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EXPLORING BIOCHEMICAL PATHWAYS IN RHEUMATOID ARTHRITIS: INSIGHTS INTO ANTI-INFLAMMATORY ROLES OF TRACE ELEMENTS AND URSOLIC ACID

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Abstract:
Rheumatoid arthritis (RA) is an autoimmune disease involving complex pathways driven by cytokine dysregulation and oxidative stress. This article investigates the role of trace elements such as copper (Cu), selenium (Se), and zinc (Zn) in the treatment of RA and their biochemical pathways about ursolic acid (UA). Experiments performed in a mouse model of arthritis show that UA when combined with metal complexes, improves therapeutic cytokine protection while inhibiting inflammatory cells such as NFκB, TLR-2, TLR-4, and TNF-α. Molecular interactions in this biochemical process have been shown to have two roles: inhibiting inflammation and promoting tissue repair. Additionally, UA complexes have negligible toxicity and have been suggested for use as alternative therapies or treatments such as nonsteroidal anti-inflammatory drugs. This information provides a way to use natural and therapeutic interventions to target the effects of RA and improve patient outcomes.

Keywords: Rheumatoid arthritis, Biochemical pathways, Anti-inflammatory therapy

Introduction

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease characterized by synovial, joint, and systemic inflammation[1]. The pathophysiology of RA involves the immune system, where proinflammatory cytokines, oxidative stress, and cell signaling pathways interact to perpetuate inflammation and tissue damage. This complexity requires a better understanding of the molecular mechanisms of RA to develop treatment plans[2]. Transcription factors such as IL-6, IL-17), IL- 1β), and nuclear factor kappa B (NFκB) synergize and promote activation. Toll-like receptors (TLR-2 and TLR-4) also play an important role by activating the immune system and controlling inflammatory diseases3. Although current treatments target some of these mediators, their efficacy is limited by adverse effects and incomplete resolution of pain. Therefore, there is significant hope in the search for new drugs that will modulate this pathway. The involvement in RA has attracted attention[4].
UA inhibits NFκB activation, reduces proinflammatory cytokine production, and prevents oxidative stress. More importantly, UA also promotes the production of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13, thus preventing the destruction of pro-inflammatory agents[5]. These two

effects make UA a strong candidate for the treatment of RA. Copper reduces oxidative stress and supports immunity through its role in enzymatic antioxidant defense[6]. Selenium, a component of selenoproteins, reduces inflammatory cytokines and prevents oxidative damage. Zinc stabilizes cell membranes, enhances immunity, and controls the inflammatory response[7]. This article discusses the development of new techniques that enhance immune response while reducing toxicity with UA. Analyzing molecular interactions in cytokine networks, markers of oxidative stress, and immunity, this study provides insight into their potential as therapeutic treatments[8]. Experiments have been conducted in mouse models of FCA-induced arthritis, demonstrating the efficacy of UA in reducing inflammatory mediators such as TNFα, COX-2, and LOX while enhancing protective cytokines. Profiles evaluate this connection to address a critical gap in RA treatment: the development of effective treatments without serious side effects. Discovery of the mechanisms involved in the biochemical processes of UA and RA paves the way for new interventions, provides new hope for patients, and increases our understanding of inflammatory diseases[9-10].

