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Harnessing Ursolic Acid Metal Complexes: Boosting Anti-Inflammatory And Immunomodulatory Effects In An Arthritic Rat Model

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Abstract:
The anti-inflammatory activities of ursolic acid (UA) a natural triterpene, copper (Cu), selenium (Se), and zinc (Zn) is well documented. This study focuses on the anti-arthritic synergistic effect of novel compounds encompassing UA+Cu, UA+Se, and UA+Zn, on Freund's complete adjuvant (FCA) arthritic rat model. Lornoxicam (LX) was used as a standard drug for comparative effects. Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to evaluate mRNA expression and enzyme-linked immunosorbent assay (ELISA), was used to determine protein levels of LOX and COX-2. Furthermore, in vitro proliferation of Concanavalin A (ConA)-stimulated splenocyte was quantified using an ELISA reader. Acute toxicity of the UA+Cu, UA+Se, and UA+Zn complexes was assessed. Combination of UA with Cu, Se, Zn resulted in significantly decreased expression of NFκB, TNF-α, TLR2, and TLR4, on the other hand IL-4, IL-10, and IL-13 expression was significantly increased. Additionally, UA complexes significantly reduced serum C-reactive protein (CRP), serum nitric oxide (NO), serum COX-2, and 5-LOX. UA+Cu, UA+Se, and UA+Zn suppressed ConA-specific splenocyte proliferation. UA+Cu, UA+Se, UA+Zn showed no hepatotoxic and nephrotoxic potential. Overall, results reflected that novel compound, UA+Cu, UA+Se, and UA+Zn have synergistic effect as compared to Cu, Se, and Zn alone, and their results were comparable with standard drug LX.

Keywords: Immunomodulatory; Arthritic model; Ursolic acid (UA); Copper (Cu); Selenium (Se); Zinc (Zn); Lornoxicam (LX)

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder that is driven by T-helper type 1 (TH-1) cells, which trigger an abnormal immune reaction resulting in both joint damage and systemic manifestations. RA involves multiple joints on both sides. It is distinguished by tendons inflammation leading to bone erosion, cartilage destruction and disability. Progressively multiple joints get involved with inclusion of likeable extraarticular symptomology (Lin, Anzaghe, & Schülke, 2020).

Although the contemporary management of RA is improving yet discovery of new drugs with better efficacy and less toxic profile has been an open challenge to the field of medicine. At present, drugs used in RA encompasses non-narcotic analgesics, glucocorticoids (GCs), disease-modifying antirheumatic drugs (DMARDs) and moreover the biological agents (Zhang et al., 2019).

The important anti-inflammatory cytokines IL-4 and IL-13 have well established role in musculoskeletal lesions. Findings from animal and ex vivo models of arthritis indicate that the anti-inflammatory properties of IL-4/IL-13 may be advantageous in the treatment of inflammatory arthritis. The activation of the IL-4 and IL-13 signaling process could represent a novel and effective approach for the therapy of inflammatory arthritis (Iwaszko, Biały, & Bogunia-Kubik, 2021).

Ursolic acid (UA) exhibits wide range of actions being effect in hepatic toxicity, inflammatory conditions, oxidative stress, glycemic control, neoplasia, dementia, immunomodulation (Sun et al., 2020). Selenium (Se) trace element is an crucial part of glutathione peroxidase (GPx), it enjoys vital role in alleviating inflammation and oxidative stress through inhibition of NF-ϰB mediated pathophysiology (Turrubiates-Hernández, Márquez-Sandoval, González-Estevez, Reyes-Castillo, & Muñoz-Valle, 2020).

Diverse trace elements, zinc (Zn), (Se) copper (Cu) are necessary for health and have an immunomodulatory role, involving both innate and adaptive immune response regulatory processes. Cu and Zn essentially linked to multiple enzymatic reactions, DNA antioxidant defense and repair, and proliferative cell processes (W. Yang et al., 2023).

One of the most integrated areas of science is the application of research about metal complexes in medicine, combination of data concerning configuration, metal complexes characteristics, plus essential processes of body regulation. The development

of novel drugs based on metal complexes is a remarkable benefaction in developing chemotherapy methods with more intrinsic activity. Novel metals are gaining great fame in clinical medicine (Demkowicz, Rachon, Daśko, & Kozak, 2016). Cu has been known for potentiating the compounds active principles clinical effectiveness, subsequently building a rationale to explore Cu-based drug complexes with enhanced improved intrinsic activity and less toxic profile (Puranik et al., 2016).
In the present experimental work, anti-arthritic effects of Cu, Se, and Zn complexes of UA were evaluated in FCA-induced arthritic rat model to explore their anti-inflammatory and immunomodulatory effects.

2. Materials and Methods

2.1. Study Settings

The investigation was carried out at Islamabad Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. The animals were acquired and housed at the National Institute of Health (NIH) in Islamabad. The synthesis of Cu, Se, and Zn complexes of UA took place at the Riphah Institute of Pharmaceutical Sciences (RIPS) at Islamabad. The research endeavors undertaken within this investigation received the ethical approval Appl # Riphah/ERC/18/0294, dated 31st July 2018 from the Ethical Review Committee (ERC) Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan.

2.2. Experimental Animals Preparation

Adult rats belonging to the Sprague Dawley strain, weighing between 0.3-0.35 kg, were procured from the NIH Animal House situated in Islamabad. The controlled laboratory setting was upheld at a temperature ranging from 20-25°C, accompanied by a relative humidity level of 50 ± 5%.
A consistent light-dark cycle of 12 hours duration was meticulously maintained for the rats throughout the study. The rats were nourished with standard rodent chow, formulated at the NIH facility, and had access to tap water via 200ml inverted bottles. The rodent chow utilized in the research was procured and sanctioned by the NIH establishment in Islamabad. Prior to commencing the experiment, a period of one week was dedicated to acclimatizing the rats, ensuring their behavioral and growth stability.

