ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

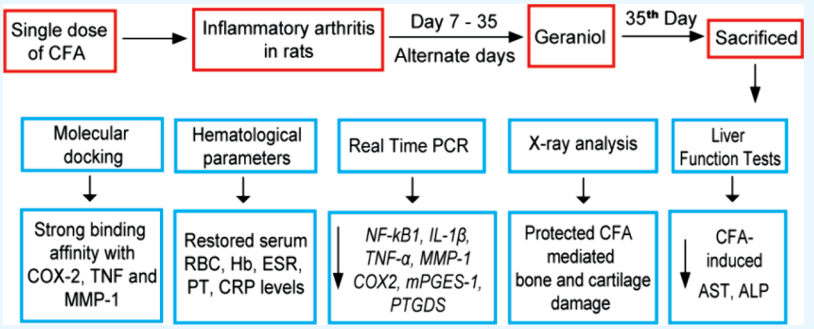
Geraniol Suppresses Oxidative Stress, Inflammation, and Interstitial Collagenase to Protect against Inflammatory Arthritis

Muhammad Nasir Hayat Malik,*[,] Muhammad Nouman Tahir, Tariq G. Alsahli, Md. Mahedi Hassan Tusher, Sami I. Alzarea, Bader Alsuwayt, Shah Jahan, Hesham A. M. Gomaa, Mohamed E. Shaker, Muhammad Ali, Irfan Anjum, Muhammad Tariq Khan, Muhammad Roman, and Ramla Shabbir

Cite This: *ACS Omega* 2023, 8, 37128−37139

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Geraniol (GER) is a plant-derived acyclic isoprenoid monoterpene that has displayed anti-inflammatory effects in numerous *in vivo* and *in vitro* models. This study was therefore designed to evaluate the antiarthritic potential of GER in complete Freund's adjuvant (CFA)-induced inflammatory arthritis (IA) model in rats. IA was induced by intraplantar injection of CFA (0.1 mL), and a week after CFA administration, rats were treated with various doses of methotrexate (MTX; 1 mg/kg) or GER (25, 50, and 100 mg/kg). Treatments were given on every alternate day, and animals were sacrificed on the 35th day. Paw volume, histopathological, hematological, radiographic, and qPCR analyses were performed to analyze the severity of the disease. GER significantly reduced paw edema after 35 days of treatment, and these results were comparable to the MTX-treated group. GER-treated animals displayed a perfect joint structure with minimal inflammation and no signs of cartilage or bone damage. Moreover, GER restored red blood cell and hemoglobin levels, normalized erythrocyte sedimentation rate, platelet, and c-reactive protein values, and also attenuated the levels of rheumatoid factor. RT-qPCR analysis demonstrated that GER decreased mRNA expression of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta. GER also down-regulated the transcript levels of cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1, prostaglandin D2 synthase, and interstitial collagenase (MMP-1). Molecular docking of GER with COX-2, TNF-α, and MMP-1 also revealed that the antiarthritic effects of GER could be due to its direct interactions with these mediators. Based on our findings, it is conceivable that the antiarthritic effects of GER could be attributed to downregulation of pro-inflammatory mediators and protease like MMP-1.

INTRODUCTION

Inflammatory arthritis (IA) is a chronic systemic autoimmune disorder that mainly damages the articular cartilage, characterized by inflammation and hyperplasia of synovium, production of autoantibodies, and cartilage/bone deformation.[1] Many risk factors like age, gender, tobacco smoking at younger age, and obesity have shown some kind of association with the onset of the disease, though the main cause remains unknown.[2] Some environmental factors such as toxic chemicals and pathogenic microorganisms have the potential to make changes in normal human protein structures,[3] and these modified proteins show an antigenic behavior and activate the immune system. This leads to the activation and proliferation of helper T and B lymphocytes, resulting in the secretion of pro-inflammatory cytokines and the production of autoantibodies such as anticitrullinated proteins antibodies (ACPA) and rheumatoid factor (RF).[4] These pro-inflammatory cytokines together with autoantibodies ultimately lead to the breakdown of cartilage and bone.[5]

Received: June 30, 2023
Accepted: September 14, 2023
Published: September 28, 2023

Overall, the goal of the therapy is to reduce pain, inflammation, and joint destruction and maintain joint function. Analgesic and anti-inflammatory drugs used in the treatment are steroids and nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs have analgesic as well as anti-inflammatory effects; they act by reducing prostaglandin production by inhibiting cyclooxygenase 1 and 2 enzymes. The chronic use of these medicines is associated with abdominal pain, ulcer, nausea, vomiting, and gastrointestinal (GI) bleeding.[6] Steroids are more potent than NSAIDs to reduce pain and inflammation, but these drugs have several adverse effects like hyperglycemia, immunosuppression, bone resorption, and weight gain.[7] Disease-modifying antirheumatic drugs (DMARDs) are also being used in the treatment to halt and slow down the progression of the disease.[8] Biological DMARDs have shown promising effects, especially when given in the early stage of the disease. DMARDs, however, have serious adverse effects including risk of infection or tuberculosis, cardiovascular toxicity, and immunosuppression, which require close monitoring of the patients.[9]

Even with the advancement in the treatment, the target of the therapy is still not fully achieved as the etiology and pathophysiology of the disease are not fully understood.[10] Currently, the drug therapy used for the treatment of IA is an empirical therapy, which is not suitable for every patient, which demands the search for new, effective, and most suitable drug.[11]

In this study, we have evaluated geraniol (GER), a monoterpene alcohol present in essential oils of various plants including *Cinnamomum tenuipilum* and *Valeriana officinalis*,[12] for the prevention/treatment of IA. Previously, GER has shown its anticancer,[13] anti-inflammatory,[14] antioxidative,[15] and antimicrobial effects.[12] Studies indicate that GER exerts anti-inflammatory as well as immunomodulatory effects by decreasing the gene expression of *C0X-2*, *NF-kB*, TNF-α, and prostaglandins.[14,16,17] GER has also shown prominent anti-inflammatory activity in numerous disease models such as colitis, hepatic ischemia-reperfusion injury, traumatic spinal cord injury, atherogenesis, lipopolysaccharide-induced acute lung injury, and imiquimod-induced psoriasis.[14,17,18] In view of previous scientific data, it was postulated that GER could have beneficial effects on the prevention or treatment of IA in rats.

MATERIALS AND METHODS

Reagents. Geraniol, dimethyl sulfoxide (DMSO), and complete Freund's adjuvant (CFA) were purchased from Sigma-Aldrich (Taufkirchen, Germany), and methotrexate was from PharmaSol (Lahore, Pakistan). All other consumables utilized in this study were of analytical grade.

Animals. Male wistar rats were purchased from the University of Veterinary and Animal Sciences, Lahore, Pakistan, and kept in the institutional animal care facility under standard conditions. The experimental study was performed according to the "Organization for Economic Cooperation and Development (OECD) guidelines", and the study protocol was approved by the institutional research ethics committee (IREC) of the Department of Pharmacology, Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan (approval number: IREC−2021−49).

CFA-Induced IA. IA was induced in rats by a single dose of (0.1 mL) CFA, which was injected via intraplantar (i.pl) route in the right hind paw. After 1 week of immunization (day 7), animals in the standard group were treated intraperitonially (ip) with MTX and treatment groups were treated (ip) with different doses of GER (25, 50, and 100 mg/kg). These treatments were repeated on alternative days from day 7 to day 35. Paw edema of rats was measured on days 7, 14, 21, 28, and 35. Rats were sacrificed on the 35th day, and blood samples were collected into EDTA and gel tubes to separate serum for analysis. Right hind paws were cut above the ankle joints and fixed in 10% formalin for histopathological analysis. X-ray analyses of ankle joints were performed to investigate the changes in bone, cartilage, and joints. A portion of liver tissues were cut, harvested in trizol, and stored at −80 °C for later mRNA analysis.[19] Animals were divided into the following groups (n = 4 in each group):

1. Control −1% DMSO
2. Disease control (CFA) − CFA followed by 1% DMSO
3. Standard group (CFA + MTX) − CFA followed by MTX (1 mg/kg)
4. CFA + GER (25 mg/kg) − CFA followed by GER (25 mg/kg)
5. CFA + GER (50 mg/kg) − CFA followed by GER (50 mg/kg)
6. CFA + GER (100 mg/kg) − CFA followed by GER (100 mg/kg)

Hematological and Biochemical Analysis. A Urit 3000 vet plus hematology analyzer was used to measure hematological parameters (red blood cell count (RBC), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), platelet count (PLT), and hemoglobin (Hb)). Biochemical parameters were measured by calorimetric analysis using standard ELISA, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). A standard latex agglutination test was used to assess RF.[19]

Antioxidant Assay. Liver samples from disease and treated groups were harvested in 1× PBS. After washing, samples were homogenized and stored overnight at −20 °C. The samples were freeze−thawed two times and centrifuged to obtain the supernatants. Supernatants were later processed according to the manufacturer's instructions to measure superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) levels using standard ELISA kits.

