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Evaluation of the Potential Anti-Inflammatory Effect of Ursodeoxycholic Acid in Treating Inducible Rheumatoid Arthritis in A Rat Model

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, tissue damage, and systemic manifestations. Although several treatment options are available, they have limitations and adverse effects. Therefore, there is a need for novel therapeutic approaches. Ursodeoxycholic Acid (UDCA), a bile acid known for its hepatoprotective properties, has shown immunomodulatory effects and potential application in autoimmune diseases. This study aims to evaluate the anti-inflammatory effect of UDCA in treating RA using a rat model.

Arthritis was induced in rats using the complete Freund's adjuvant (CFA), and the results of UDCA were compared to Diclofenac, a commonly used anti-inflammatory drug. The arthritic score, body weight, and paw edema size were evaluated to assess the effectiveness of UDCA in controlling RA. Hematological parameters were analyzed to evaluate the impact of UDCA on inflammation.

UDCA administration significantly reduced paw edema size, decreased Tumor Necrosis Factor alpha(TNF-α) and Rheumatoid factor(RF) release, and improved hematological parameters compared to the CFA-induced non-treated group. The arthritic score and paw edema size were significantly smaller in the UDCA-treated group compared to the CFA-induced non-treated group. Body weight was significantly improved in the UDCA-treated group, indicating a beneficial effect. Hematological parameters showed improvement, with a decrease in White Blood Cells count and Erythrocyte sedimentation rate and an increase in Red Blood Cell count in the UDCA-treated group.

Conclusion: This study demonstrates the potential anti-inflammatory effect of UDCA in treating RA in a rat model. UDCA reduced paw edema size, decreased TNF-α and RF levels, and improved hematological parameters compared to the CFA-induced non-treated group.

Keywords: Rheumatoid arthritis (RA), Ursodeoxycholic acid (UDCA), Inflammation, In vivo model.

تقييم التأثير المضاد للالتهاب المحتمل لحمض الاورسوديوكسيكولك في علاج التهاب المفاصل الروماتويدي المستحدث في نموذج الجرذ

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الخلاصة

التهاب المفاصل الروماتويدي هو مرض مناعي ذاتي مزمن يتميز بالتهاب المفاصل وتلف الأنسجة ومظاهر جهازية. على الرغم من توفر العديد من خيارات العلاج ، إلا أن لها قيودًا وتأثيرات ضارة. لذلك ، هناك حاجة إلى مناهج علاجية جديدة. الاورسوديوكسيكولك حمض الصفراء المعروف بخصائصه الواقية للكبد ، أظهر تأثيرات مناعية وإمكانية تطبيقه في أمراض المناعة الذاتية. تهدف هذه الدراسة إلى تقييم التأثير المضاد للالتهابات لمادة حمض الاورسوديوكسيكولك في علاج التهاب المفاصل الروماتويدي باستخدام نموذج الجرذان.

تم تحريض التهاب المفاصل في الجرذان باستخدام محرض فروند الكامل ، وتمت مقارنة نتائج حمض الاورسوديوكسيكولك مع ديكلوفيناك ، وهو دواء شائع الاستخدام كمضاد للالتهابات. تم تقييم درجة التهاب المفاصل ووزن الجسم وحجم وذمة المخلب لتقييم فعالية حمض الاورسيديوكسيكولك في السيطرة على التهاب المفاصل الروماتويدي. تم تحليل المتغيرات الدموية لتقييم تأثير حمض الاوسوديوكسيكولك على الالتهاب.

تناول حمض الاورسوديوكسيكولك قلل بشكل كبير من حجم وذمة المخلب ، وانخفض عامل نخر الورم الفا وعامل الروماتيد ، وتحسن معاملات الدم مقارنة بمجموعة محرض فروند الكامل غير المعالجة. كانت درجة التهاب المفاصل وحجم وذمة المخلب أصغر بكثير في المجموعة المعالجة بحمض

الاورسوديوكسيكولك مقارنة بالمجموعة غير المعالجة الناتجة عن محرض فروند الكامل. تحسن وزن الجسم معنوياً في المجموعة المعالجة بحمض الاورسوديوكسيكولك مما يشير إلى تأثير مفيد. أظهرت معاملات الدم تحسنا مع انخفاض في تعداد كرات الدم البيضاء ومعدل ترسيب كرات الدم الحمر وزيادة في عدد كرات الدم الحمراء في مجموعة حمض الاورسوديوكسيكولك المعالجة.

توضح هذه الدراسة التأثير المحتمل المضاد للالتهابات لحمض الاورسوديوكسيكولك في علاج التهاب المفاصل الروماتويدي في نموذج الجرذان. حمض الاورسوديوكسيكولك انخفاض حجم وذمة المخلب انخفاض عامل نخر الورم الفا ومستويات عامل الروماتير ، وتحسين معاملات الدم مقارنة مع مجموعة محرض الفريوند الكامل غير المعالجة

الكلمات المفتاحية: التهاب المفاصل الروماتويدي. حمض أورسوديوكسيكوليك. الالتهاب في نموذج الجسم الحي.