2.3. Drugs and Dosage

Cu 3 mg/kg i.p. (Puranik et al., 2016), Se 0.5 mg/kg i.p. (Sengul, Gelen, Yildirim, Tekin, & Dag, 2021), , UA 5 mg/kg i.p. (S. Dai et al., 2021), Zn 5 mg/kg i.p. (Skrajnowska & Bobrowska-Korczak, 2019), UA+Cu 5 mg/kg, UA+Se 5 mg/kg, UA+Zn 5 mg/kg, LX 1.3 mg/kg i.p. (Helmy et al., 2017).
2.3.1. Preparation of UA+Cu, UA+Se, UA+Zn
UA+Cu, UA+Se, UA+Zn complexes were prepared at Pharmaceutical department of Riphah at Islamabad, following protocol mentioned (Batool, Shahid, & Muddasir, 2015).

2.4. Arthritic induction

Arthritis was initiated on day 0 through the administration of Freund's complete adjuvant (FCA) containing 0.5 mg of Mycobacterium butyricum suspended in 0.1 ml of paraffin oil into the subplantar region of the left hind paw of each subject under light anesthesia induced by a combination of ketamine/xylazine (80:10 mg/kg, ip). On the 15th day, the animals were randomly allocated into ten experimental groups, each consisting of 6 rats. The first group (Group I) received an injection of paraffin oil (0.1 ml) in the left hind paw; the second group (Group II) received 0.1 ml of adjuvant (FCA) in the left hind paw; Groups III, IV, V, VI, VII, VIII, and IX were designated as test treatment groups and were administered Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, intraperitoneally. Group X served as the reference treatment group and received LX. The animals received daily doses of vehicle and treatment for fourteen consecutive days, commencing on day 15 and continuing until day 28 post-injection (Kumar, Dhaliwal, Dhaliwal, Dharavath, & Chopra, 2020).

2.5. Collection of Blood Sample

On day 29, light ether vapors anesthesia was given to all animals followed by aspiration of blood through cardiac puncture, later they were sacrificed. Blood samples collection was done in EDTA vacutainers for blood parameters analysis. For serum separation, the collection of samples was also taken in gel containing vacutainers.

2.6. Blood inflammatory cells

For blood TLC automated hemocytometer (Sysmex XT-1800i) was employed.
2.7. Proinflammatory and anti-inflammatory cytokines mRNA expression detection
The isolation of total RNA from lung tissue was conducted using a total RNA isolation kit from Favorgen in Ping-Tung, Taiwan. The assessment of RNA purity and yield was performed using a Nanodrop 2000C from Thermo Scientific in the United States. The RevertAid First Strand cDNA Synthesis kit from Thermo Scientific in the USA was utilized for cDNA synthesis from the RNA. Real-time PCR was employed for the absolute quantification of proinflammatory cytokines. (Table 1). The prescribed protocol provided by the manufacturer for thermal cycling with SYBR green was adhered to during the PCR reaction. This procedure was conducted utilizing the ABI Prism 7000 sequence detection system manufactured by Applied Biosystems in the United States.

2.8. ELISA LOX and COX-2

The levels of serum LOX and COX-2 were measured following the manufacturer's protocol for the Elabscience ELISA kit. The samples were added to pre-coated wells of a 96-well microtiter plate, followed by the addition of COX-2 and 5-LOX antibodies and streptavidin-horseradish peroxidase. Subsequently, the chromogen solution was added sequentially. An incubation period of 10 minutes was maintained at 37 degrees Celsius in the absence of light. The optical density was determined at a wavelength of 450 nm after introducing a stop solution.

2.9. C-reactive protein detection

C-reactive protein (CRP) levels were assessed using the agglutination technique provided by a commercial kit and following the manufacturer's guidelines (Antec diagnostic products-UK). The evaluation is based on the immunological interaction between serum CRP and CRP antisera attached to inactive latex particles. Increased levels of CRP in the serum sample resulted in observable agglutination due to the reaction with the antisera. The determination of CRP levels followed a semiquantitative approach as per the specified protocol.

2.10. FCA-linked splenocyte proliferation

The spleens underwent a thorough rinsing process with a solution composed of Hank's balanced salt and antibiotics. Subsequently, the tissue was finely crushed within a 5ml solution of Hank's balanced salt and passed through a 45µm nylon membrane syringe filter. An equal volume of ammonium chloride solution was utilized for the lysis of red blood cells. Following centrifugation at 1000 rpm for 10 minutes, the resulting cell mixture was plated in a 96-well flat-bottom culture plate with culture media containing RPMI-1640 enriched with 10% heat-inactivated fetal bovine serum and 2% antibiotics. Stimulation of cell proliferation was achieved by adding 10 µg of ConA to the wells with the cells and culture media. The plate was then placed in a water-jacketed incubator set at 37°C with 5% CO2 for 72 hours. Splenocyte proliferation was assessed using a BrdU cell proliferation assay kit, with absorbance readings taken at 450 nm using an ELISA plate reader.

2.11. Nitric oxide analysis

Measurement of serum nitric oxide (NO) was carried out utilizing the colorimetric assay kit (BioVision Incorporated USA), with the absorbance being assessed at a wavelength of 540 nm. Serum NO was detected using the Griess method.