Real Time-qPCR Analysis. The trizol technique was used to extract the total RNA from the homogenized liver samples. Subsequently, reverse transcription of RNA was performed using a WizScript cDNA synthesis kit (Wizbio solutions, New Mexico, USA). To analyze samples in RT-qPCR, the reaction mixture was prepared according to the standard protocol of SYBR Green qPCR mix (Zokeyo, Wuhan, China). Relative mRNA expression levels were measured by a ddCT method using the following conditions: initial denaturation at 94 °C for 2 min followed by 40 cycles of denaturation at 94 °C for 1 min and annealing at 59 °C for 2 min. Hypoxanthine−guanine phosphoribosyl transferase (HPRT) was used as an internal control. The list of primers used for qPCR analysis is provided in Table S1.[19]

Molecular Docking Analysis. *Retrieval of COX-2, TNF-α, and MMP-1 Structures from Protein Data Bank.* The three-dimensional (3D) structures of COX-2, TNF-α, and MMP-1 from *Homo sapiens* were accessed from Protein Data Bank (PDB) (www.rcsb.org) with PDB IDs of 5KIR, 2AZ5, and 4AUO, respectively. The target proteins were prepared for docking analysis using the Autodock Tools program. The

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

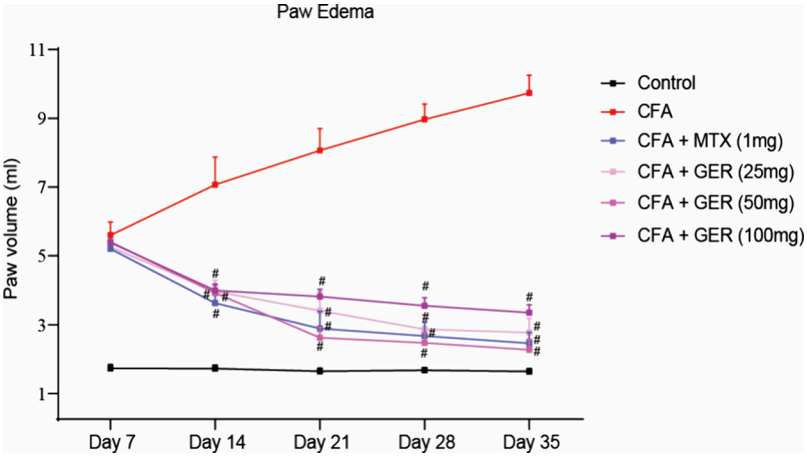


Figure 1. GER abated CFA-induced paw inflammation. IA was induced by injecting 100 μL of CFA in the right hind paw of the rats. After 7 days, treatments were given with the mentioned doses of MTX and GER. Paw volume was measured with a digital plethysmometer to check the edema at the mentioned intervals of time. GER at 25 and 50 mg/kg reduced paw edema, which was comparable to the MTX-treated group. # $\leq$ 0.001 (two-way ANOVA followed by a Bonferroni multiple comparison test); significant acronyms (#) represent the comparison of treatment groups with the disease group (CFA); n = 7.

proteins were energy-minimized, and Gasteiger charges were added and saved in pdbqt format. The hydrophobicity and Ramachandran graphs were generated by Discovery Studio 4.1 Client (2012). The protein architecture and statistical percentage values of helices, β-sheets, coils, and turns were accessed by VADAR 1.8.[20]

Ligand Molecular Docking. The compounds GER and MTX were drawn in Discovery Studio Client and saved in pdb format as ligands after energy minimization. Autodock Tools was used for the preparation of the ligand in their most stable conformation. The ligand was saved in pdbqt format after the addition of the Kolman and Gasteiger charges. The molecular docking experiment was used for the synthesized ligand (GER) against COX-2, TNF-α, and MMP-1 by a PyRx[20] virtual screening tool with an Auto Dock VINA Wizard approach.[21]

Statistical Analysis. This study was performed in biological replicates, and results were shown in terms of mean $\pm$ standard deviation (SD) unless stated otherwise. Data were analyzed by one or two-way ANOVA followed by posthoc analyses using GraphPad prism 8.0 (Graphpad Software, Inc., San Diego, USA). Probability values of less than 0.05 were deemed significant using the following acronyms: # $\leq$ 0.001, ** $\leq$ 0.01, * $\leq$ 0.05.

RESULTS

GER Reduced CFA-Induced Paw Inflammation. CFA administration induced symmetrical inflammation similar to the inflammation in IA. The paw volume kept on increasing day by day, and the paws of the disease group were severely inflamed on the 35th day. On the seventh day of the CFA immunization, the right hind paws of all groups showed marked inflammation. GER treatments reduced paw inflammation after the first week of administration. Over the next 2 weeks (14th to 28th day), GER showed prominent reduction in the paw volume, and this anti-inflammatory effect actually reduced with the increase in dose except at 100 mg/kg. MTX-treated animals also displayed a reduction in inflammation and paw volume over the course of therapy, and interestingly, GER at a dose of 50 mg/kg showed comparable efficacy to MTX treatment. On the 35th day, the hind paws of GER- and MTX-treated groups seemed normal just like the hind paws of the control group, while disease control groups' rats displayed severe inflammation and tissue necrosis (Figure 1).

Radiographic Analysis Revealed a Protective Effect of GER against CFA-Induced IA. X-ray analysis of the control group showed normal joint and cartilage architecture with no arthritic changes or inflammation. In the disease group, cartilage and bone were severely damaged, and there was clear evidence of soft and hard tissue swelling. Tarsal bones had become smaller, and all the phalangeal joints were misaligned with an increase in their joints' spaces. The MTX group showed a nearly normal joint structure, and there was no evidence of periosteal reaction in the joints. X-ray analysis of the GER-treated groups (25 and 50 mg/kg) showed a marked reduction in the inflammation of the joints along with no changes in bone and cartilage. However, animals treated with GER (100 mg/kg) showed mild soft tissue inflammation with minor degenerative changes (Figure 2).

Effects of GER on Hematological and Biochemical Parameters. To evaluate the hematological effects of GER, we quantified different hematological and biochemical variables. Hematological analysis showed that ESR, CRP, TLC and PLT values were higher in the disease group. In the MTX group, these values decreased and approached levels close to the control group. In GER-treated groups, GER effectively reduced ESR, CRP, and PLT values, which were comparable to the MTX group. In addition, RBCs and Hb values were lowered in the disease control group, while MTX- and GER-treated groups restored RBCs and Hb values (Figure 3). As we know, MTX causes LFT abnormalities, so we measured serum AST, ALT, and ALP levels. MTX as expected produced a significant increase in AST levels, while ALT and ALP levels were the same as the control group. Interestingly, GER at 100 mg/kg elevated AST, ALT, and ALP values, and it was higher than the MTX-treated group, indicating that it could have toxic effects on the liver at this particular dose. However, lower doses of GER (25 and 50 mg/kg) showed a better safety profile as all the LFT parameters were comparable to the control group (Figure 3).

Moreover, qualitative analysis of autoantibodies production also showed that the rheumatoid factor (RF) was positive in the disease group, while it was negative in MTX- and GER (25

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

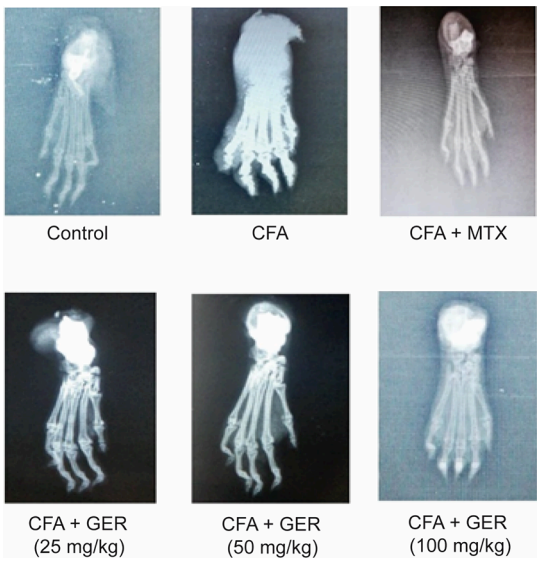


Figure 2. X-ray images of the right hind paws of the rats exhibiting a reduction in the severity of IA by MTX and GER. IA was induced by injecting 0.1 mL of CFA, and a week after CFA administration, rats were treated with the mentioned doses of MTX or GER. On every other day, treatments were repeated until day 35 and animals were sacrificed. The hind paws were cut and fixed in 10% (buffered) solution of formaldehyde. X-ray films of the right hind paws were developed, which revealed a clear reduction in inflammation and erosion of bone and cartilage by MTX and GER (n = 3). "Photographs courtesy of Ishtiaq Ahmed, University of Veterinary and Animal Sciences, Lahore; Copyright 2022".

and 50 mg/kg)- treated groups. GER at 100 mg/kg showed a weak positive RF, indicating that at higher doses, GER might not be effective in treating/preventing CFA-induced IA (Table 1).