Introduction

Rheumatoid arthritis (RA) is a bilateral, chronic disease that affects the joints, characterized by joint hyperplasia, joint and surrounding tissue inflammation, and bone and cartilage damage [(1)]. As a systemic disease, RA affects all body parts, including blood vessels, lungs, skin, eyes, and joints on both sides of the body [(2)]. Inflammatory cytokines, including interleukin (IL-6), IL-1, and tumor necrosis factor (TNF), contribute to the pathophysiology of RA [(3,4)]. TNF- is a proinflammatory cytokine that contributes to several biological processes involving the control of cell proliferation, differentiation, and apoptosis; and is a cell signaling protein implicated in the systemic inflammatory response [(5)]. TNF-α is released in many diseases, such as Non-Alcoholic Fatty Liver Disease (NAFLD) and RA [(6)] . These cytokines encourage persistent inflammatory synovitis leading to the interruption of nearby tissue in joints leading to RA. The suppression of overproduced cytokines was found to reduce patients' symptoms of RA [(7)].

Some treatments are available to help manage the disease and improve symptoms. Disease-modifying anti-rheumatic medications (DMARDs), including sulfasalazine, hydroxychloroquine, methotrexate, and leflunomide, are often used to stop joint damage and reduce the progression of RA. These medications work by decreasing inflammation and targeting the immune system, but they take weeks or months to start effecting. Steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are also commonly used to treat RA, with NSAIDs helping to reduce inflammation and pain and steroids helping to reduce inflammation and relieve symptoms quickly [(8)]. By blocking the action of these cytokines, medications can reduce inflammation, slow or halt joint damage, and provide symptomatic relief for patients with RA. Despite the effectiveness of the above mentioned treatments for RA, their long-term use is limited by their adverse effects, such as liver and bone marrow damage, heart problems, and stomach discomfort [(9)].

Genetic research on RA has led to the development of novel, better-tolerated medications called biologics (monoclonal antibodies), such as infliximab, rituximab, sarilumab, tocilizumab, and tofacitinib. These drugs can target immune system components that lead to inflammation-induced joint and tissue damage [(10)]. However, several investigations have shown that individuals taking these biologics are more susceptible to infections of the genitourinary system, skin, soft tissue, and joints than those taking conventional medications [(11)]. As no drug has been effective in reducing the effects of RA up until now, it is desirable to develop new drugs that can overcome these limitations. Ursodeoxycholic acid (UDCA), also known as ursodiol (USAN), is a secondary bile acid produced in the liver from primary bile acids, such as chenodeoxycholic acid (CDCA).

UDCA can also be made by certain bacteria in the intestines through a process called bacterial 7 dehydroxylation. UDCA constitutes approximately 5% of human bile acids and is known for its hepatoprotective and choleretic properties. It treats many hepatic illnesses, including primary biliary cholangitis, a chronic autoimmune liver condition characterized by the gradual destruction of the liver's tiny bile ducts [(12,13)]. Nevertheless, it prevents dendritic cell activation via the farnesoid X receptor and reduces eosinophil-mediated inflammation [(14)], as evidenced by the interaction between dendritic cells and T cells and its impact on T cell performance. Recent investigations have suggested that UDCA may be an immunomodulator in autoimmune diseases and potentially reduce monocyte expression of TNF-induced IL-8 [(15)], thus, having a great therapeutic potential towards inflammatory diseases. This study aims to investigate the potential of UDCA in treating RA by assessing the anti-arthritis effect of UDCA in a Complete Freund Adjuvant (CFA)-induced RA in a Rat model.

Materials and methods

Chemicals, Drugs

Complete Freund Adjuvant (CFA) was bought from Sigma Aldrich (country of origin), Ursodeoxycholic Acid (UDCA) was purchased from Picasso (China), Diclofenac (50 mg) was purchased from Novartis (Switzerland), and Sodium bicarbonate was purchased from Ishtar (Iraq).

Reagents

TNF-α ELISA test kit and RF were obtained from Pars Biochem (China).

Preparation of UDCA Solution

UDCA solution in sodium bicarbonate (15 mg/ml in 5% sodium bicarbonate) was prepared by adding 100g of chemical grade sodium bicarbonate powder to 1800ml of sterile water in a 2500ml volumetric flask placed on a hot plate stirrer. After solubilization, the sodium bicarbonate solution was rapidly boiled. Then, 30g of UDCA was accurately weighed, and 7.5g of UDCA was added to the boiling sodium bicarbonate solution. At a 10 min interval, the remaining UDCA was gradually added to the solution. The final UDCA solution was diluted to 2000 ml by adding sterile hot water and then poured into amber containers and kept at 4°C until further use, following a published protocol [16].

Induction of arthritis in rats

The left hind paw footpad of the rats received an intradermal injection of 0.1 ml CFA-heat-killed Mycobacterium tuberculosis suspended in paraffin oil and mannide monooleate 1mg/ml under ether anesthesia. A 0.1 cc booster subcutaneous injection was administered around the tail the same day and the day after. A similar RA induction protocol was reported elsewhere [17].

Experimental animals

Twenty-four adult Wistar Albino rats of either sex, weighing 180-230g, were obtained from the animal house at the University of Baghdad, College of Pharmacy, and were kept on standard circumstances for temperature, humidity, and the cycle of light and dark with water and standard diet free access *ad libitum***.** The following groups of rats were designed : **(1)** the control group rats that received sodium bicarbonate for 21 days **(2)** the induction group that received CFA injection intradermally at the foot-pad of the left hind paw and remained untreated for 21 days; **(3)** Diclofenac group given a CFA injection with orally administered Diclofenac (5 mg/kg) for 21 days (18), **(4)** UDCA group given a CFA injection with orally administered UDCA (100 mg/kg) for 21 days ([19]).