2.12. Hematological and biochemical analysis

To evaluate the deleterious effects of the metal complexes in combination with UA, the levels of creatinine, urea, Hb, and hepatic enzymes (ALT, ALP) were measured using commercially available kits (Analyticon Biotechnologies AG, Germany) (Humalyzer 3500). The Sysmex XT-1800i was used to measure platelet and RBC counts.

2.13. Statistical Analysis

GraphPad version 6 was applied for data analysis. A p-value of less than or equal to 0.05 was deemed statistically significant. One-way ANOVA was applied to analyze the quantitative variables and see how each group differed from the others. Post hoc Tukey's test and t-test were subsequently employed for additional data examination when deemed suitable.

3. Results

3.1.1. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on WBC count

The findings showed that the FCA group WBC count was significantly elevated as compared with the NC group. While when compared with FCA group, the WBC count was significantly decreased after treatment with Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX (Figure. 1a).

3.1.2. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on serum CRP

The findings showed that the FCA group serum CRP was significantly elevated. In comparison to FCA group, the serum CRP was significantly decreased after treatment with Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX (Figure. 1b).

3.1.3. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on serum NO

The findings showed that the FCA group serum NO was significantly elevated as compared with the NC group. While when compared with FCA group, the serum NO was significantly decreased after the administration of Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX except Cu alone (Figure. 1c).

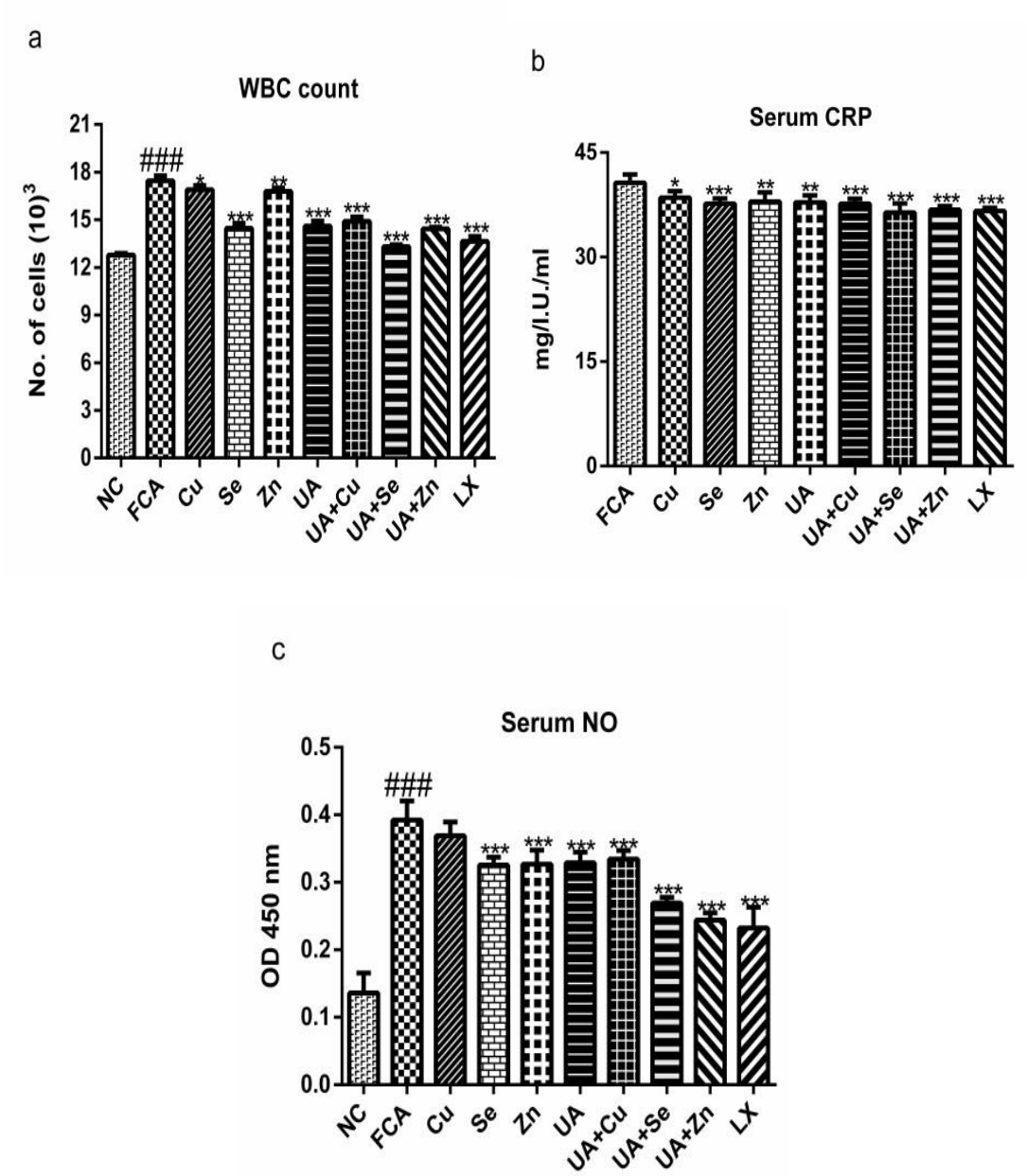


Figure. 1. (a) Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly decreased blood TLC. **(b)** Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly decreased serum CRP. **(c)** Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly decreased serum NO except Cu alone. Findings are represented as Mean ± SD. *, **, *** denotes P <0.05, P <0.01, P <0.001 respectively when drug-treated groups compared to FCA group while ### denotes P <0.001 when FCA group compared to NC group.