GER Reduced CFA-Induced Oxidative Stress. Since CFA is known to induce oxidative stress, we therefore measured antioxidant levels in liver homogenates. As expected, CFA significantly reduced the levels of SOD and GSH and induced the levels of MDA. Treatments with MTX and GER significantly restored the levels of antioxidants, indicating their antioxidant potentials as well (Figure 4).

GER Reduced the Transcript Levels of Pro-inflammatory Mediators. Inflammation is a key point in the initiation and progression of the IA. To explore the molecular mechanism of antiarthritic activity of GER against CFA-induced arthritis, the relative mRNA expression of different biomarkers responsible for the onset of inflammation, disease initiation, and disease progression was measured.[22−25]

Our results revealed that the transcript levels of nuclear factor kappa-B1 (*NF-kB1*), tumor necrosis factor-alpha (TNF-α), and interleukin 1 beta (*IL-1*β) were effectively down-regulated by GER treatment, and these results were comparable to MTX. In contrast, the disease group showed high levels of these cytokines, which might be due to the inflammation caused by CFA. Interstitial collagenase/matrix metalloproteinase-1 (MMP-1) is known to cause bone and cartilage erosion,[26,27] and its transcript levels were significantly reduced by GER treatment. In addition, cycloxigenase-2 (*COX-2*), microsomal Prostaglandin E Synthase-1 (*mPGES-1*), and prostaglandin D2 synthase (*PTGDS*) enzymes are associated with chronic inflammation and immune response in IA. Our findings demonstrated that the levels of these enzymes were significantly reduced by GER and MTX treatments (Figure 5).

GER Displayed Strong Binding Affinity with COX-2 and Modest Affinity with TNF-α and MMP-1. The affinity among the protein targets and the ligand (GER) was investigated by molecular docking. The AutoDock Vina program was used for the docking analysis through the PyRx user interface. The *E*-value (kcal/mol) was used to assess the affinity of protein and best docked pose complex. It provided prediction of the binding free energy and binding constant for docked ligands. The results obtained from the docking studies of GER against COX-2, TNF-α, and MMP-1 were in conjuction with their phamracological activities. Binding affinities of GER with COX-2, TNF-α and MMP-1 were −6.2, −3.7, and −4.6 kcal/mol, respectively. These findings indicate that GER-induced antiarthritic effects could be due to its direct interaction with COX-2, TNF-α, and MMP-1 (Figure 6). The standard drug (MTX) was also docked with the target proteins mentioned above, and MTX displayed binding affinities comparable to GER, i.e., MTX with COX-2 (−6.1 kcal/mol), MTX with TNF-α (−6.0 kcal/mol), and MTX with MMP-1 (−5.5 kcal/mol) (Figure 7).

DISCUSSION

Since GER has been known for its anti-inflammatory activity,[28] we therefore designed this study to evaluate its antiarthritic activity in a CFA-induced IA model. CFA-induced IA in rats is one of the remarkable models to assess the antiarthritic activity of different plant extracts or compounds. CFA induces persistent immunological and pathophysiological features, immune system dysfunction (including the involvement of inflammatory mediators), and bone/joint erosion just like IA.[29] The circulating levels of immune cells (including activated B-cells and activated T-cells) and autoantibodies (RF, ACPA) also increase in IA, which in turn activate various immune cells like monocytes, ultimately resulting in severe inflammation owing to release of pro-inflammatory mediators such as IRAK1, NF-kB1, TNF-α, IL-1β, and IL-17.[30] If these inflammatory conditions persist, the inflammatory environment exposes new self-antigens to the immune system, which leads to the endless cycle.[31] Moreover, due to this vicious cycle, pro-inflammatory cytokines produced by immune cells further activate fibroblast-like synoviocytes (FLS) and these activated FLS proliferate continuously and ultimately lead to synovial hyperplasia. FLS together with other immune cells present in the joint release mediators that result in pannus formation and osteoclast transformation. All these inflammatory conditions induce joint swelling, bone erosion, and cartilage destruction.[23,32] In addition to these pro-inflammatory cytokines, prostaglandins are also produced in a large amount in the synovium of the joint. Prostaglandin (PG)D2 and PGE2 are produced in large quantities due to increased expression of COX-2, MPGES, and PTGDS. These prostaglandins are also responsible for pain, inflammation, swelling, and degradation of the joint.[25,33]

In this study, we used MTX as a standard drug because MTX is still considered a gold standard for the treatment of IA as well as rheumatoid arthritis. Our findings showed that GER at doses of 25 and 50 mg/kg decreased paw inflammation from the 14th day to 35th day and all the treated paws exhibited complete healing. Histopathological evaluation of the ankle and metacarpophalangeal joints also reiterated the antiarthritic activity of GER. Animals treated with a higher dose of GER,

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

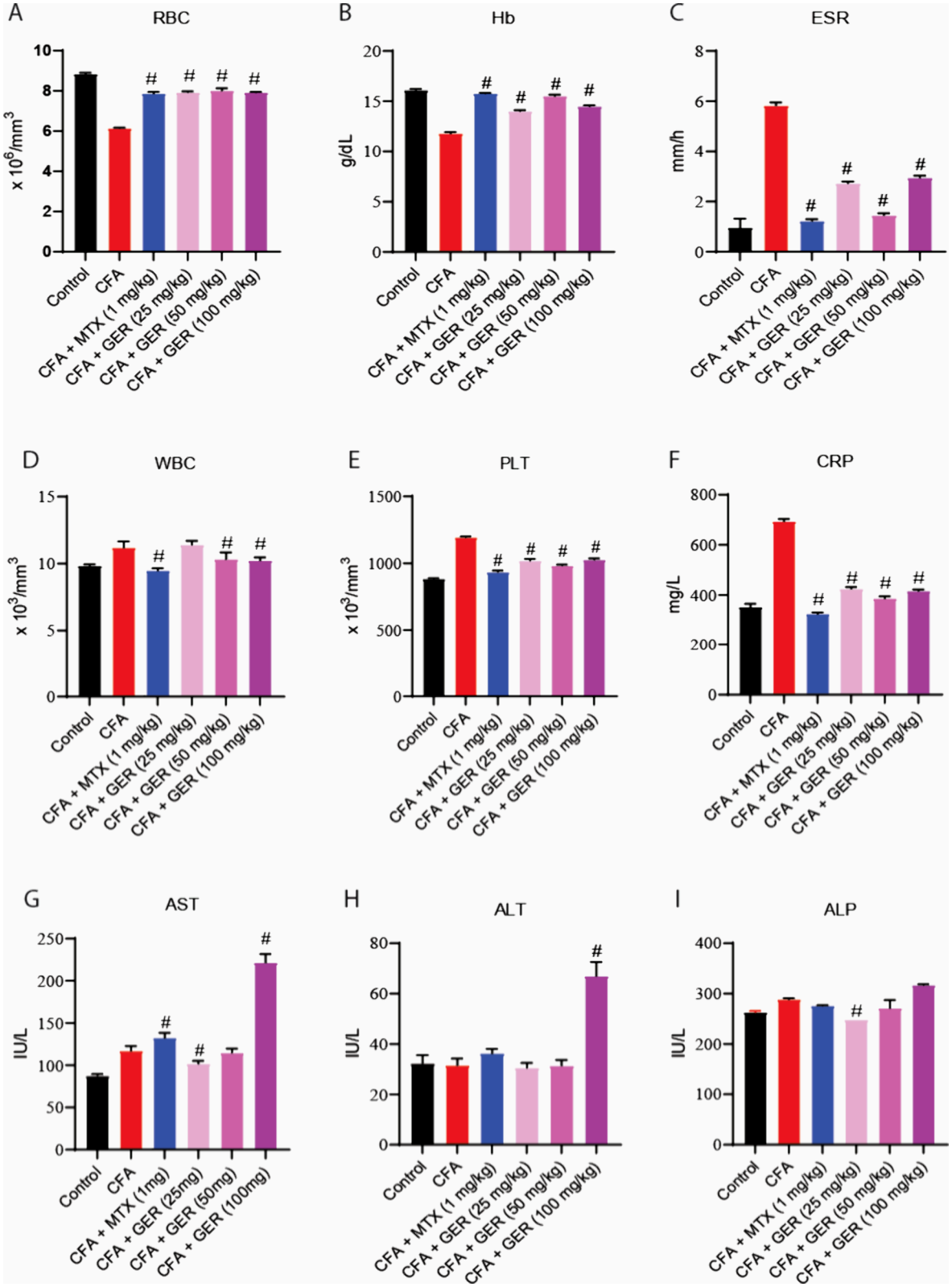


Figure 3. Hematological and biochemical analysis revealed protective effects of GER against CFA-induced IA damage. IA was induced by injecting 100 μL of CFA, and after a week of CFA administration, rats were treated with the mentioned doses of MTX or GER. Treatments were given every other day until day 35, and later, animals were sacrificed. Biochemical and hematological parameters were analyzed using an automatic hematology and biochemical analyzer machine. GER successfully restored (A) RBC, (B) Hb, (C) ESR, (D) WBC, (E) PLT, and (F) CRP levels. GER (100 mg/kg) induced (G) AST, (H) ALT, and (I) ALP, displaying its hepatotoxic potential at higher doses. # $\leq$ 0.001 (one-way ANOVA followed by the Tukey's multiple comparison test); significant acronyms (#) represent the comparison of treatment groups with the disease group (CFA); n = 7.