Arthritis score

Using a method developed by Zhang *et al.*, (2009), the arthritis score was determined (20). On a 5-point scale, swelling and erythema of the paws (hind and fore) were graded as follows: 0, no signs of swelling or erythema; 1, swelling or erythema in the ankle or wrist; 2, swelling or erythema in the ankle and tarsal or wrist and carpal; 3, swelling or erythema extending to the metatarsals or metacarpals; and 4, signs of swelling or erythema involving the entire hind or fore paw. The highest possible score was 8.

Body Weight

The body weight of each animal was measured on the day the CFA administration and then every 7 days, up to 21 days. For each drug-treated group, the mean percentage change in body weights from the day of CFA administration was determined and compared to the disease control group.

Paw Edema

The hind limbs' paw thickness post-CFA injection was measured on days 1, 7, 14, and 21 using Image J software (Figure 1).

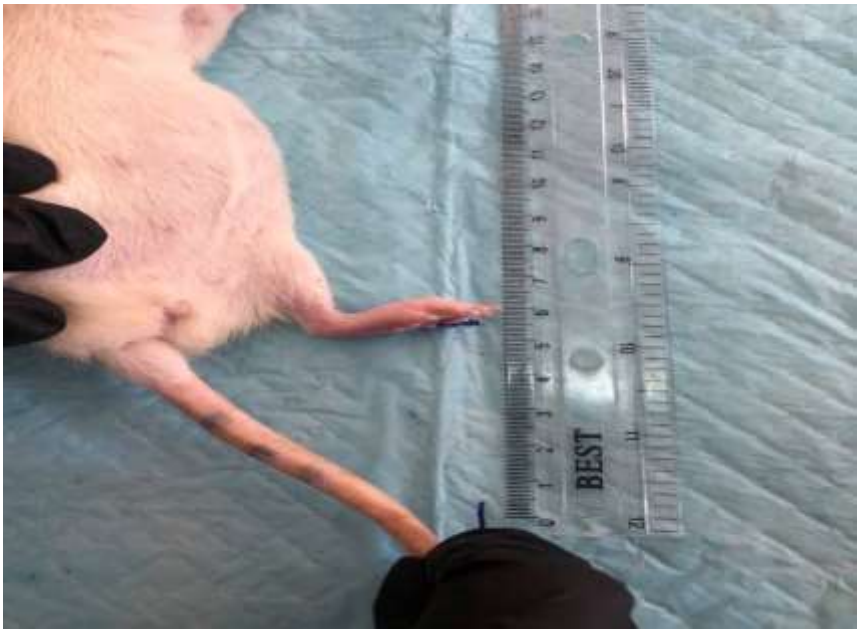

Figure 1. The paw thickness measurement by Image J software.

Hematological Parameters and Biochemical Analysis

Blood samples were collected after 21 days under ether anesthesia in (1) EDTA tube for the determination of RBC, WBC, Hb, and ESR; (2) Gel tubes, where the blood samples left to stand for 30 min to allow clotting, centrifuged for 30 min to obtain clear serum then stored at -20°C until the day of analysis. Stored sera were used for the estimation of serum ~~activities~~ ~~Of~~ TNF-α and Rheumatoid Factor (RF) using sandwich qualitative ELISA technique.

Statistical analysis

The results of six animals were expressed as stander error mean (±SEM). One-way ANOVA was used to assess the statistical difference in mean followed by Turkey's multiple comparison tests, where P<0.05 was regarded as statistically significant.

Result

Effect of UDCA on the arthritic score

The CFA-induced non-treated group showed a significant increase in arthritic scores up until day 21 compared to other treatment groups and controls. On the other hand, the arthritic score measured on days 14 and 21 were significantly decreased in Diclofenac- and UDCA-treated groups compared to the induction group (**Figure 2**).

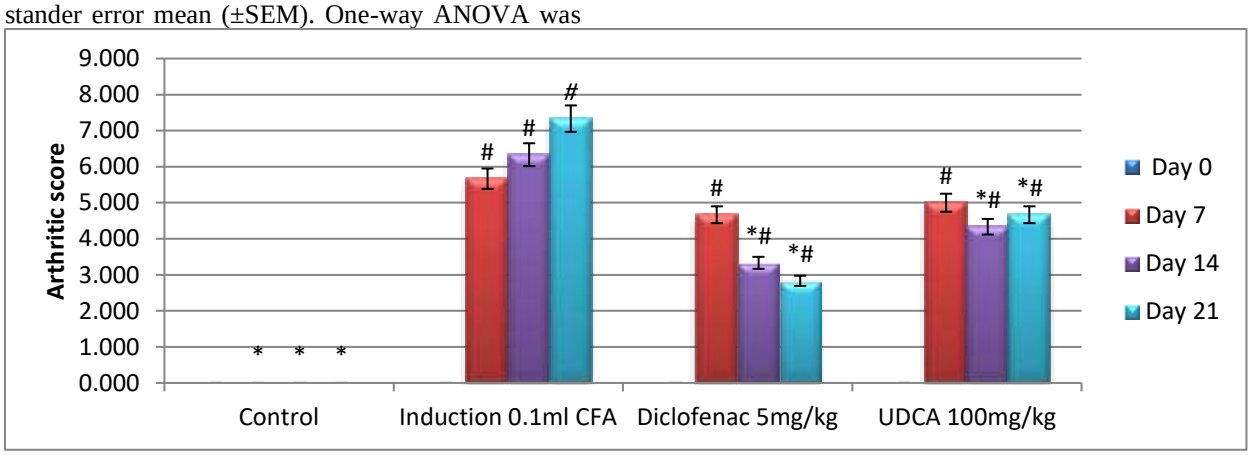

Figure 2. The effect of UDCA on the arthritic score. Values are shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). *Denotes significant difference compared to the CFA-induced non-treated group (P<0.05).