3.1.4. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on serum LOX and COX-2

The findings showed that in comparison to the NC group, the FCA group exhibited significantly raised COX-2. While comparison to the FCA group, there was reduction of COX-2 significantly with the administration of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX. (Figure. 2a). The findings showed that in comparison to the NC group, the FCA group exhibited significantly raised LOX. While in comparison to FCA group, there was reduction of LOX significantly due to the administration of Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX, Cu alone effect was found to be insignificant. (Figure. 2b).

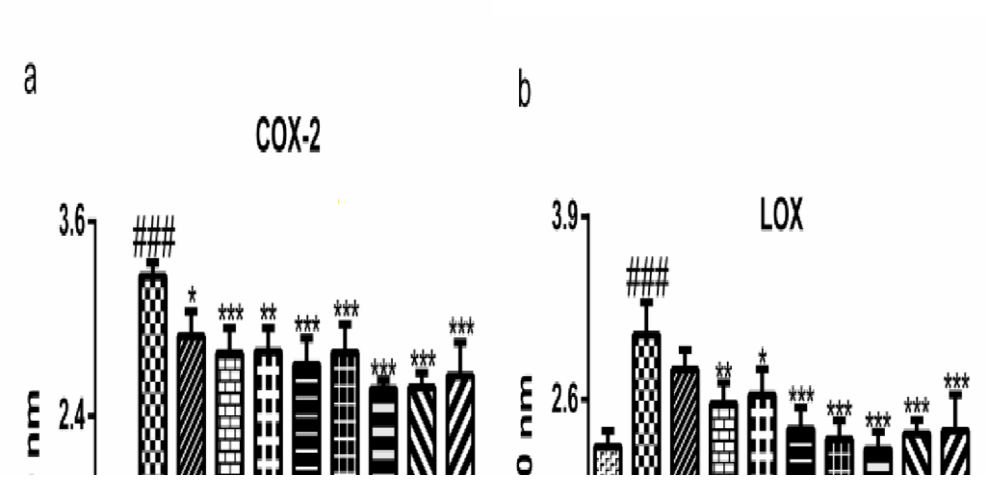
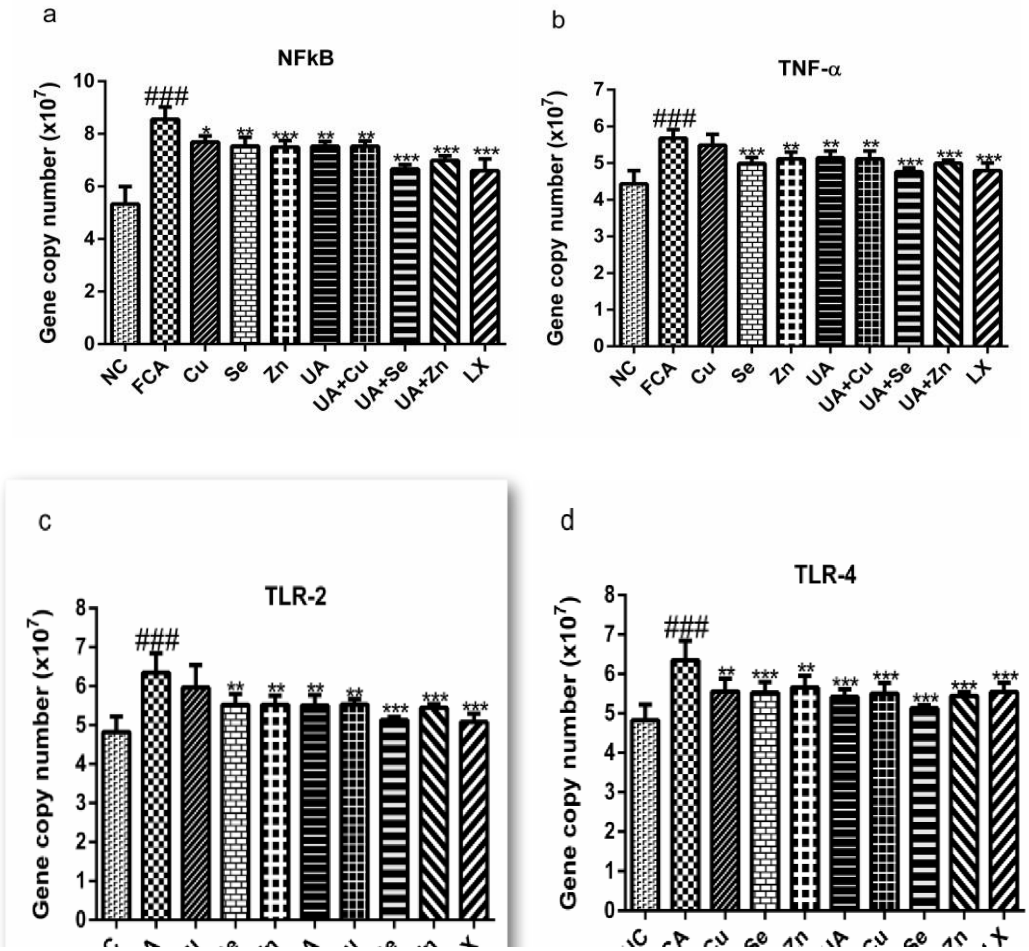


Figure. 2. (a) Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly reduced serum COX2. (b) Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly reduced serum LOX except Cu alone. Findings are represented as Mean ± SD. *, **, *** denotes P <0.05, P <0.01, P <0.001 respectively when drug-treated groups compared to FCA group while ### denotes P <0.001 when FCA group compared to NC group.