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

Table 1. GER Reduced the Development of RF in the CFA-Induced IA Model

	control	CFA	CFA + MTX (1 mg/kg)	CFA + GER (25 mg/kg)	CFA + GER (50 mg/kg)	CFA + GER (100 mg/kg)
RF	negative	positive	negative	negative	negative	weak positive

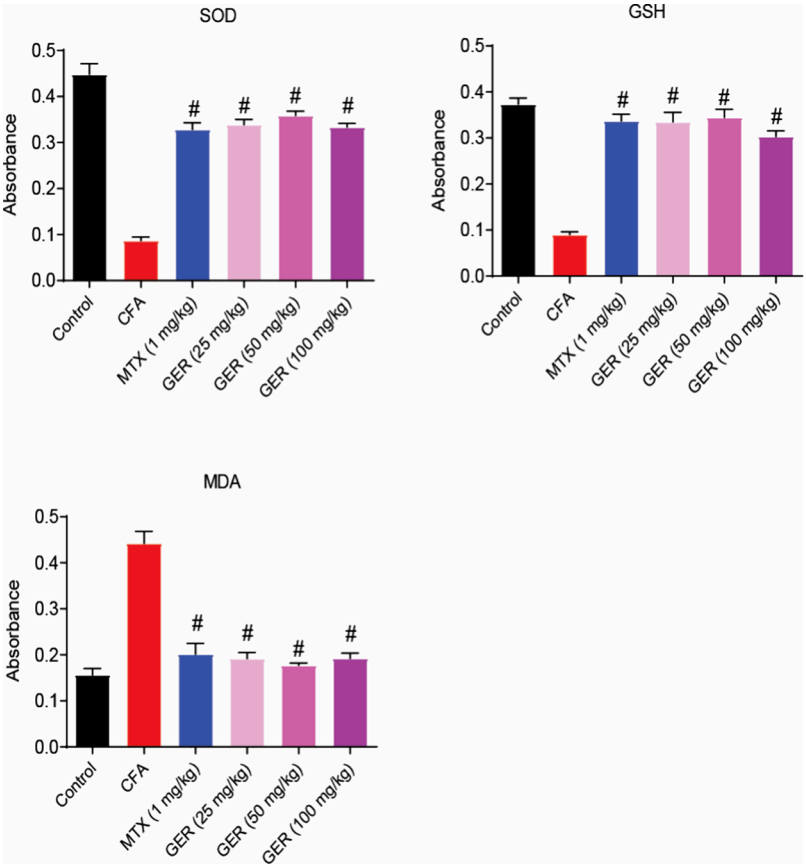


Figure 4. GER displayed an antioxidant effect. GER increased serum levels of SOD and GSH and reduced the levels of MDA, illustrating its beneficial antioxidant effects. # ≤ 0.001 (one-way ANOVA followed by the Tukey's multiple comparison test); significant acronyms (#) represent the comparison of treatment groups with the disease group (CFA); $n = 6$.

i.e, 100 mg/kg showed mild cartilage damage and inflammation, indicating that this dose might not be effective in treating/preventing IA. In contrast, histopathological analysis of the disease group showed infiltration of the inflammatory cells, cartilage and bone erosion, presence of dead tissue, and synovial hyperplasia. Moreover, X-ray analysis of the treated groups showed the reduction in the inflammation of the joints, bones, and soft tissue. There was no inflammation and cartilage erosion at lower doses (25 and 50 mg/kg) of GER. The group treated with 100 mg/kg showed some inflammation in soft tissue, but no erosion of bone and cartilage was witnessed.

IA is known to modulate hematological and biochemical parameters in the body.[34−36] Studies have shown that chronic IA patients develop anemia because of the large production of cytokines, which inhibit the utilization of iron and cause structural changes in bone marrow, which leads to depletion in RBCs. These cytokines also interfere with the release of erythropoietin from the kidney.[35−37] TLC also increases because of the raised levels of cytokines. Moreover, the ESR level is always elevated during any inflammatory condition. RBCs settle down at a faster rate in inflammatory conditions and slowly in normal physiological conditions.[35,38] CRP levels also get elevated, which represent the inflammatory condition in the body and it also has been used as a diagnostic biomarker of systemic inflammation in IA.[39] To assess these changes, we measured the levels of Hb, ESR, RBCs, TLC, CRP, and PLT in blood serum. As expected, our findings revealed that CFA altered the abovementioned parameters, while GER restored their levels, indicating its protective effects against CFA-induced pathological changes.

Abnormal LFTs are also accompanied by IA, and IA patients are always at risk to develop autoimmune liver disease. Furthermore, since MTX is also associated with the raised AST and ALT levels,[36,40] we therefore measured the effects of GER on LFT levels. Our finding showed that CFA did not significantly cause abnormal LFT levels but MTX and GER at 100 mg/kg significantly raised LFTs. GER at low doses (25 and 50 mg/kg) showed a greater hepatic safety profile than MTX treatment.

IA is a complex autoimmune disease that requires more specific and more sensitive disease biomarkers for the progression of the disease and also for the establishment of the severity of the disease.[41] RFs are the autoantibodies that are produced by the body's immune system in response to the modified protein of the body and has almost 75% sensitivity to the autoimmune disease such as IA.[42] It has been proven that IA patients have abnormally elevated RF values.[43] However, many patients that have positive RF results do not have IA