Effect of UDCA on body weight

The body weight in the CFA-induced non-treated group was significantly lower than the control group. The body weight in the Diclofenac- and UDCA-treated groups was significantly higher (improved) than in the CFA-induced non-treated group on days 14 and 21 (**Figure 3**).

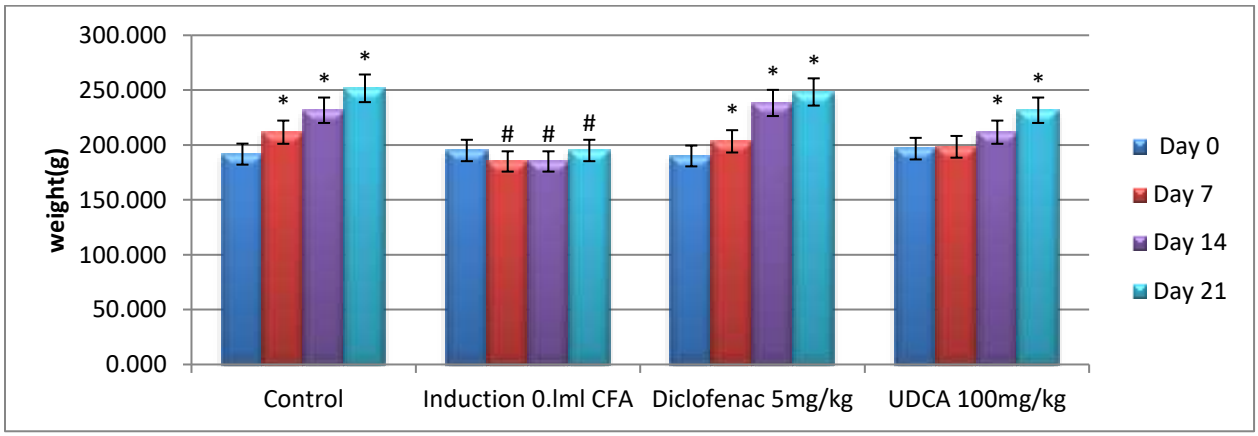

Figure 3. The effect of UDCA on body weight. Values were shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). * Denotes a significant difference compared to the CFA-induced non-treated group (P<0.05).

Effect of UDCA on paw edema size

In the CFA-induced non-treated group, the paw edema showed a significant increase in size from day 7 until day 21 compared to the control group. Furthermore, the paw edema size was significantly smaller in the Diclofenac- and UDCA-treated groups than in the CFA-induced non-treated group on day 21 (**Figure 4 & Figure 5**).

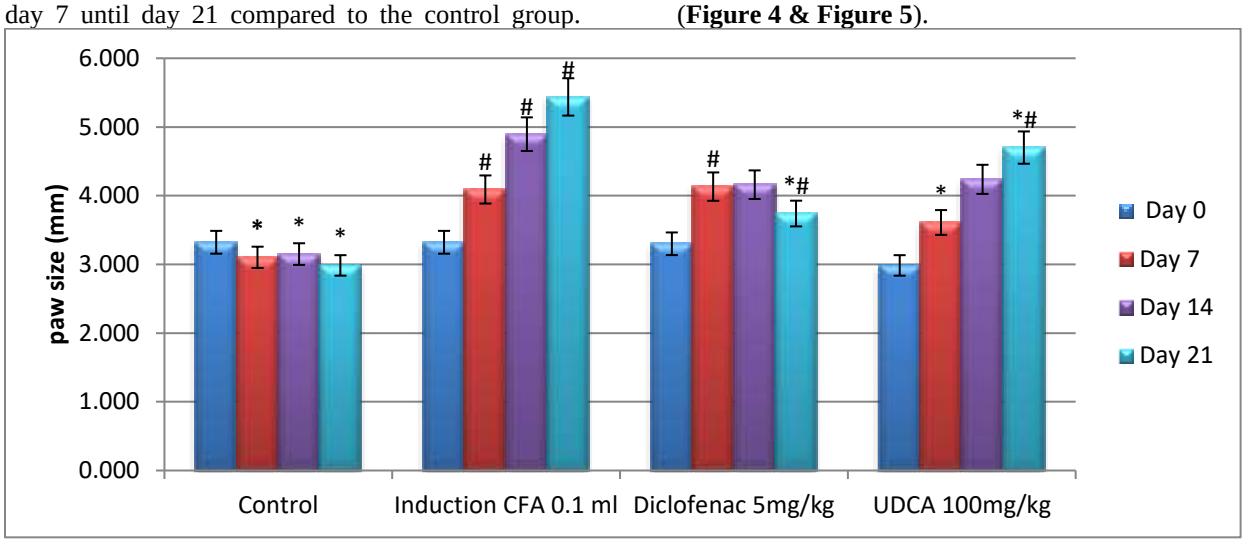

Figure 4. Effect of UDCA on paw edema size. Values were shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). *Denotes a significant difference compared to the CFA-induced non-treated group (P<0.05).