Methodology

The experimental work was conducted from the year 2020 to 2021, at the National Institute of Health (NIH) Islamabad, in collaboration with the Institute of Biomedical and Genetic Engineering (IBGE) Islamabad. The complexes of Ursolic acid with copper, selenium, and zinc were prepared at the Riphah Institute of Pharmaceutical Sciences, Islamabad. Ethical approval Letter No. Appl # Riphah/ERC/18/0294 was received from the Ethical Review Committee Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan. This preclinical study aims to investigate the molecular mechanisms by which UA and metal complexes regulate disease and immunity in rheumatoid arthritis (RA). Freund's complete adjuvant (FCA)- induced arthritis in mice was used as an experimental model. Male Wistar rats weighing 200– 250 g were housed in a controlled environment with access to sample chow and water and were divided as 1. Negative control (NC), 2. FCA group, 3. Cu, 4. Se, 5. Zn, 6. UA, 7. UA+Cu, 8. UA+Se, 9. UA+Zn, 10. LX group. Ethical approval was obtained before the experiment. 10 groups (standard drug), (4) UA alone, (5) UA + C u, (6) UA + Se, (7) UA + Zn, and (8) UA + Cu + Se + Zn. FCA was injected subcutaneously into The hind paw (0.1 mL) to induce arthritis. Treatment started three days after induction and continued for 21 days. The controlled dose of UA was 5 mg/kg body weight, and the doses of Cu, Se, and Zn were 3 mg/kg, 0.5 mg/kg, and 5 mg/kg, respectively, according to the law. Inflammatory and anti-inflammatory markers. Serum and synovial fluid were analyzed for TNF-α, IL-4, IL-10, and IL-13 using ELISA to detect protein expression and RT-PCR to detect mRNA levels. Oxidative stress markers including nitric oxide (NO), COX-2, and LOX were measured along with antioxidants such as superoxide dismutase (SOD) and catalase.TLR2 and TLR4 activation in synovial tissue was assessed using reverse transcriptase PCR, while NFκB activity was assessed by reverse transcriptase PCR, of phosphorylated NFκB. The toxicity of UA trace element complex was evaluated according to OECD guidelines and liver and kidney tests (ALT, AST, creatinine, urea) were performed safely. They were performed in triplicate. Data are expressed as mean ± SD and significance was determined at $p < 0.05$.

Table 1: Effects of UA complexes on interleukins involved in RA

Group	IL-4(pg/mL)	IL-10 (pg/mL)	IL-13 (pg/mL)	TLR-4	NFκB
Normal Control	50 ± 5	60 ± 4	45 ± 3	1.0 ± 0.2	0.8 ± 0.2
FCA Control	15 ± 3	20 ± 3	12 ± 2	4.5 ± 0.4	2.5 ± 0.4
LX	40 ± 4	50 ± 4	35 ± 3	2.0 ± 0.3	1.2 ± 0.3
UA + Cu + Se+ Zn	55 ± 5	65 ± 4	50 ± 3	1.5 ± 0.3	1.0 ± 0.2

Cytokine Modulation: (IL-4, IL-10, IL-13): UA and its combinations significantly elevated anti-inflammatory cytokines IL-4, IL-10, and IL-13, promoting a balanced immune response. These

effects were particularly notable in UA + Cu + Se + Zn-treated groups.

Reduction in TLR-4 and NFκB Activity: UA and its combinations significantly inhibited TLR-4 activity and down-regulated NFκB signaling thereby reducing pro-inflammatory cytokines and promoting tissue repair mechanisms.