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

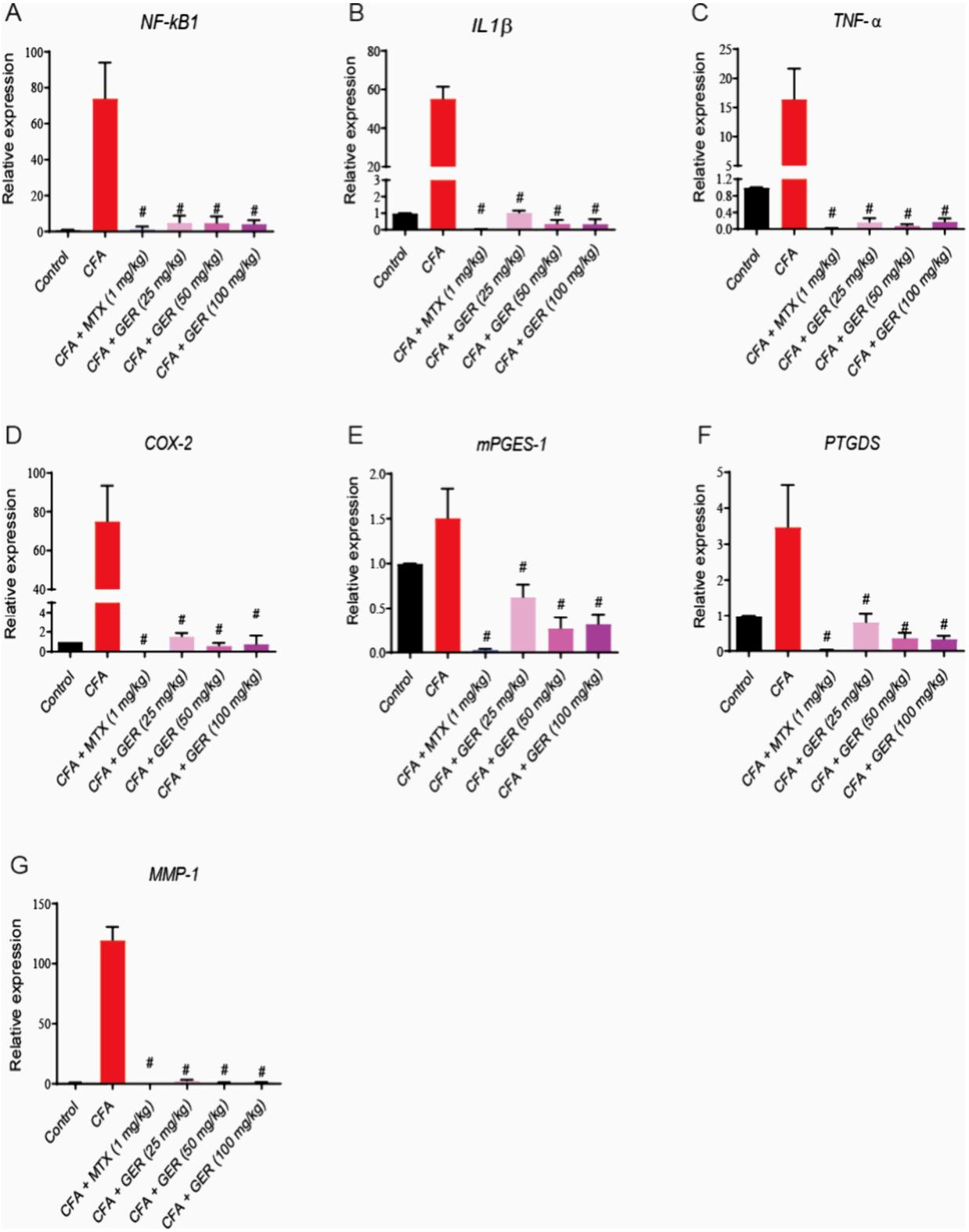


Figure 5. GER attenuated the mRNA levels of CFA-induced pro-inflammatory mediators. IA was induced by injecting 0.1 mL of CFA, and after 7 days of CFA administration, rats were treated with the abovementioned doses of MTX or tested compound (GER). On alternative days, treatments were repeated until day 35. Relative mRNA expressions of indicated genes were measured from liver homogenates. GER significantly reduced (A) *NF-kB1*, (B) *IL-1β*, (C) TNF-*α*, (D) *COX-2*, (E) *mPGES-1*, (F) *PTGDS*, and (G) *MMP-1*. # ≤ 0.001 (one-way ANOVA followed by the Tukey's multiple comparison test); RT-qPCR. Significant acronyms (#) represent the comparison of treatment groups with the disease group (CFA); $n = 6$.

disease because of less specificity.[44] Our findings indicated that GER effectively reduced RF values that were stimulated by CFA. However, RF values did not decline to the normal levels because when the body starts producing RF, the small amount of RF keeps on circulating in the blood even after the remission of the disease.[45]

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

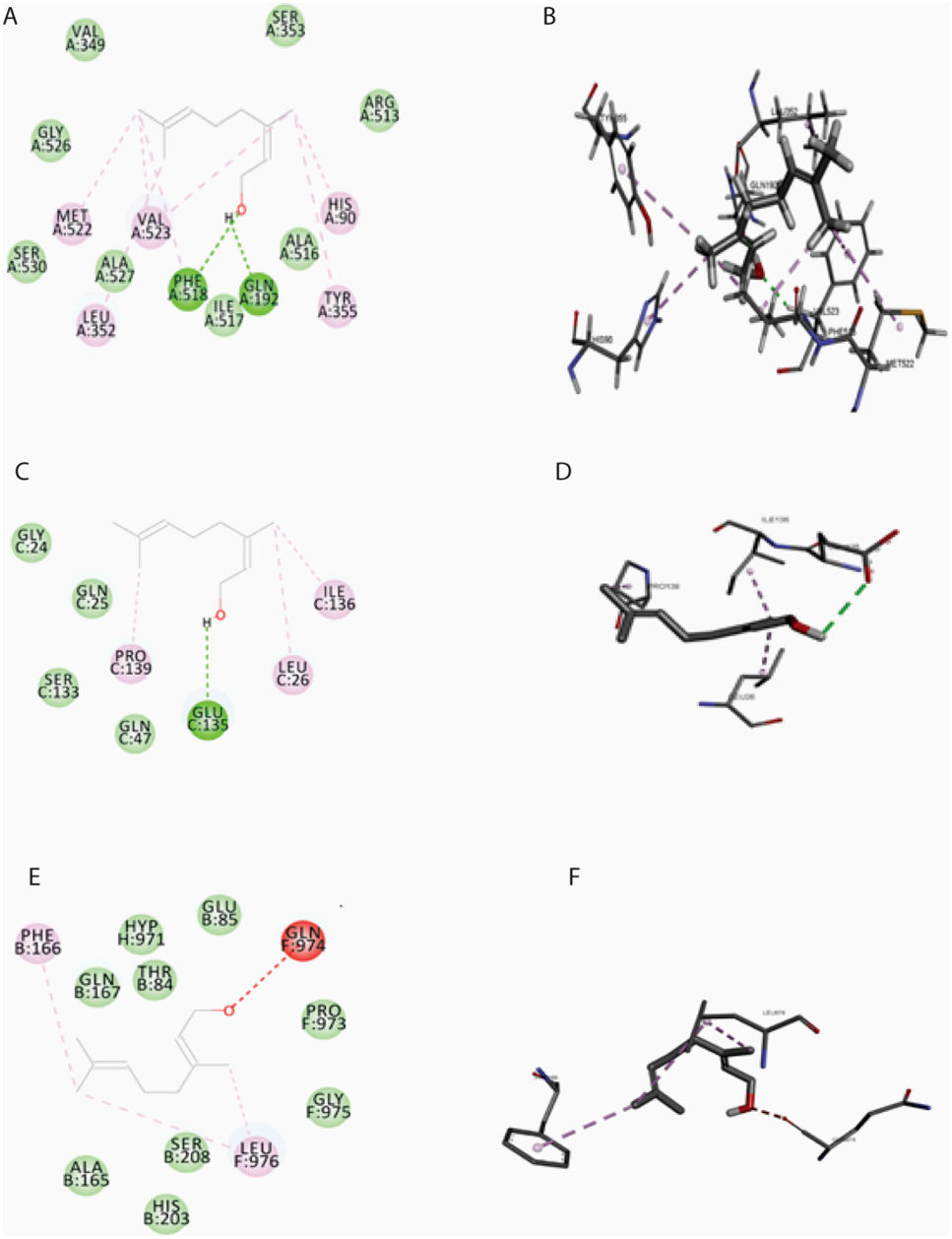


Figure 6. Binding interactions of GER with COX-2, TNF-α, and MMP-1. 2D and 3D presentation of binding interactions of GER with the amino acid residues of the binding site of (A, B) COX-2, (C, D) TNF-α, and (E, F) MMP-1.

To scrutinize the molecular mechanism of GER, we measured different pro-inflammatory mediators, prostaglandin synthases, and MMP-1. GER effectively reduced the pro-inflammatory cytokines (*IL-1β* and TNF-α). Different studies have demonstrated their role in upregulation of inflammation in IA.[46,47] IL-1β and TNF-α are the highly studied cytokines due to their main role in the progression of inflammation. IL-1β is released from both immune and nonimmune cells and is involved in the communication among many cells in the joint. Moreover, it is also involved in bone and cartilage destruction.[46,48] TNF-α is responsible for inflammation, production of other pro-inflammatory cytokines, immune cell recruitment, and their activation. Studies have demonstrated that inhibition of TNF-α reduces the inflammation and production of other cytokines.[24,49]

COX-2 is an enzyme that induces pain and inflammation in the joint by producing prostaglandins in the synovium. Pro-inflammatory cytokines like IL-1β and TNF-α increased the expression of COX-2 in the synovial tissue, which produces prostaglandins, mainly PGE2. Our qPCR investigation demonstrated that GER effectively reduces the expression of *COX-2*, *mPGES-1* (produces PGE2), and *PTGDS* (produces PGD2). Previous studies have also shown that elevated levels of PGD2 are present in synovial fluid and play a key role in the development of vasodilation, edema, and pain in the synovial tissue. PGE2 with other mediators also cause destruction of cartilage and bone.[25,33] Moreover, synoviocytes producing PGE2 alter the metalloproteinase balance.[50]

Apart from pro-inflammatory mediators and prostaglandin synthase, metalloproteinase and other proteases also play crucial roles in the pathogenesis of IA. IL-1β and TNF-α