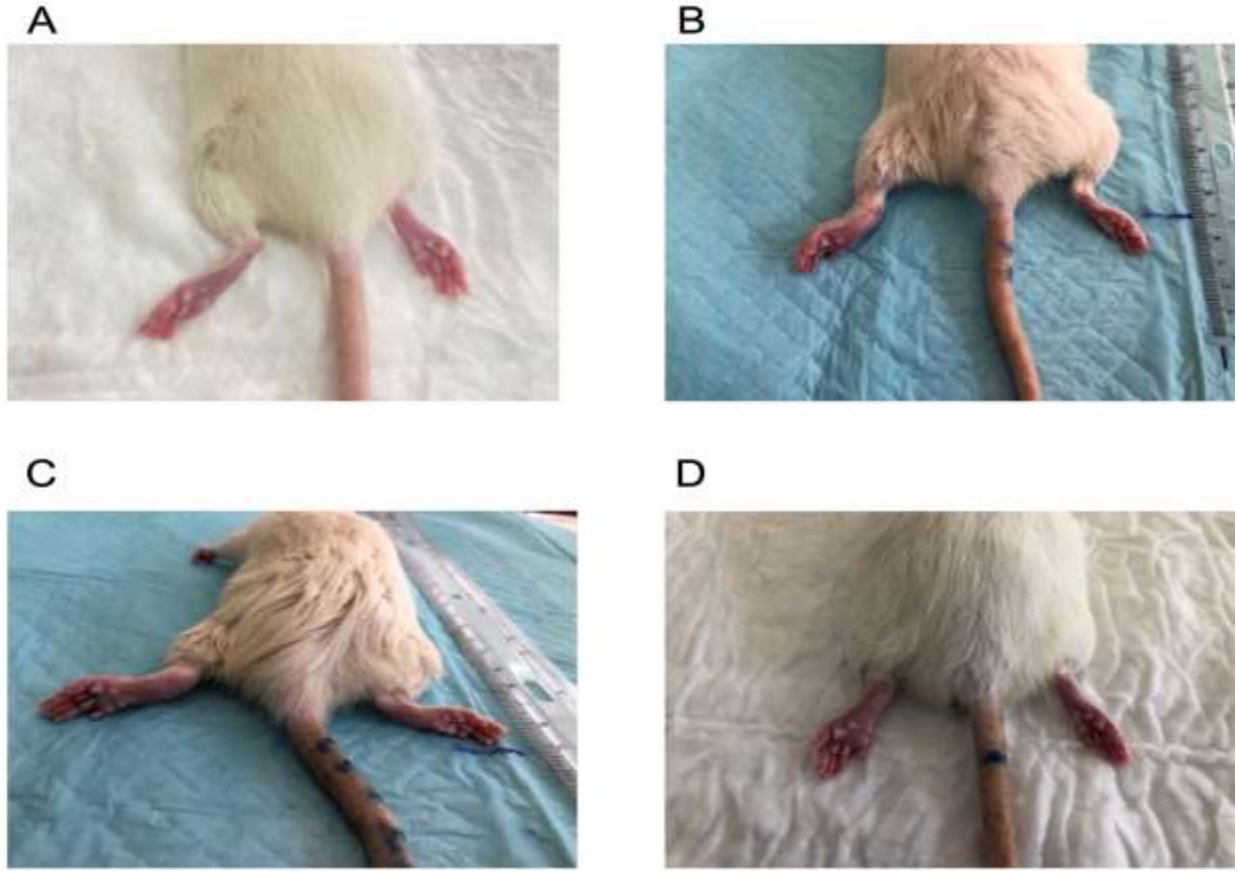

Figure 5. Images of the visual change in the edema on day 21. A) Control. B) CFA-induced non-treated group. C) Diclofenac-treated group. D) UDCA-treated group.

Effect of UDCA on TNF-α levels in rat models

The TNFα level was significantly higher in the CFA-induced non-treated group compared to treatment groups and controls, where the TNFα level was significantly lower (**Figure 6**). In addition, the UDCA-treated group showed no significant difference from the control group in promoting the TNFα levels.

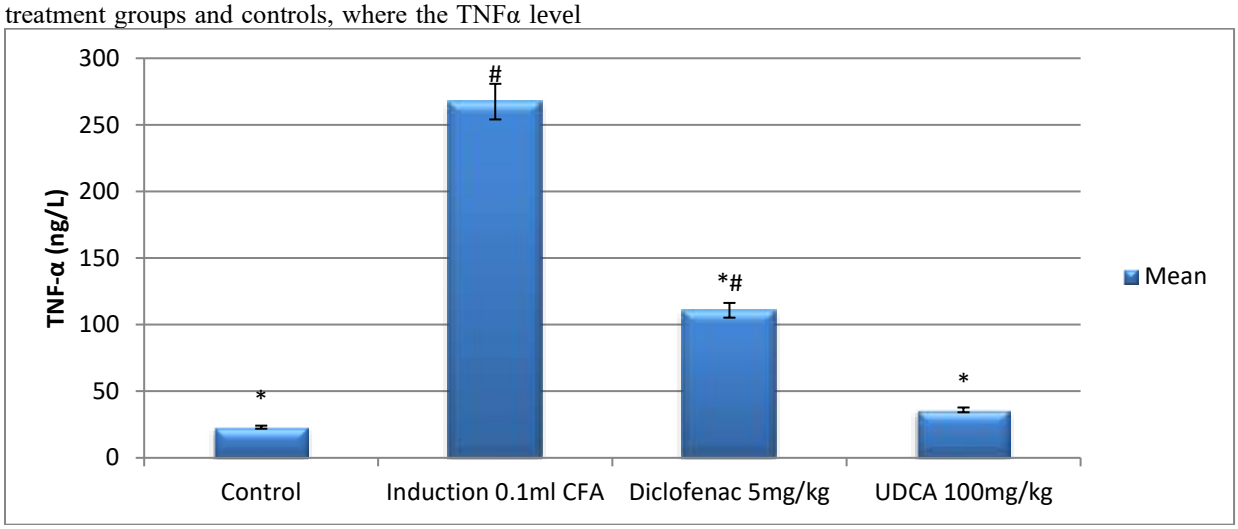

Figure 6. The effect of UDCA on TNFα level. Values were shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). * Denotes a significant difference compared to the CFA-induced non-treated group (P<0.05).