3.1.5. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on pro and anti-inflammatory cytokines

Our findings showed significant increase in the gene expression of pro-inflammatory cytokines NFκB, TNF-α, TLR-2, TLR-4, IL-17 and significant decreased in the gene expression anti-inflammatory genes IL-4, IL-10, and IL-13 in FCA group in comparison NC group. Results indicate a significant decrease in expression of anti-inflammatory cytokines IL-4, IL-10, and IL-13 of FCA group as compared to NC group respectively. Treatment with Cu, Se, Zn, UA+Cu, UA+Se, UA+Zn and LX significant increased expression of IL-4, IL-10, and IL-13 on the other hand significant decreased expression of NFκB, TNF-α, TLR-2, TLR-4, IL-17 as compared to FCA group. Cu alone significantly decreased expression of NFκB, TLR-4 whereas results were insignificant in case of TNF-α, TLR-2 and IL-17 (Figure. 3a-h).



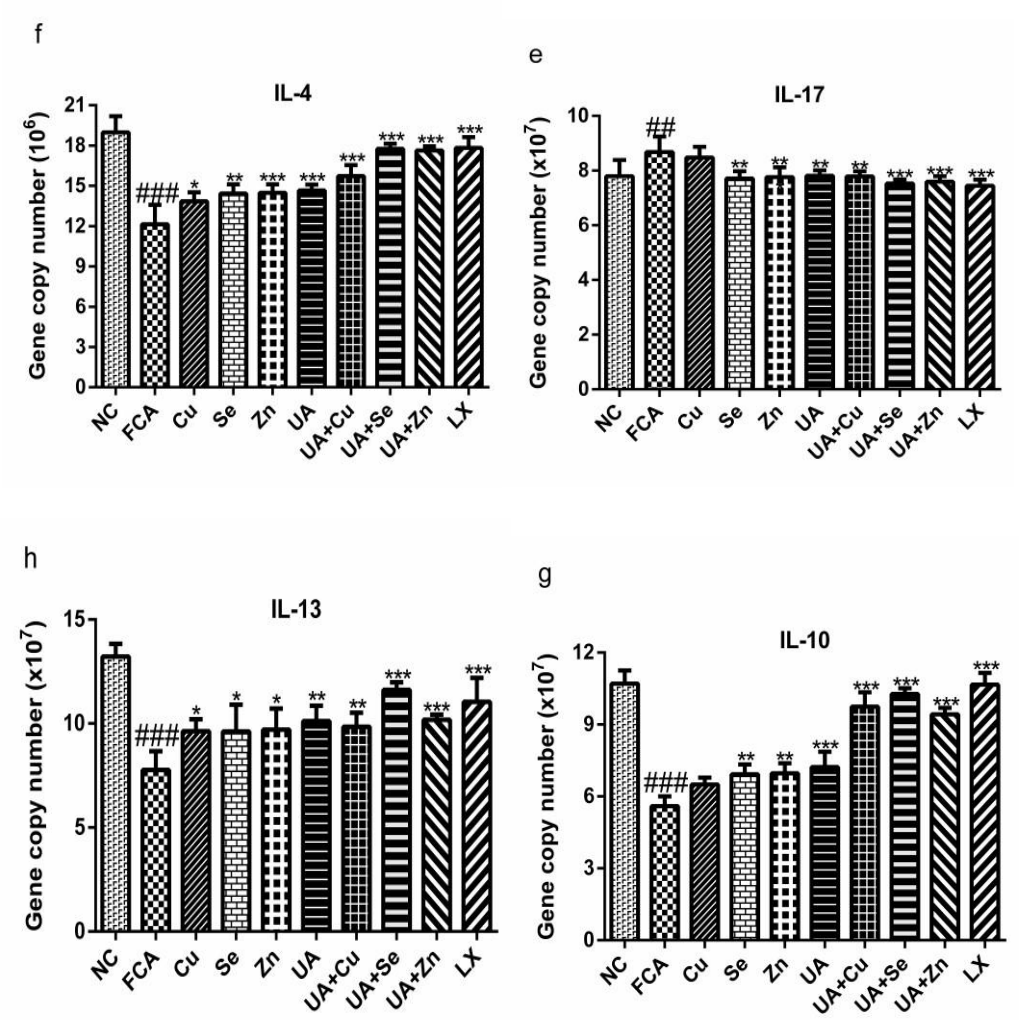
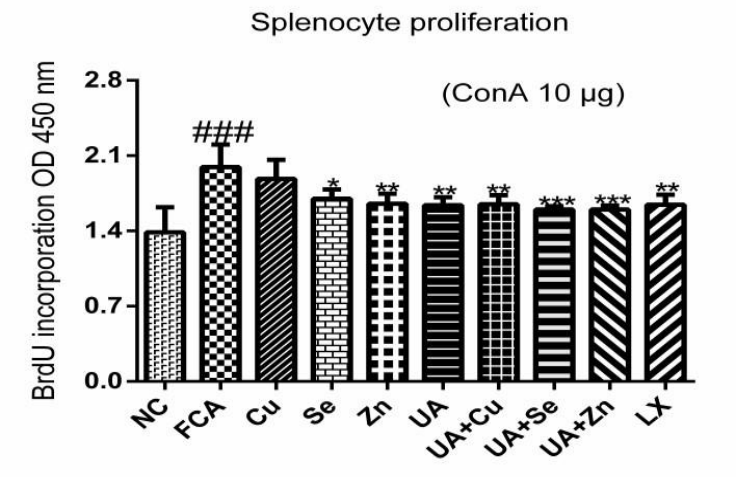


Figure. 3. Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, UA+Zn, LX significantly reduced expression of (a) NFκB, (b) TNF-α, (c) TLR-2, (d) TLR-4, (e) IL-17, (f) IL-4, (g) IL-10, and (h) IL-13. With exception to IL-17, TNF-α, TLR-2, IL-10, Cu alone significantly reduced NFκB, TLR-4, IL-4, and IL-13. Results are indicated as Mean ± SD. *, **, *** indicates $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drugtreated groups compared to FCA group and ##, ### denotes $P < 0.01$, $P < 0.001$ respectively when FCA group compared to NC group.