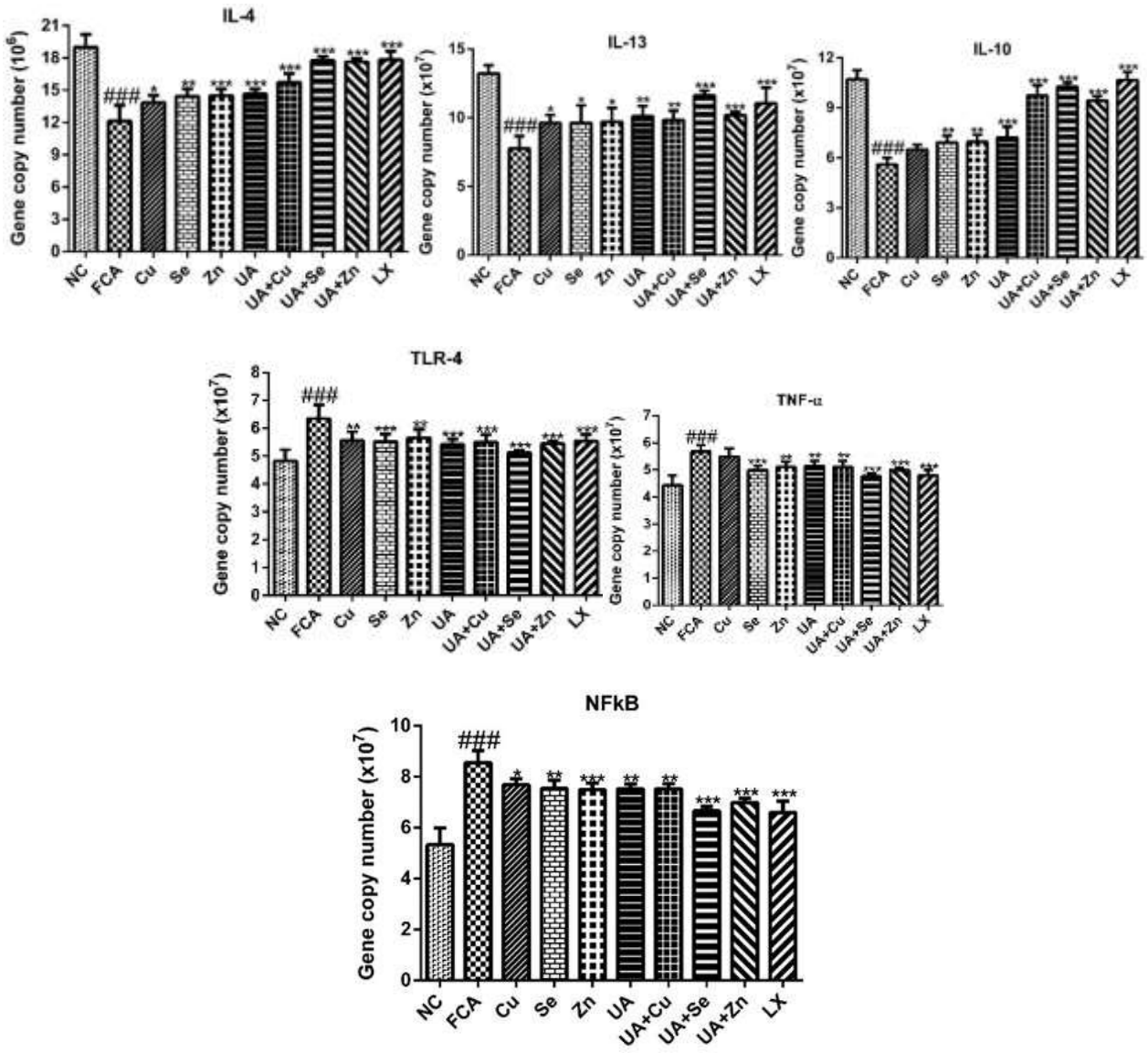

Figure 1: Illustrate mRNA expression levels of cytokines in all groups.

The anti-inflammatory and immune-modulatory effects of UA (ursolic acid) combined with trace elements (Se, Zn, Cu) in rheumatoid arthritis (RA). It demonstrates a significant increase in anti-inflammatory cytokines IL-4, IL-10, and IL-13, with UA + Se showing the most prominent effects on IL-13, highlighting its role in immune regulation and joint tissue repair. Additionally, it reveals a reduction in pro-inflammatory markers, including TNF-α and NFκB activity, with UA combinations outperforming standalone treatments. The figure also depicts suppressed TLR-4 activity, emphasizing how UA combinations inhibit innate immune activation. These findings collectively support the efficacy of UA and trace element combinations in reducing RA-related inflammation and enhancing immune balance.