Effect of UDCA on serum RF levels in rat models

Likewise, the serum RF level was significantly higher in the CFA-induced non-treated group compared to treatment groups and controls (**Figure 7**). The UDCA-treated group also showed no significant difference from the control group in promoting the serum RF levels.

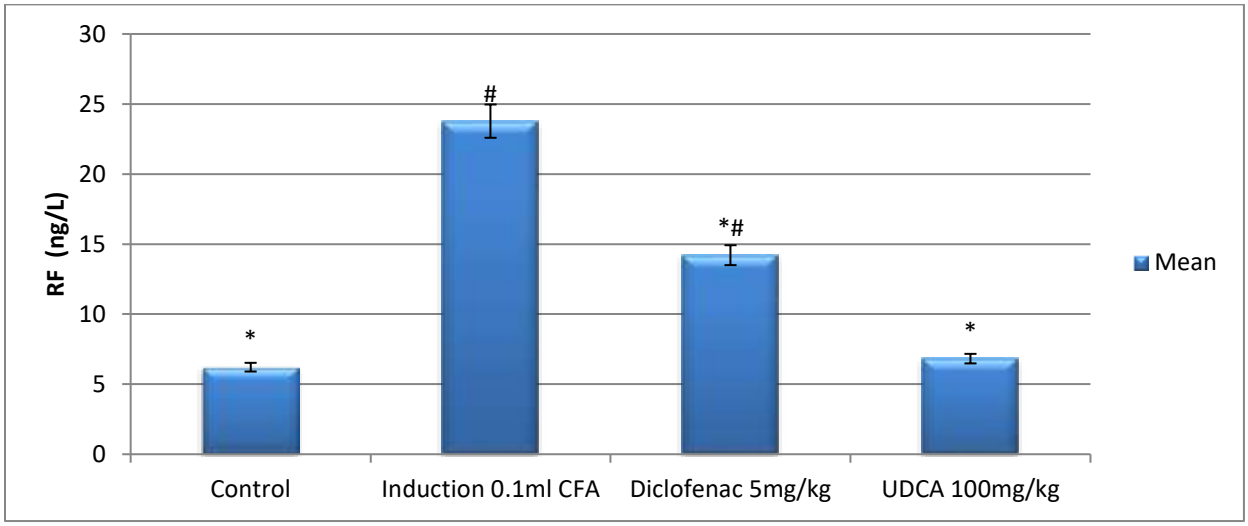

Figure 7. The effect of UDCA on the serum RF level. Values were shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). * Denotes a significant difference compared to the CFA-induced non-treated group (P<0.05).

Effect of UDCA on Hematological Parameters.

The RBC count was significantly reduced, while the WBC count and ESR of the CFA-treated group were significantly increased compared to the control group. RBC count showed a significant increase in the Diclofenac- and UDCA-treated groups. Furthermore, the WBC and ESR showed a significant decrease in the UDCA-treated group compared to the CFA-induced non-treated group.

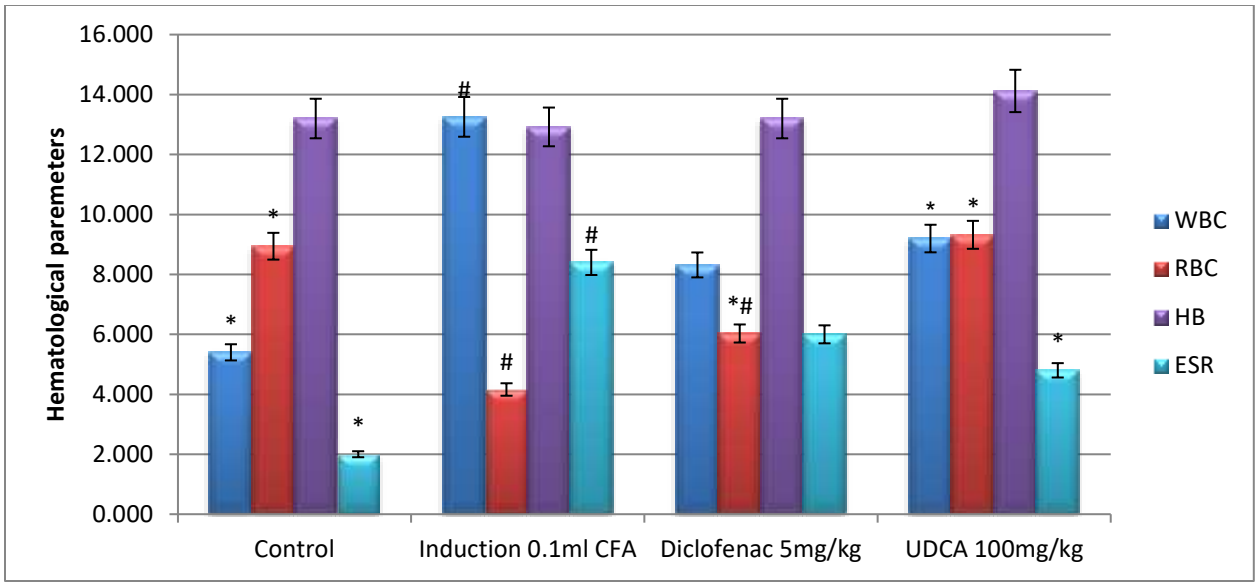

Figure 8. The effect of UDCA on Hematological Parameters. Values were shown as mean ± SEM and N=6 for each group. # Denotes significant difference compared to the control group (P<0.05). * Denotes significant difference compared to the CFA-induced non-treated group (P<0.05).