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

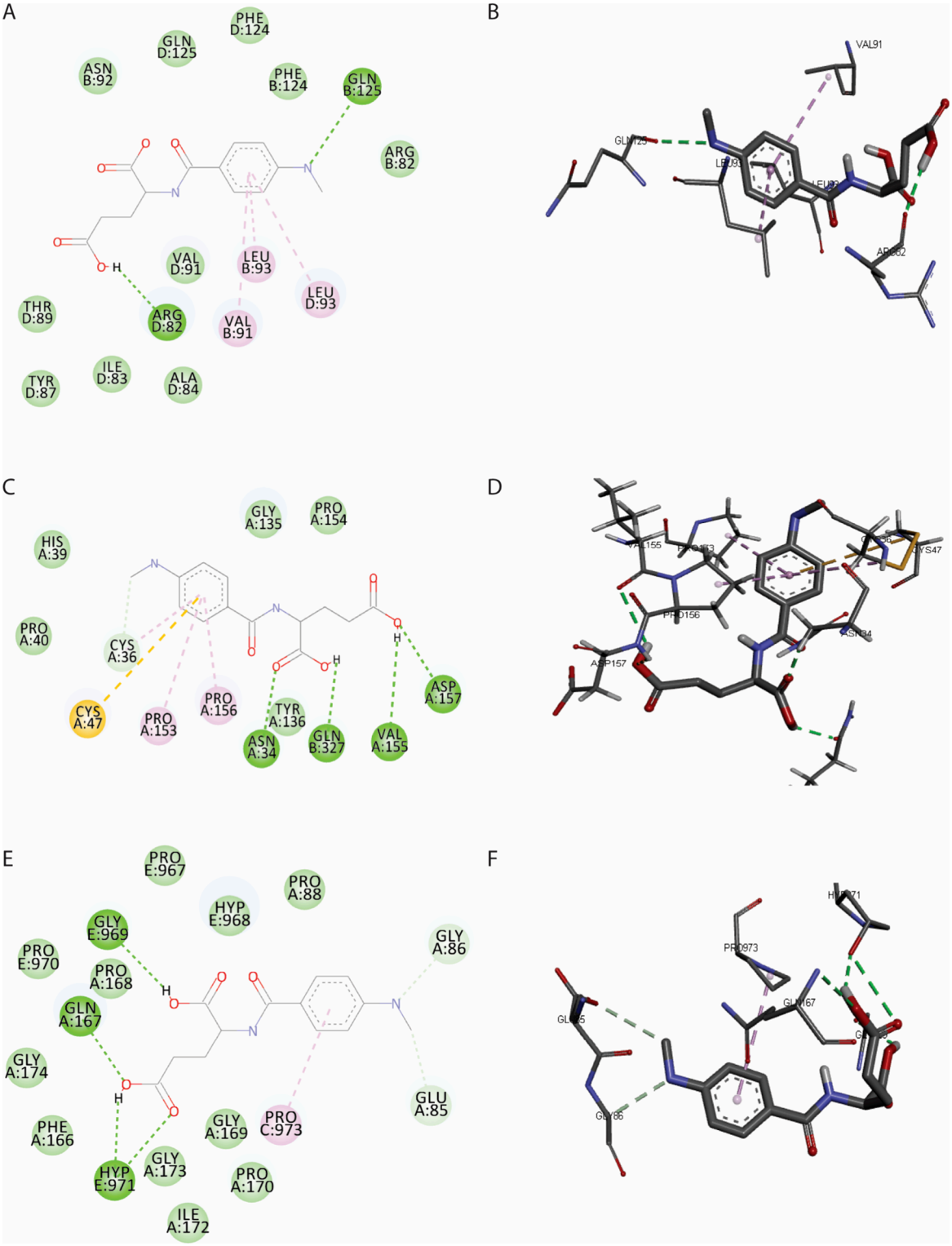


Figure 7. Binding interactions of MTX with COX-2, TNF-α, and MMP-1. 2D and 3D presentation of binding interactions of GER with the amino acid residues of the binding site of (A, B) COX-2, (C, D) TNF-α, and (E, F) MMP-1.

stimulate the production of MMPs that are responsible for the destruction of bone and cartilage. Their levels are used to correlate with tissue damage.[27,51] Our tested compound (GER) showed a downregulation of *MMP-1* expression, which indicates the protective effect of GER against bone and cartilage damage.

CONCLUSIONS

It can be concluded from this study that GER protects against CFA-induced IA in rats via the suppression of pro-inflammatory cytokines, prostaglandins, and MMP1. However, further studies are required to explore the detailed mechanism and to translate these findings in a human model.

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

■ ASSOCIATED CONTENT

Data Availability Statement

Any other relevant material or data can be provided on request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04684.

List of primers for RT-qPCR (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Muhammad Nasir Hayat Malik − *Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad 44000, Pakistan; Faculty of Pharmacy, The University of Lahore, Lahore 54000, Pakistan;* orcid.org/0000-0002-2657-9810; Phone: +92-3348466407; Email: Nasir.hayat@cust.edu.pk

Authors

Muhammad Nouman Tahir − *Faculty of Pharmacy, The University of Lahore, Lahore 54000, Pakistan*

Tariq G. Alsahli − *Department of Pharmacology, College of Pharmacy, Jouf University, Sakaka, Aljouf 72341, Saudi Arabia*

Md. Mahedi Hassan Tusher − *Department of Pharmacology, Faculty of Basic Sciences, Bangladesh University of Health Sciences, Dhaka 1216, Bangladesh*

Sami I. Alzarea − *Department of Pharmacology, College of Pharmacy, Jouf University, Sakaka, Aljouf 72341, Saudi Arabia*

Bader Alsuwayt − *Department of Pharmacy Practice, College of Pharmacy, University of Hafr Al-Batin, Hafr Al-Batin 31991, Saudi Arabia*

Shah Jahan − *Department of Immunology, University of Health Sciences, Lahore 54000, Pakistan*

Hesham A. M. Gomaa − *Department of Pharmacology, College of Pharmacy, Jouf University, Sakaka, Aljouf 72341, Saudi Arabia*

Mohamed E. Shaker − *Department of Pharmacology, College of Pharmacy, Jouf University, Sakaka, Aljouf 72341, Saudi Arabia*

Muhammad Ali − *Faculty of Pharmacy, The University of Lahore, Lahore 54000, Pakistan*

Irfan Anjum − *Faculty of Pharmacy, The University of Lahore, Lahore 54000, Pakistan; Shifa College of Pharmaceutical Sciences, Shifa Tameer-e-Millat University, Islamabad 44000, Pakistan;* orcid.org/0000-0001-5884-8950

Muhammad Tariq Khan − *Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad 44000, Pakistan*

Muhammad Roman − *Department of Microbiology, University of Health Sciences, Lahore 54000, Pakistan*

Ramla Shabbir − *Faculty of Pharmacy, The University of Lahore, Lahore 54000, Pakistan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04684

Author Contributions

◆M.N.H.M., M.N.T., and T.G.A. contributed equally. M.N.H.M., M.N.T., T.G.A., and M.M.H.T. performed the experiments. H.A.M.G. and M.E.S. analyzed the histopathological and X-ray data, while S.J., M.R., S.I.A., M.A., B.A., I.A., and R.S. analyzed the qPCR, hematological, and biochemical data. M.T.K. performed the molecular docking analysis. All authors participated in editing of manuscript. T.G.A. managed to get project funding from the Ministry of Education, Saudi Arabia. M.N.T. wrote the first version of the manuscript. M.N.H.M. designed and supervised the project and edited the final version of the manuscript.

Funding

The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number 223202.

Notes

The authors declare no competing financial interest.

Experiments were performed according to guidelines of OECD and were approved by Institutional Research Ethics Committee of Faculty of Pharmacy, The University of Lahore (approval number: IREC-2021-49).

■ ACKNOWLEDGMENTS

We would like to thank Dr. Ahsan Sattar (Institute of Molecular Biology, The University of Lahore) for providing us the platform for qPCR studies.

■ REFERENCES

(1) McInnes, I. B.; Schett, G. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* **2011**, *365* (23), 2205−2219.

(2) El-Mashharawi, H.; Alshawwa, I.; Elkahlout, M.; Abu-Naser, S. An Expert System for Arthritis Diseases Diagnosis Using SL5 Object. *Int. J. Acad. Health Med. Res.* **2019**, 3 (4), 28−35.

(3) Guo, Q.; Wang, Y.; Xu, D.; Nossent, J.; Pavlos, N. J.; Xu, J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res.* **2018**, *6*, 15.

(4) Szekanecz, Z.; Pakozdi, A.; Szentpetery, A.; Besenyei, T.; Koch, A. E. Chemokines and angiogenesis in rheumatoid arthritis. *Front. Biosci.* **2009**, *1* (1), 44−51. Klareskog, L.; Ronnelid, J.; Lundberg, K.; Padyukov, L.; Alfredsson, L. Immunity to citrullinated proteins in rheumatoid arrthritis. *Annu. Rev. Immunol.* **2008**, *26*, 651−675.

(5) Kinne, R. W.; Brauer, R.; Stuhlmuller, B.; Palombo-Kinne, E.; Burmester, G. R. Macrophages in rheumatoid arthritis. *Arthritis Res.* **2000**, *2* (3), 189−202. Deleuran, B. W.; Chu, C. Q.; Field, M.; Brennan, F. M.; Mitchell, T.; Feldmann, M.; Maini, R. N. Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. Implications for local actions of tumor necrosis factor alpha. *Arthritis Rheum.* **1992**, *35* (10), 1170−1178. Gravallese, E. M. Bone destruction in arthritis. *Ann. Rheum. Dis.* **2002**, *61* (Suppl 2), ii84−86.

(6) Ong, C. K.; Lirk, P.; Tan, C. H.; Seymour, R. A. An evidence-based update on nonsteroidal anti-inflammatory drugs. *Clin. Med. Res.* **2007**, *5* (1), 19−34. From Nlm.

(7) Combe, B.; Landewe, R.; Daien, C. I.; Hua, C.; Aletaha, D.; Alvaro-Gracia, J. M.; Bakkers, M.; Brodin, N.; Burmester, G. R.; Codreanu, C.; et al. 2016 update of the EULAR recommendations for the management of early arthritis. *Ann. Rheum. Dis.* **2017**, *76* (6), 948−959. From Nlm.