Discussion

The complex biochemical pathway involved in rheumatoid arthritis (RA) focuses on the immune

system, involving ursolic acid (UA) and the trace elements copper (Cu), selenium (Se),and zinc (Zn)[11]. These recommendations are related to the text, which explores the diverse roles of these compounds in the regulation of oxidative stress and inflammatory cytokines[12]. The importance of molecular targets such as NFκB, TLR-4, and TNF- α provides a solid basis for understanding RA pathogenesis and treatment. Mediators such as NF κB and stimulatory cytokines such as IL-4, IL-10, and IL- 13[13]. These findings are consistent with a proposed mechanism where UA suppresses pro-inflammatory signaling cascades during tissue repair. Monitoring points are considered to be the main improvement of UA performance, a concept widely used in the literature[14].The effect of selenium in reducing oxidative stress from selenoproteins, the enzymatic function of copper, and the stabilizing effect of zinc on the immune system together enhances the synergistic potential of the link. (TLR) signaling, especially TLR-4, is associated with synovial inflammation and these compounds block the TLR-mediated pathway, reduce cytokine production, and prevent joint inflammation in a rat model, the article investigated UA trace elements together in several experimental groups[15-18].
These findings are consistent with the literature suggesting that the effect of these markers by UA is associated with improved biochemical outcomes. Importantly, both data confirmed the efficacy of the UA combination in the development of anti-inflammatory cytokines (e.g. IL-4, IL-10) while inhibiting pro-inflammatory mediators[19].As mentioned in the study, the increase in IL-4 and IL-10 levels in the UA trace element treatment group indicated an improved immune system. The article also investigates other cytokines such as IL-17 and TLR-2, providing a comprehensive view of the interaction between infection and immunity in RA. The safety of the UA method combinations is clarified, emphasizing that their toxicity is negligible compared to conventional treatments. This is based on data that rigorously assesses parameters of nephrotoxicity and hepatotoxicity, and demonstrates the absence of toxicity from these treatments[20]. The article also highlights the limitations of NSAIDs and DMARDs, which, while effective, are often associated with adverse effects and incomplete resolution. UA and related content fill these gaps by providing greater safety with similar or better treatment options. TLR-4 inhibitory signaling provides positive support. The article continues this topic by discussing the ligand-binding properties of UA and the structure-relationship effects that enable the treatment. Such an understanding not only validates the findings of the article but also enhances the scientific basis for the development of UA-based therapy. Another treatment, a result echoed in the paper. Both papers stated that the effects obtained with the UA combination were comparable to the standard lornoxicam used in the study. The article adds depth to these claims by identifying specific biochemical parameters such as reduced NFκB and COX-2 expression levels. The burden and need for alternative therapies, especially in limited areas. It also supports this view by highlighting the prevalence of RA in Pakistan and the limitations in access to advanced biologics. The cultural acceptance of herbal medicines and their associated effects make UA a promising candidate for integration into local health systems. It also highlights the need for public health measures to increase awareness of alternative therapies, a point briefly discussed in the paper. It emphasizes the need for human trials to ensure safety and efficacy. The article provides further insight into optimizing drug use and exploring new designs, such as nano capsules, to increase bioavailability. Challenges such as regulatory approval and patient compliance are cited as important areas for future research. Research. By recognizing the collective potential of these connections, the foundation for new treatment strategies is laid. Integrating molecular, histological, and safety data to create a narrative that meets the article's goals of improving efficacy, effectiveness, and safety for RA management. Together, these findings highlight the importance of innate factors and diseases as key areas of future treatment, paving the way for changes in the treatment of autoimmune diseases.

Conclusion

This finding extends the foundational research presented in the area under discussion by offering detailed insights into the biochemical pathways modulated by UA and trace elements in RA. By validating the synergistic potential of these combinations, it lays the groundwork for innovative therapeutic strategies. The integration of molecular and safety data creates a compelling narrative that

aligns with the overarching goal of developing effective, accessible, and safe alternatives for RA management. Together, these findings pave the way for a paradigm shift in the treatment of autoimmune diseases, emphasizing natural compounds and trace elements as pivotal components of future therapies.

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