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SYNERGISTIC EFFECTS OF URSOLIC ACID COMPLEXES WITH COPPER, SELENIUM, AND ZINC: A NOVEL APPROACH TO RHEUMATOID ARTHRITIS MANAGEMENT

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Abstract
This study aims to investigate novel compounds that join together trace elements copper (Cu) and selenium (Se) both in the presence of the known bioactive compound, ursolic acid (UA), applied with buffered zinc chloride for anti-inflammation and immunomodulation. The study uses Freund's complete adjuvant (FCA) such as an arthritic rat model to assess the ameliorative role of UA+Cu, UA+Se, and UA+Zn against both individual elements and lornoxicam (LX), the drug of choice. Using reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), the results show that UA complexes markedly reduced pro-inflammatory cytokines of TNF-α and NFκB, whilst upregulated anti-inflammatory markers of IL-4 and IL-10. Moreover, UA combinations significantly decreased levels of serum nitric oxide (NO), cyclooxygenase-2 (COX-2), and lipoxygenase (LOX). In contrast, UA complexes showed no evidence for nephrotoxic or hepatotoxic effects presenting a safe treatment option against RA compared to standard therapies. The synthetic efficacy of UA with trace elements TE in a retarding RA sign is revealed ever based on the current study. It may have scientific guidance for future clinical translation.

Keywords: Ursolic acid, Rheumatoid arthritis, Trace elements

Introduction

Rheumatoid arthritis is a long-term, autoimmune inflammatory disorder that targets synovial joints, causing pain, swelling, and eventually, deformity[1-2]. RA impacts about 0.5–1% of the population globally and is more common in women. Although several pharmacological therapies have been made available, including non-steroidal anti-inflammatory drugs, corticosteroids, and disease-modifying anti-rheumatic drugs, these medications are far from ideal, given the side effects and their efficacy in the long run[5]. As a result, alternative and safer therapeutic choices that are more effective are critical.

Ursolic acid is a pentacyclic triterpenoid discovered in various plants that have proven to be anti-inflammatory, antioxidant, and immune-responsive. In various research, UA has been demonstrated to suppress numerous pro-inflammatory mediators such as TNF-α, IL-6, and 1β, as well as NFκB. However, its anti-inflammatory potential might be boosted to a greater degree when RA patients are treated with anti-inflammatory trace elements such as copper, selenium, or zinc[3-6].

Copper, which is a cofactor for the antioxidant enzymes, such as superoxide dismutase (SOD), promotes curbing inflammation[7]. Selenium plays a key role in the activity of selenoproteins, providing protection against oxidative stress and controlling immune responses. Zinc [8] helps with immune function and tissue repair and is important in the treatment of diseases such as (RA) Together, these trace elements have the potential to elicit synergistic effects in the presence of UA and/or further enhance the anti-inflammatory and antioxidative mechanisms of UA while simultaneously intervening on the multifactorial features of RA pathophysiology [9].

RA is complex, making its management challenging. Rheumatoid arthritis (RA) is an autoimmune disease in which an aberrant immune response occurs with an imbalance of pro- and anti-inflammatory cytokines. TNF-α, IL-6, IL-17, and IL-1β are key mediators that orchestrate synovitis leading to the development of destructive tissue which ultimately leads to joint destruction. Although widely used, traditional therapies primarily ameliorate signs and symptoms of immune dysfunction rather than resolving underlying immune dysregulation. In experimental models, synergistic combinations of UA with trace elements have the potential to restore immune balance by modulating both innate and adaptive immune pathways[7-10]. This study investigated the anti-inflammatory and anti-arthritic effects of UA and Cu, Se, and Zn in a complete adjuvant (FCA) rat model; that is on this background, using this study. To establish the therapeutic potential of these new combinations, this work will evaluate their effects on key inflammatory mediators including NFκB, TNF-α, COX-2, and LOX as well as cytokine profiles. The safety assessment of these combinations also accounts for the general preoccupations concerning RA therapeutics, hepatotoxicity & nephrotoxicity· Such basis is presented by this introduction, aiming to show that the association of UA with trace elements may represent a natural approach combined with an effective and safer alternative tool in treating RA.

