rheumatoid arthritis.

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