3.1.6. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on Splenocyte proliferation

Our results demonstrate FCA group exhibited a significant enhanced BrdU uptake as compared to NC group. Furthermore, we observed a significant decrease in BrdU uptake following treatment with Se, Zn, UA, UA+Cu, UA+Se, UA+Zn, and LX compared to the FCA group. Cu alone displayed insignificant findings **(Figure. 4)**



3.1.7. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on hematological parameters

RBC count, platelet count and Hb findings were unremarkable in relevance to the effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX (Figure. 5 a-c).

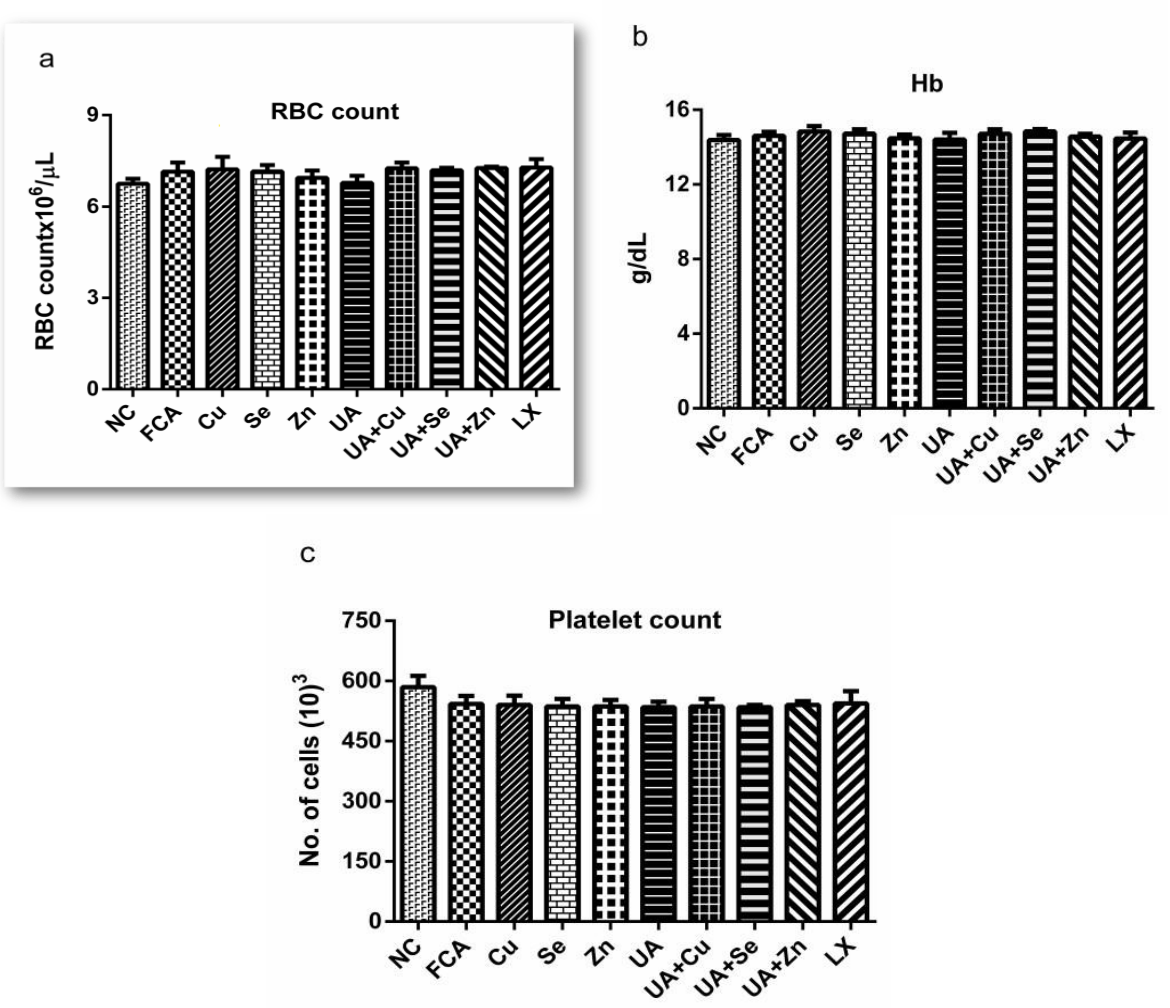
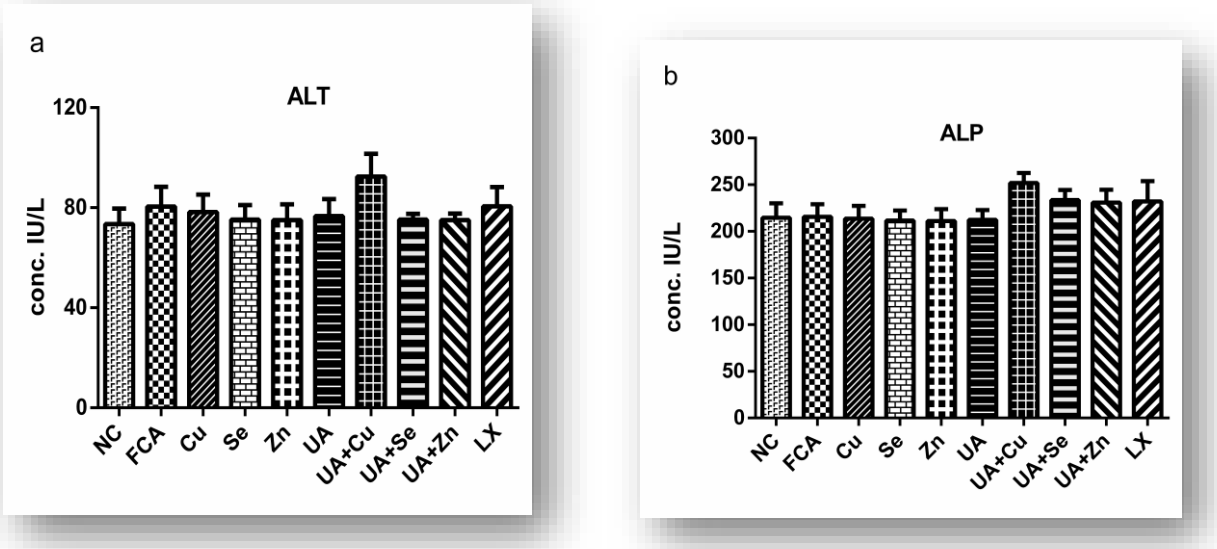