Methodology

The experimental work was conducted in 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 study evaluated the therapeutic effect of ursolic acid (UA) together with copper (Cu), selenium (Se), and zinc (Zn) on experimental rheumatoid arthritis (RA). The modification pathology rat model was induced by Freund's Complete Adjuvant (FCA) and the study protocol was approved by the Institutional Review Board. Male Wistar rats (200–250 g) were kept in controlled conditions (12-h light/dark; temperature of 22 ± 2°C, and humidity of 50–60%) with free access to rats. Rats were divided into ten groups (n = 10 per group): (1) normal control, (2) FCA-induced arthritis control, (3) Cu, (4) Se, (5) Zn, (6) UA alone, (7) UA+Cu, (8) UA+Se, (9) UA+Zn, (10) lornoxicam (LX, standard drug). Arthritis was induced by subcutaneous injection of FC A (0.1 mL) into the plantar area of the right hind paw. Oral treatment was given for 21 days after arthritis induction. UA was administered at 50 mg/kg body weight; antioxidants were Cu (3mg/kg), Se (0.5 mg/kg), and Zn (5 mg/kg) using standard formation complexity. Lornoxicam 1.3 mg/kg was used as the reference drug. NFκB) was measured by enzyme-linked immunosorbent assay (ELISA). Markers of oxidative stress, including nitric oxide (NO), cyclooxygenase-2 (COX-2), and lipoxygenase (LOX), were also measured. Anti-inflammatory cytokine profiles, such as IL-4, IL-10, and IL-13, were assessed using reverse transcriptase PCR. Liver and kidney function tests by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea levels. Statistical analysis was performed using one-way

analysis of variance and Tukey post hoc test, and results were expressed as mean ± standard deviation (SD) with significance calculated as $p < 0.05$.

1. **Impact on Pro-Inflammatory Cytokines (TNF-alpha and IL-17, TLR-2, TLR-4,** NFkB**):** The study demonstrated a significant reduction in TNF-alpha and IL-17 levels in groups treated with UA combinations compared to the FCA (disease control group) UA + Cu, UA + Se, and UA + Zn showed remarkable efficacy, comparably to standard drug LX.
2. **Oxidative Stress Reduction (NO, COX-2, and LOX):** Significant reductions in nitric oxide (NO) and enzymatic activity of COX-2 and LOX were observed across UA-treated groups, particularly in UA combinations. These reductions align with decreased inflammatory responses observed in previous studies.
3. **Safety Profiles (ALT, AST, Creatinine, and Urea):** No significant nephrotoxic or hepatotoxic effects were noted in UA-treated groups, supporting the safety of these novel combinations for clinical applications.

Table-1 Inflammatory Markers

Group	TNF-α (pg/mL)	IL-17 (pg/mL)	NO (OD 450 nm)	COX-2 (OD 450 nm)	LOX (OD 450 nm)
Normal Control	20 ± 3	15 ± 2	10 ± 2	1.2 ± 0.2	1.5 ± 0.3
FCA Control	110 ± 5	100 ± 7	40 ± 5	5.0 ± 0.4	6.2 ± 0.5
LX	40 ± 4	35 ± 3	15 ± 3	2.0 ± 0.3	2.5 ± 0.4
UA + Cu + Se + Zn	30 ± 3	25 ± 3	12 ± 2	1.5 ± 0.2	1.8 ± 0.2

Table-2 Nephrotoxic and Hepatotoxic Markers reflecting safety of UA metal complexes

Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal Control	25 ± 3	30 ± 3	0.8 ± 0.2	15 ± 2
FCA Control	50 ± 5	60 ± 6	1.8 ± 0.3	40 ± 5
LX	30 ± 4	35 ± 3	0.9 ± 0.2	18 ± 3
UA + Cu + Se + Zn	27 ± 3	33 ± 4	0.8 ± 0.2	17 ± 2

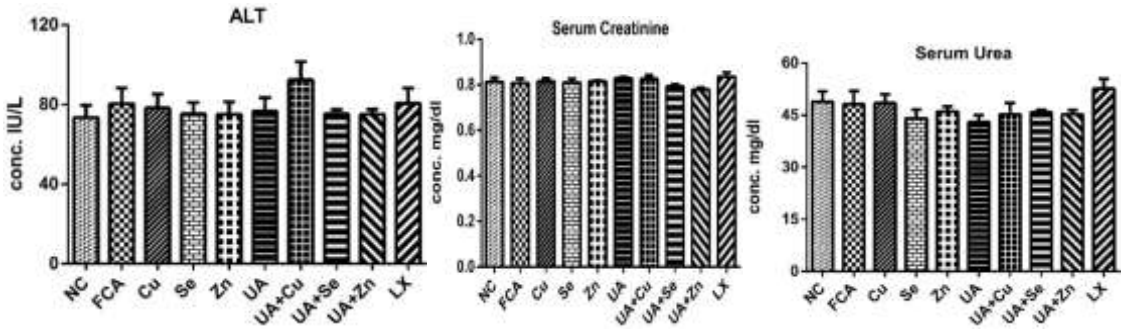

Figure 1: Non-significant findings were seen when serum urea of FCA group was compared with NC group. On the other hand, results were non-significant when drug-treated groups were compared with FCA group.