Discussion

Using the CFA-induced arthritis rat model, post-CFA injection, arthritis was slowly started appearing in several joints in the rats on day 7, reaching a peak on days 14 and 21 (**Figure 2**). There was a significant increase in the arthritic score in the CFA-induced non-treated group as compared to the control group ($p<0.05$) between days 7 and 21, and there was a significant decrease in the arthritic score in the Diclofenac- and UDCA-treated group on days 14 and 21, as compared with CFA-induced non-treated group. The effect of UDCA on the arthritic score may refer to the anti-inflammatory activity of UDCA (20), which lead to reduced swelling and erythema of rat paws.

The body weight was significantly decreased in the CFA-induced non-treated group compared to the control group, in front of a significant increase in body weight between day 7 and day 21 in the treatment groups. Weight loss is a common indicator of advanced chronic illnesses, including RA. It may be due to a combination of factors, including decreased appetite, increased metabolic rate, and loss of lean body mass. In RA, weight loss has been linked to increased levels of inflammatory cytokines, such as TNF-α and IL-6, which promote a catabolic state and muscle wasting (22). The UDCA-treated group showed a significant increase in body weight between days 14 and 21 compared to the CFA-induced non-treated group (**Figure 3**). The results indicate that UDCA could increase appetite and body weight (23).

A significant increase in paw edema size was also shown in the CFA-induced non-treated group compared to the control group. There was, however, a significant decrease in paw edema size in the treatment groups. Joint inflammation and edema develop within a few days post-CFA injection to peak after 14 days (17). The severity and duration of joint inflammation can vary depending on the CFA dose and the specific strain of rats used in the experiment. In general, joint swelling and inflammation development are hallmark features of RA (24,25). Interestingly, the UDCA-treated group showed a significant decrease in edema size on days 7 and 21 compared to the CFA-induced non-treated group (**Figure 4** and **Figure 5**), which aligns with a decrease in the arthritic scores.

The production of cytokines by immune cells in the synovium leads to a chronic inflammatory response, which destroys joint tissues and develops characteristic symptoms, such as joint pain, swelling, and stiffness. Current RA treatments aim to control inflammation by targeting specific cytokines or other molecules involved in the inflammatory cascade (4,26). A significant increase in TNF-α levels was also observed in the CFA-induced non-treated group compared to the treatment groups and control (**Figure 6**). Our results showed that UDCA causes a significant reduction in TNF-α levels as compared to the CFA-induced non-treated group and becomes non-significant when compared to the control group, which means that UDCA could control inflammation and the targeting of TNF-α post-RA induction. On the other hand, RF is a specific type of autoantibody that targets the Fc region of immunoglobulin G (IgG) antibodies. RF can be detected in the blood of some patients with RA, and it is a diagnostic marker of the disease

(27,28). The results showed that Diclofenac and UDCA cause a significant reduction of RF compared to the CFA-induced non-treated group (**Figure 7**).

In the presence of inflammation, the concentration of acute-phase proteins, including fibrinogen and C-reactive protein (CRP), increases in the blood. These proteins promote the formation of rouleaux, or stacks of RBCs, which settle more quickly than individual RBCs. As a result, the ESR is elevated in conditions associated with inflammation, including RA. In our results, the arthritic rats in the CFA-induced non-treated group showed a decreased RBC count, which is consistent with the chronic inflammatory conditions of RA (**Figure 8**). Overall, the ESR and RBC count are useful diagnostic features in patients with chronic arthritis and can provide valuable information on the presence and severity of inflammation and related complications (29). In the CFA-induced group treated with UDCA, the RBC count was significantly increased compared to the CFA-induced non-treated group. At the same time, ESR and WBC were significantly decreased, indicating the high therapeutic potential of UDCA in treating RA.

Conclusion

The study demonstrates that ursodeoxycholic acid (UDCA) exhibits potential anti-inflammatory effects in a rat model of rheumatoid arthritis (RA). The administration of UDCA effectively reduced paw edema size, decreased TNF-α levels, and modulated RF levels in the experimental animals. UDCA treatment also improved hematological parameters suggesting that UDCA has promising therapeutic potential for managing RA and its associated inflammation. The anti-inflammatory properties of UDCA make it a promising candidate for further investigation as a potential therapeutic agent in the treatment of RA. Future studies should explore the underlying mechanisms by which UDCA exerts its beneficial effects and evaluate its long-term safety and efficacy in RA patients. Overall, the results support the potential use of UDCA as a novel therapeutic approach for rheumatoid arthritis treatment.

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Ethics Statements

It was approved by the Ethical Committee of the College of Pharmacy/University of Baghdad before the start of the study.

Conflict of Interest

The authors have no conflict of interest.

Author contributions

The first author did the practical work and result analysis. The second author supervised the whole work.