(8) Smolen, J. S.; Landewe, R.; Breedveld, F. C.; Dougados, M.; Emery, P.; Gaujoux-Viala, C.; Gorter, S.; Knevel, R.; Nam, J.; Schoels, M.; et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann. Rheum. Dis.* **2010**, *69* (6), 964−975. From Nlm.

(9) Radu, A. F.; Bungau, S. G. Management of Rheumatoid Arthritis: An Overview. *Cells* **2021**, *10* (11), 2857. From Nlm. Tanaka, Y. Recent progress in treatments of rheumatoid arthritis: an overview of developments in biologics and small molecules, and remaining unmet needs. *Rheumatology* **2021**, *60* (Suppl 6), vi12−vi20. From Nlm. Singh, J. A.; Wells, G. A.; Christensen, R.; Tanjong Ghogomu, E.;

Maxwell, L.; Macdonald, J. K.; Filippini, G.; Skoetz, N.; Francis, D.; Lopes, L. C.; et al. Adverse effects of biologics: a network meta-analysis and Cochrane overview. *Cochrane data. Sys. Rev.* **2011**, *2011* (2), CD008794.
(10) Emery, P. Treatment of rheumatoid arthritis. *Bmj* **2006**, *332* (7534), 152−155. Smolen, J. S.; Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat. rev. Rheum.* **2015**, *11* (5), 276−289.
(11) Fleischmann, R. M. Is there a need for new therapies for rheumatoid arthritis? *J. Rheumatol.* **2005**, *73*, 3−7.
(12) Lei, Y.; Fu, P.; Jun, X.; Cheng, P. Pharmacological Properties of Geraniol - A Review. *Planta Med.* **2019**, *85* (1), 48−55.
(13) Cho, M.; So, I.; Chun, J. N.; Jeon, J. H. The antitumor effects of geraniol: Modulation of cancer hallmark pathways (Review). *Int. J. Oncol.* **2016**, *48* (5), 1772−1782.
(14) Wang, J.; Su, B.; Zhu, H.; Chen, C.; Zhao, G. Protective effect of geraniol inhibits inflammatory response, oxidative stress and apoptosis in traumatic injury of the spinal cord through modulation of NF-kappaB and p38 MAPK. *Exp. Ther. Med.* **2016**, *12* (6), 3607−3613.
(15) Hosseini, S. M.; Hejazian, L. B.; Amani, R.; Siahchehreh Badeli, N. Geraniol attenuates oxidative stress, bioaccumulation, serological and histopathological changes during aluminum chloride-hepato-pancreatic toxicity in male Wistar rats. *Environ. Sci. Pollut. Res. Int.* **2020**, *27* (16), 20076−20089.
(16) Murbach Teles Andrade, B. F.; Conti, B. J.; Santiago, K. B.; Fernandes Junior, A.; Sforcin, J. M. Cymbopogon martinii essential oil and geraniol at noncytotoxic concentrations exerted immunomodulatory/anti-inflammatory effects in human monocytes. *J. Pharm. Pharmacol* **2014**, *66* (10), 1491−1496. Murbach Teles Andrade, B. F.; Conti, B. J.; Santiago, K. B.; Fernandes, A.; Sforcin, J. M. Cymbopogon martinii essential oil and geraniol at noncytotoxic concentrations exerted immunomodulatory/anti-inflammatory effects in human monocytes. *J. Pharm. Pharmacol.* **2014**, *66* (10), 1491−1496. (acccessed 12/15/2022). Su, Y. W.; Chao, S. H.; Lee, M. H.; Ou, T. Y.; Tsai, Y. C. Inhibitory effects of citronellol and geraniol on nitric oxide and prostaglandin E(2)production in macrophages. *Planta medica* **2010**, *76* (15), 1666−1671.
(17) Jayachandran, M.; Chandrasekaran, B.; Namasivayam, N. Geraniol attenuates fibrosis and exerts anti-inflammatory effects on diet induced atherogenesis by NF-kappaB signaling pathway. *Eur. J. Pharmacol.* **2015**, *762*, 102−111. Ye, C. J.; Li, S. A.; Zhang, Y.; Lee, W. H. Geraniol targets K(V)1.3 ion channel and exhibits anti-inflammatory activity in vitro and in vivo. *Fitoterapia* **2019**, *139*, No. 104394.
(18) Jiang, K.; Zhang, T.; Yin, N.; Ma, X.; Zhao, G.; Wu, H.; Qiu, C.; Deng, G. Geraniol alleviates LPS-induced acute lung injury in mice via inhibiting inflammation and apoptosis. *Oncotarget* **2017**, *8* (41), 71038−71053. Medicherla, K.; Sahu, B. D.; Kuncha, M.; Kumar, J. M.; Sudhakar, G.; Sistla, R. Oral administration of geraniol ameliorates acute experimental murine colitis by inhibiting pro-inflammatory cytokines and NF-kappaB signaling. *Food Funct.* **2015**, *6* (9), 2984−2995. El-Emam, S. Z.; Soubh, A. A.; Al-Mokaddem, A. K.; Abo El-Ella, D. M. Geraniol activates Nrf-2/HO-1 signaling pathway mediating protection against oxidative stress-induced apoptosis in hepatic ischemia-reperfusion injury. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2020**, *393* (10), 1849−1858.
(19) Zubair, A. M.; Malik, M. N. H.; Younis, W.; Malik, M. A. H.; Jahan, S.; Ahmed, I.; Yuchi, A.; Mushtaq, M. N.; Tahir, R.; Sarwar, M. B.; et al. Novel acetamide derivatives of 2-aminobenzimidazole prevent inflammatory arthritis in rats via suppression of pro-inflammatory mediators. *Inflammopharmacology* **2022**, *30* (3), 1005−1019.
(20) Willard, L.; Ranjan, A.; Zhang, H.; Monzavi, H.; Boyko, R. F.; Sykes, B. D.; Wishart, D. S. VADAR: a web server for quantitative evaluation of protein structure quality. *Nucleic Acids Res.* **2003**, *31* (13), 3316−3319.
(21) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31* (2), 455−461.
(22) Paleolog, E. M. Angiogenesis in rheumatoid arthritis. *Arthritis Res.* **2002**, *4* (Suppl 3), S81−90. Makarov, S. S. NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res.* **2001**, *3* (4), 200−206. Schiff, M. H. Role of interleukin 1 and interleukin 1 receptor antagonist in the mediation of rheumatoid arthritis. *Ann. Rheum. Dis.* **2000**, *59* (Suppl 1), i103−108. Wang, M.; Zhou, Y.; Huang, W.; Zeng, Y.; Li, X. Association between matrix metalloproteinase-1 (MMP-1) protein level and the risk of rheumatoid arthritis and osteoarthritis: a meta-analysis. *Braz. J. Med. Biol. Res.* **2020**, *54* (2), No. e10366. Woods, J. M.; Mogollon, A.; Amin, M. A.; Martinez, R. J.; Koch, A. E. The role of COX-2 in angiogenesis and rheumatoid arthritis. *Exp. Mol. Pathol.* **2003**, *74* (3), 282−290.
(23) Paleolog, E. M.; Fava, R. A. Angiogenesis in rheumatoid arthritis: implications for future therapeutic strategies. In *Springer seminars in immunopathology*, 1998; Springer: Vol. *20*, pp 73−94.
(24) Farrugia, M.; Baron, B. The role of TNF-alpha in rheumatoid arthritis: a focus on regulatory T cells. *J. Clin. Transl. Res.* **2016**, *2* (3), 84−90.
(25) Fattahi, M. J.; Mirshafiey, A. Prostaglandins and rheumatoid arthritis. *Arthritis* **2012**, *2012*, No. 239310.
(26) Mohammed, F. F.; Smookler, D. S.; Khokha, R. Metalloproteinases, inflammation, and rheumatoid arthritis. *Ann. Rheum. Dis.* **2003**, *62* (Suppl 2), ii43−ii47.
(27) Green, M. J.; Gough, A. K.; Devlin, J.; Smith, J.; Astin, P.; Taylor, D.; Emery, P. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology* **2003**, *42* (1), 83−88. Burrage, P. S.; Mix, K. S.; Brinckerhoff, C. E. Matrix metalloproteinases: role in arthritis. *Front. Biosci.* **2006**, *11*, 529−543.
(28) Santos, P. L.; Matos, J.; Picot, L.; Almeida, J.; Quintans, J. S. S.; Quintans-Junior, L. J. Citronellol, a monoterpene alcohol with promising pharmacological activities - A systematic review. *Food Chem. Toxicol.* **2019**, *123*, 459−469, DOI: 10.1016/j.fct.2018.11.030.
(29) Snekhalatha, U.; Anburajan, M.; Venkatraman, B.; Menaka, M. Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Comparison of thermography and histopathology. *Zeitschrift fur Rheumatologie* **2013**, *72* (4), 375−382. Almarestani, L.; Fitzcharles, M. A.; Bennett, G. J.; Ribeiro-da-Silva, A. Imaging studies in Freund's complete adjuvant model of regional polyarthritis, a model suitable for the study of pain mechanisms, in the rat. *Arthritis Rheum.* **2011**, *63* (6), 1573−1581. Shirani, K.; Iranshahi, M.; Askari, V. R.; Gholizadeh, Z.; Zadeh, A. A.; Zeinali, M.; Hassani, F. V.; Taherzadeh, Z. Comparative evaluation of the protective effects of oral administration of auraptene and umbelliprenin against CFA-induced chronic inflammation with polyarthritis in rats. *Biomed. Pharmacother.* **2021**, *139*, No. 111635.
(30) El-Ghazaly, M. A.; Fadel, N. A.; Abdel-Naby, D. H.; Abd El-Rehim, H. A.; Zaki, H. F.; Kenawy, S. A. Potential anti-inflammatory action of resveratrol and piperine in adjuvant-induced arthritis: Effect on pro-inflammatory cytokines and oxidative stress biomarkers. *Egyptian Rheumatologist* **2020**, *42* (1), 71−77.
(31) Fehrenbacher, J. C.; Vasko, M. R.; Duarte, D. B. Models of inflammation: carrageenan-or complete freund's adjuvant (CFA)−induced edema and hypersensitivity in the rat. *Current protocols in pharmacology* **2012**, *56* (1), 5.4. 1−5.4. 4.
(32) Paleolog, E. M. Angiogenesis in rheumatoid arthritis. *Arthritis Res. Ther.* **2002**, *4* (3), 1−10. Woolley, D. E. Cartilage Destruction. *Mech. Models Rheumatoid Arthritis* **1995**, *115*. Jung, Y. K.; Kang, Y. M.; Han, S. Osteoclasts in the Inflammatory Arthritis: Implications for Pathologic Osteolysis. *Immune network* **2019**, *19* (1), No. e2.
(33) Crofford, L. J.; Wilder, R. L.; Ristimaki, A. P.; Sano, H.; Remmers, E. F.; Epps, H. R.; Hla, T. Cyclooxygenase-1 and −2 expression in rheumatoid synovial tissues. Effects of interleukin-1 beta, phorbol ester, and corticosteroids. *J. Clin. Invest.* **1994**, *93* (3), 1095−1101.
(34) Bowman, S. J. Hematological manifestations of rheumatoid arthritis. *Scand J. Rheumatol* **2002**, *31* (5), 251−259. Baer, A. N.;