Figure. 5. Non-significant difference was observed in all groups when drug-treated groups compared to NC group for the determination of **(a)** RBC count, **(b)** Hb and **(c)** Platelet count respectively.

3.1.9. Effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn on renal and hepatic parameters

Cu, Se, and Zn complexes of UA did not exhibit hepatotoxic as well as nephrotoxic properties (Figure. 6a-d).



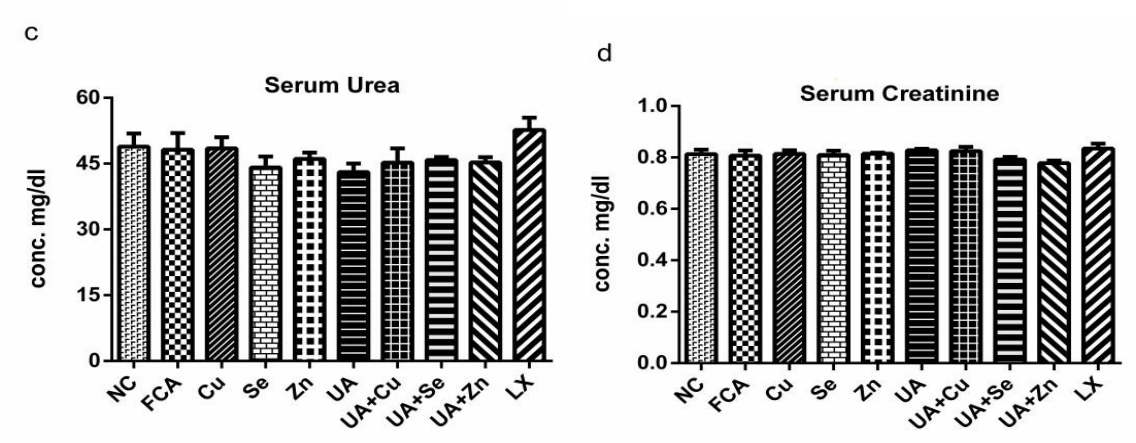


Figure. 6. Non-significant difference was observed in all groups when drug-treated groups compared to NC group for the determination of serum **(a)** ALT, **(b)** ALP, **(c)** urea, and **(d)** creatinine.

3.1.10. Test for acute toxicity of new metal complexes

The acute toxicity assessment of newly developed drug complexes was conducted following the guidelines set forth by the Organization for Economic Co-operation and Development (OECD). A total of nine female rats were assigned to three groups (UA+Cu, UA+Se, and UA+Zn), each consisting of three rats. These groups were gradually subjected to a dosage higher than the therapeutic dose of UA+Cu, UA+Se, and UA+Zn for a period of two weeks (Deyno et al., 2020). Throughout this period, the rats were closely monitored for any indications of changes in behavior, food intake, water consumption, body weight, as well as mortality rates (Table 2). Notably, no significant findings were recorded in any of the specified parameters. Based on our findings, it may be reflected that the administration of UA+Cu, UA+Se, and UA+Zn through the oral route in rats appears to be relatively safe.

Table 2. UA+Cu, UA+Se, UA+Zn acute toxicity test

Novel complex	Total Rats (09)	(Two weeks duration)				
		Body weight	Food intake	Water intake	Behavioral change	Change skin/fur in
UA+Cu	Rat 1-3	Unchanged	+	+	Alert	Nil
UA+Se	Rat 4-6	Unchanged	+	+	Alert	Nil
UA+Zn	Rat 7-9	Unchanged	+	+	Alert	Nil

4. Discussion

In the scope of our present study, an inquiry was conducted to evaluate the immunomodulatory and anti-inflammatory characteristics of copper (Cu), selenium (Se), and zinc (Zn) compounds of UA in a rheumatoid inflammatory model induced by FCA exposure. It is a well-established fact that the bonding of metal complexes to organic drugs has the potential to augment the biological activities of these drugs, including their anti-inflammatory, cancer chemotherapy, anti-microbial effects (Santos et al., 2022).

We employed a rat model of FCA-induced arthritis, and lornoxicam (LX), a medication often prescribed for rheumatoid arthritis was utilized as the standard treatment.

The anti-inflammatory effects of UA are attributed to its ability to downregulate inflammatory cytokines and upregulate anti-inflammatory cytokines through various signaling cascades (Zhao et al., 2023).