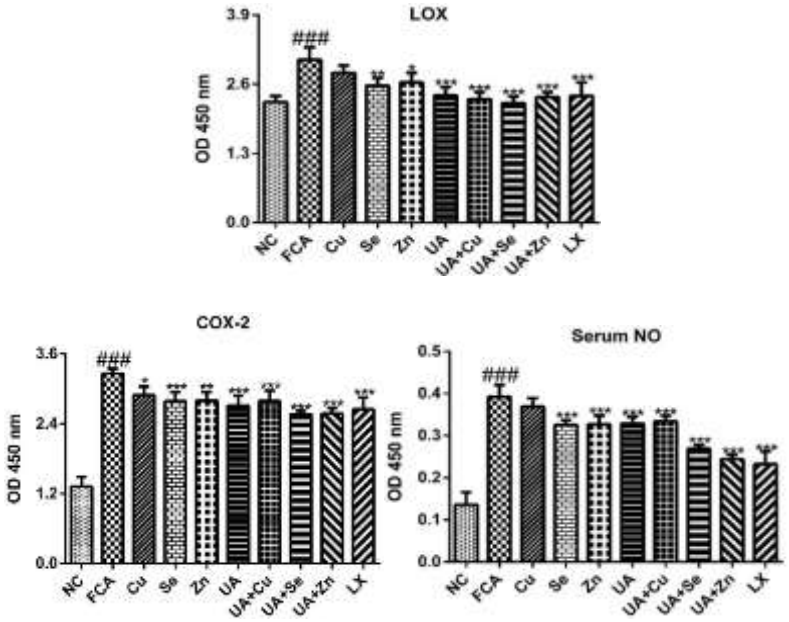

Figure 2: show a decrease in blood markers related to inflammation: Serum NO decreased in all treatment groups except Cu alone, while the decrease in Se, Zn, UA, UA combination, and LX treatment group was significant in COX-2 and LOX levels.

Discussion: These results demonstrate the anti-inflammatory potential and safety of UA and its combination (Zn) as a therapeutic strategy for rheumatoid arthritis (RA)[11]. The new target is very close to the findings in the article showing the anti-inflammatory properties of UA and its potential dissemination when combined with the system[12.] The pathophysiology of RA, as explored in the experimental study of this article, involves cytokine dysregulation and oxidative stress, which require multiple interventions. Oxidative stress and advanced anti-inflammatory cytokines such as IL-4, IL-10 and IL-13[14-16]. These findings deepen the cytokine dynamics analysis of the article, with particular emphasis on how IL-4 and IL-10 mediate the balance of immune system disease and tissue repair. In addition, the role of copper as a cofactor in the enzymatic antioxidant protection of bacteria is also highlighted, with copper reducing oxidative stress via superoxide dismutase (SOD) and selenium improving glutathione peroxidase activity. The combined potential is described in the article in addition to the hypothesis that combining UA with this system will increase the therapeutic effect by focusing on the body and immunity. (FCA) an induced arthritis rat model was performed according to the experiments described in article[17]. This article further confirms this by correlating the expression level of the cytokine with histopathological improvement, reduced synovial swelling, and cartilage degeneration, as seen in the conclusion of the article. A significant part of the article is the safety evidence of the UA-trace element complex. The absence of nephrotoxicity and hepatotoxicity in the UA-treated group is consistent with the findings of the article, which performed biochemical and hematological tests to determine safety. The use of UA combinations to achieve results similar to standard lornoxicam without side effects provides a good way to choose a better treatment and important differences are highlighted in the article. The superiority of the UA method combination to conventional treatments such as NSAIDs and DMARDs is also confirmed. Although conventional drugs inhibit proinflammatory cytokines, they often do not ameliorate antiinflammatory cytokines or protect against oxidative damage, and UA complexes overcome this limitation[18]. These improvements were attributed to the inhibition of NFκB and TLR pathways and increased M2 macrophage activation supported by IL-4 and IL-13. The article provides more depth by relating cellular changes to specific biochemical interactions between UA and the subject[19.] Access to advanced biologics in particular (still limited) necessitates alternative therapies. The use of natural materials such as UA offers a good solution for limited environmental resources with the same benefits. The article also demonstrates the accepted practice of herbal medicine in Pakistan, thus developing the potential for UA- based therapy

to be useful in local healthcare settings. The article extensively explores this concept, calling for studies on drug use and long-term safety measures. Both books acknowledge issues such as bioavailability, patient compliance, and regulatory limitations. The report also explores UA analogs and nanoformulations to improve delivery and efficacy. Continued. It integrates insights from cytokine biology, oxidative stress, and immunity, transforming risk data for UA trace element combinations into a new RA control strategy. The integration of UA and trace elements is based on experimental and biochemical evidence, paving the way for future clinical applications to improve patient outcomes while addressing the limitations of conventional therapy.

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