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139

Dessypris, E. N.; Goldwasser, E.; Krantz, S. B. Blunted erythropoietin response to anaemia in rheumatoid arthritis. *Br. J. Hamaetol.* **1987**, *66* (4), 559−564.
(35) Tishkowski, K.; Gupta, V. Erythrocyte Sedimentation Rate. In *StatPearls [Internet]*; StatPearls Publishing, 2021.
(36) Curtis, J. R.; Beukelman, T.; Onofrei, A.; Cassell, S.; Greenberg, J. D.; Kavanaugh, A.; Reed, G.; Strand, V.; Kremer, J. M. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. *Ann. Rheum. Dis.* **2010**, *69* (01), 43−47.
(37) Bowman, S. Hematological manifestations of rheumatoid arthritis. *Scand. J. Rheumatol.* **2002**, *31* (5), 251−259. Tatro, D. *Textbook of therapeutics, drug and disease management*. William & Wilkins: Baltimore 1996, 33−44. Baer, A. N.; Dessypris, E. N.; Goldwasser, E.; Krantz, S. B. Blunted erythropoietin response to anaemia in rheumatoid arthritis. *Br. J. Haematol.* **1987**, *66* (4), 559−564. Baer, A. N.; Dessypris, E. N.; Krantz, S. B. The pathogenesis of anemia in rheumatoid arthritis: a clinical and laboratory analysis. *Semin. Arthritis Rheum.* **1990**, *19* (4), 209−223.
(38) Harrison, M. Erythrocyte sedimentation rate and C-reactive protein. *Australian prescriber* **2015**, *38* (3), 93−94.
(39) Giles, J. T.; Bartlett, S. J.; Andersen, R.; Thompson, R.; Fontaine, K. R.; Bathon, J. M. Association of body fat with C-reactive protein in rheumatoid arthritis. *Arthritis Rheum.* **2008**, *58* (9), 2632−2641. van Leeuwen, M. A.; van Rijswijk, M. H.; van der Heijde, D. M.; Te Meerman, G. J.; van Riel, P. L.; Houtman, P. M.; van De Putte, L. B.; Limburg, P. C. The acute-phase response in relation to radiographic progression in early rheumatoid arthritis: a prospective study during the first three years of the disease. *Br J. Rheum.* **1993**, *32* (suppl_3), 9−13.
(40) Radovanović-Dinić, B.; Tešić-Rajković, S.; Zivkovic, V.; Grgov, S. Clinical connection between rheumatoid arthritis and liver damage. *Rheumatol. Int.* **2018**, *38* (5), 715−724. Hartmann, U.; Schmitt, S.; Reuss-Borst, M. Elevated liver enzymes in rheumatoid arthritis: differential diagnostic considerations based on a case report. *Zeitschrift fur Rheumatologie* **2008**, *67* (5), 440−444. Conway, R.; Carey, J. J. Risk of liver disease in methotrexate treated patients. *W. J. Hepa.* **2017**, *9* (26), 1092−1100.
(41) Bugatti, S.; Manzo, A.; Montecucco, C.; Caporali, R. The Clinical Value of Autoantibodies in Rheumatoid Arthritis. *Front Med.* **2018**, *5* (339), 339.
(42) van Boekel, M. A.; Vossenaar, E. R.; van den Hoogen, F. H.; van Venrooij, W. J. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res.* **2002**, *4* (2), 87−93.
(43) Ingegnoli, F.; Castelli, R.; Gualtierotti, R. Rheumatoid factors: clinical applications. *Dis Markers* **2013**, *35* (6), 727−734.
(44) Aggarwal, R.; Liao, K.; Nair, R.; Ringold, S.; Costenbader, K. H. Anti-citrullinated peptide antibody (ACPA) assays and their role in the diagnosis of rheumatoid arthritis. *Arthritis Rheum.* **2009**, *61* (11), 1472. Kurowska, W.; Kuca-Warnawin, E. H.; Radzikowska, A.; Maslinski, W. The role of anti-citrullinated protein antibodies (ACPA) in the pathogenesis of rheumatoid arthritis. *Cent Eur. J. Immunol* **2017**, *42* (4), 390−398.
(45) Ingegnoli, F.; Castelli, R.; Gualtierotti, R. Rheumatoid factors: clinical applications. *Disease markers* **2013**, *35* (6), 727−734.
(46) Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. NF-κB signaling in inflammation. *Signal Transduction Targeted Ther.* **2017**, *2* (1), 17023.
(47) Wang, Y.; Mao, L.; Zhang, L.; Zhang, L.; Yang, M.; Zhang, Z.; Li, D.; Fan, C.; Sun, B. Adoptive Regulatory T-cell Therapy Attenuates Subarachnoid Hemor-rhage-induced Cerebral Inflammation by Suppressing TLR4/NF-B Signaling Pathway. *Curr. Neurovasc. Res.* **2016**, *13* (2), 121−126.
(48) Zhang, J. M.; An, J. Cytokines, inflammation, and pain. *Int. Anesthesiol Clin* **2007**, *45* (2), 27−37. Kay, J.; Calabrese, L. *The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. Rheumatology* (Oxford, England), 2004, *43* Suppl 3, iii2-iii9. DOI: 10.1093/rheumatology/keh201. Schiff, M. H. Role of interleukin 1 and interleukin 1 receptor antagonist in the mediation of rheumatoid arthritis. *Ann. Rheum. Dis.* **2000**, *59* (Suppl 1), i103−108.
(49) Manara, M.; Sinigaglia, L. Bone and TNF in rheumatoid arthritis: clinical implications. *RMD open* **2015**, *1* (Suppl1), No. e000065.
(50) Baratelli, F. E.; Heuze-Vourc'h, N.; Krysan, K.; Dohadwala, M.; Riedl, K.; Sharma, S.; Dubinett, S. M. Prostaglandin E2-dependent enhancement of tissue inhibitors of metalloproteinases-1 production limits dendritic cell migration through extracellular matrix. *J. Immunol.* **2004**, *173* (9), 5458−5466.
(51) Mohammed, F.; Smookler, D.; Khokha, R. Metalloproteinases, inflammation, and rheumatoid arthritis. *Ann. Rheum. Dis.* **2003**, *62* (suppl 2), iie43−ii47.

https://doi.org/10.1021/acsomega.3c04684
ACS Omega 2023, 8, 37128−37139