References

1. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum. 2006;54(9):2793–806.
2. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet. 2009; 373 (9664) :659 –72.
3. Firestein GS, McInnes IB. Immunopathogenesis of Rheumatoid Arthritis. Immunity. 2017;46(2):183–96.
4. Burska A, Boissinot M, Ponchel F. Cytokines as biomarkers in rheumatoid arthritis. Mediators Inflamm. 2014;2014.
5. 5. Majeed IA, Al-Shawi NN. Effects of omega-3 co-administered with therapeutic dose of lornoxicam on male rats' liver. Iraqi J Pharm Sci. 2019;28(2):159–64.
6. Nafeer SA, Zalzala MH. Possible amelioration of the severity of nutritional steatohepatitis by guggulsterone in mice. Iraqi J Pharm Sci. 2019;28(1):17–23.
7. Sweeney SE, Firestein GS. Rheumatoid arthritis: regulation of synovial inflammation. Int J Biochem Cell Biol. 2004;36(3):372–8.
8. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389(10086):2338–48.
9. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16(5):821–47.
10. Scott DL. Biologics-based therapy for the treatment of rheumatoid arthritis. Clin Pharmacol Ther. 2012;91(1):30–43.
11. Horton SC, Nam JL, Buch MH. Safety of biologics in rheumatoid arthritis. Int J Clin Rheumtol. 2012;7(4):425–51.
12. Hofmann AF, Roda A. Physicochemical

properties of bile acids and their relationship to biological properties: An overview of the problem. J Lipid Res. 1984;25(13):1477–89.

13. Hofmann AF, Mysels KJ. Bile acid solubility and precipitation in vitro and in vivo: the role of conjugation, pH, and Ca2+ ions. J Lipid Res. 1992;33(5):617–26.

14. Willart MAM, Van Nimwegen M, Grefhorst A, Hammad H, Moons L, Hoogsteden HC, et al. Ursodeoxycholic acid suppresses eosinophilic airway inflammation by inhibiting the function of dendritic cells through the nuclear farnesoid X receptor. Allergy Eur J Allergy Clin Immunol. 2012;67(12):1501–10.

15. O'Dwyer AM, Lajczak NK, Keyes JA, Ward JB, Greene CM, Keely SJ. Ursodeoxycholic acid inhibits TNFα-induced IL-8 release from monocytes. Am J Physiol - Gastrointest Liver Physiol. 2016;311(2):G334–41.

16. Okan A, Astarcioglu H, Tankurt E, Sagol O, Altekin E, Astarcioglu I, et al. Effect of Ursodeoxycholic Acid on Hepatic Steatosis in Rats. Dig Dis Sci. 2002;47(11):2389–97.

17. Weng W, Wang F, He X, Zhou K, Wu X, Wu X. Protective effect of Corynoline on the CFA induced Rheumatoid arthritis via attenuation of oxidative and inflammatory mediators. Mol Cell Biochem. 2021;476(2):831–9.

18. Sharma M, Chaudhary D. Exploration of bromelain laden nanostructured lipid carriers: An oral platform for bromelain delivery in rheumatoid arthritis management. Int J Pharm. 2021;594:120176.

19. Olausson M, Mjörnstedt L, Wramner L, Persson H, Karlberg I, Friman S. Adjuvant treatment with ursodeoxycholic acid prevents acute rejection in rats receiving heart allografts BT - Transplant International Official Journal of the European Society for Organ Transplantation. In: Kootstra G, Opelz G, Buurman WA, van Hooff JP, MacMaster P, Wallwork J, editors. Berlin, Heidelberg: Springer Berlin Heidelberg; 1992. p. 539–41.

20. Zhang R-X, Fan AY, Zhou A-N, Moudgil KD, Ma Z-Z, Lee DY-W, et al. Extract of the Chinese herbal formula Huo Luo Xiao Ling Dan inhibited adjuvant arthritis in rats. J Ethnopharmacol. 2009;121(3):366–71.

21. Hunneyball IM, Crossley MJ, Spowage M. Pharmacological studies of antigen-induced arthritis in BALB/c mice I. Characterization of the arthritis and the effects of steroidal and non-steroidal anti-inflammatory agents. Agents Actions. 1986;18(3):384–93.

22. Martín AI, Castillero E, Granado M, López-Menduiña M, Villanúa MA, López-Calderón A. Adipose tissue loss in adjuvant arthritis is associated with a decrease in lipogenesis, but not with an increase in lipolysis. J Endocrinol. 2008;197(1):111–9.

23. Tatsumura T, Sato H, Yamamoto K, Ueyama T. Ursodeoxycholic acid prevents gastrointestinal disorders caused by anticancer drugs. Jpn J Surg. 1981;11(2):84–9.

24. Duer-Jensen A, Hørslev-Petersen K, Hetland ML, Bak L, Ejbjerg BJ, Hansen MS, et al. Bone edema on magnetic resonance imaging is an independent predictor of rheumatoid arthritis development in patients with early undifferentiated arthritis. Arthritis Rheum. 2011 Aug;63(8):2192–202.

25. McGonagle D, Conaghan PG, O'Connor P, Gibbon W, Green M, Wakefield R, et al. The relationship between synovitis and bone changes in early untreated rheumatoid arthritis: A controlled magnetic resonance imaging study. Arthritis Rheum. 1999 Aug;42(8):1706–11.

26. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007;7(6):429–42.

27. Westwood OMR, Nelson PN, Hay FC. Rheumatoid factors: what's new? Rheumatology. 2006 Apr;45(4):379–85.

28. Song YW, Kang EH. Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies. QJM An Int J Med. 2010 Mar;103(3):139–46.

29. Ramsay ES, Lerman MA. How to use the erythrocyte sedimentation rate in paediatrics. Arch Dis Child Educ Pract Ed. 2015;100(1):30–6.