Lower levels of copper (Cu), selenium (Se), and zinc (Zn) may lead to transient immune impairment or even disrupt the regulation of systemic inflammation in the long term. Therefore, comprehending the mechanisms and ensuring the adequate supply of these essential minerals hold significant importance (Weyh, Krüger, Peeling, & Castell, 2022).

Current insights propose that interleukin-4 (IL-4) and interleukin-13 (IL-13) may play a pivotal role in suppressing the inflammatory processes underlying the pathology of rheumatoid arthritis (RA) and positively modulating the disease progression (Iwaszko et al., 2021). Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on IL-4 and IL-13 were detected.

Current study indicated that Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly raised IL-4 and IL-13 as compared to FCA group. The findings of our research are consistent with prior experimental studies (Shaaban et al., 2022) (Haikal et al., 2019).

Cytokine (IL-10), is recognized for its role as an anti-inflammatory role within the synovial tissue of arthritic problem and its capacity to hinder cytokine production by obstructing NF-ϰB activity. (Lee et al., 2019).

Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on IL-10 were detected. Current study indicated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly raised IL-10 as compared to FCA group with exception to Cu alone,

which displayed non-significant result. The findings of our research are consistent with prior experimental studies (Yuba et al., 2021).

IL-17 is involved in both early and established RA diseases. It promotes activation of fibroblast-likesynoviocytes (FLS), osteoclast genesis, recruitment and activation of neutrophils, macrophages and B cells (Kondo, Kuroda, & Kobayashi, 2021). Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on IL-17 were detected. Present study demonstrated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated IL-17 except Cu. The findings of our research are consistent with prior experimental studies (Chrastina et al., 2022).

The pathogenesis of RA in the joints is influenced by infiltrating immune cells, with TNF-αactivated rheumatoid arthritis synovial fibroblasts (RASFs) being key contributors (Siegel et al., 2022). Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on TNF-α were detected. Present study demonstrated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated TNF-α except Cu. The findings of our research are consistent with prior experimental studies (Shen et al., 2022).

NF-ϰB pathway regulation has the ability to modulate the systemic immune inflammatory response, which leads to a reduction in inflammation, as well as the erosion of articular cartilage and bone tissue (Wang, Zha, Ruan, Yao, & Zhang, 2022).

Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on NF-κB were detected. Present study demonstrated that Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated NF-κB. The findings of our research are consistent with prior experimental studies (Shu et al., 2022). Earlier work has indicated that toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4) play crucial roles in the pathogenesis of arthritic model, with upregulation of these proinflammatory cytokines within the synovium (Q. Dai, Li, Wang, Li, & Li, 2020).

Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on TLR-2 and TLR-4 were detected. Present study demonstrated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated both TLR-2 and TLR-4, Cu showed significant reduction of TLR-4 but in case of TLR-2 result was non-significant. The findings of our research are consistent with prior experimental studies (Roome et al., 2019).

The upregulation of prostanoids and lipoxins derived from arachidonic acid is believed to have a role in the pathogenesis of rheumatic diseases, and targeting this pathway could result in more effective treatment approaches (Sano et al., 2020).

Present study demonstrated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated COX-2 and LOX, Cu showed significant reduction of COX-2 but in case of LOX result was nonsignificant. The findings of our research are consistent with prior experimental studies (L. Yang et al., 2020).

Nitric oxide (NO) plays a significant role in the pathophysiology of rheumatoid arthritis (RA) and is closely related to oxidative stress and inflammation in the disease process (Hajizadeh & Azizi, 2021). Effects of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on NO were detected. Present study demonstrated that Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX significantly attenuated NO except Cu.

The findings of our research are consistent with prior experimental studies (Cui et al., 2020).

We identified the effect of Cu, Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX on splenocyte proliferation. Our findings showed that a significant increase BrdU uptake in FCA group as compared to negative control on the other hand BrdU uptake was significantly reduced in Se, Zn, UA, UA+Cu, UA+Se, UA+Zn and LX except Cu. The findings of our research are consistent with prior experimental studies (X. Dai et al., 2020).

Our study revealed insignificant alterations in red blood cell count (RBC) and hemoglobin (Hb) levels, while a notable difference was observed in platelet count when comparing the FCA group to the drug-treated groups. Additionally, a substantial increase in WBC count was noted in the FCA group compared to the UA+Cu, Zn, UA+Se, UA+Zn groups, where values were normalized. These outcomes align with previous experimental investigations. To assess the safety profile of UA+Cu, Zn, UA+Se, UA+Zn, we conducted analyses of serum ALT, ALP, creatinine, and urea, indicating the absence of significant hepatotoxic and nephrotoxic effects associated with these compounds.

Conclusion

Our study demonstrated a significant reduction in FCA-induced arthritis in rats, reflecting the potential anti-arthritis properties of UA+Cu, UA+Se, and UA+Zn reflecting their anti-inflammatory and immunomodulatory mechanism of action. The combined effects of UA+Cu, UA+Se, and UA+Zn exhibited synergistic effects as compared to Cu, Se, and Zn alone, the results were comparable to the standard drug LX.

Author Contributions:

Conceptualization, F.A. and A.J.; methodology, F.A., A.J, M.S.Z.; validation, A.K.N., F.A, and M.S.Z.; formal analysis, F.A., R.Y. and A.J.; investigation, F.A, A.J. M.S.Z., R.Y. and A.K.N; writing—original draft preparation, F.A. A.J. A.S..; writing—review and editing, F.A, A.J, A.K.N, and A.S; supervision, A.K.N, A.J.; project administration, A.K.N, M.S.Z., and R.Y.

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