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Original Article

A Combination of *Thymus vulgaris*, *Oliveria Decumbens*, and *Anthemis Hyaline* Extracts Alleviate Rheumatoid Arthritis in a Rat Model

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ABSTRACT

It has been shown that herbal medicine can be a promising therapeutic option for rheumatoid arthritis (RA). The purpose of this study was to investigate the effectiveness of a combination of *Thymus vulgaris*, *Oliveria decumbens*, and *Anthemis hyaline* in relieving both articular and extra-articular symptoms of RA in rats. The animals were divided into three groups: a healthy control without RA or any treatment, RA animals treated with a combination extract injection, and RA animals without any intervention. After disease induction, a combination of hydroethanolic plant extract was injected intra-articularly. Levels of inflammatory factors, rheumatoid factor (RF), C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, and IL-17 were measured. The activities of enzymes involved in oxidative stress, catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were also assessed. The RA animals treated with the combination extract showed improvements in body weight, reductions in arthritis score, and reduction in hind paw volume. Histological assessments revealed decreased bone destruction and inflammatory cell infiltration after treatment with the combination extract. Histopathological observations indicated that the combination extract led to improvements in RA-induced alterations in kidney and liver tissues. The levels of liver health markers, aspartate aminotransferase (AST) and alanine transaminase (ALT), as well as renal markers, urea and creatinine, decreased with combination extract treatment. These findings demonstrate the anti-inflammatory and antioxidant properties of the combination extracts of *T. vulgaris*, *O. decumbens*, and *A. hyaline*, proposing that they could be further investigated as potential therapeutic agents to alleviate the severity and complications of RA.

Keywords: Rheumatoid arthritis, Herbal medicine, *Thymus vulgaris*, *Oliveira decumbens*, *Anthemis hyaline*

INTRODUCTION

Rheumatoid arthritis (RA) stands as one of the main global health issues that imposes high costs on healthcare systems worldwide [1]. This systemic chronic inflammatory disorder affects approximately 1% of adults globally [2]. Although the exact cause of RA is not yet fully understood, the etiology of RA, it is widely accepted that the disease's pathogenesis is linked to a dysfunctional immune system, including dysregulated cytokine/chemokine networks and immune complex-mediated complement activation [3,4]. Pharmacological options are commonly used to regulate the aberrant immune responses in RA patients [5]. However, these treatments often face limitations, such as serious hepatorenal toxicity [6], incomplete response or drug resistance [7], and the high costs of biologicals [8]. Therefore, there is a need for the development of safer, more effective, accessible, and affordable therapeutics to ameliorate and treat RA.

Recently, there has been a growing interest in herbal medicine for managing RA. This attention has arisen from the perceived safety and potential effectiveness of herbal remedies [9-11]. Patients with RA have turned to medicinal plants in the hope of either curing the disease or alleviating symptoms such as pain, edema, inflammation, swelling, joint stiffness, and loss of mobility (as reviewed in sources [12,13]). Bioactive compounds found in plants such as terpenoids, flavonoids, alkaloids, and polyphenols, have shown promising antioxidant, anti-inflammatory, and analgesic properties [14]. Therefore, they can be considered for controlling and improving RA.

The genus *Anthemis* is found in the Mediterranean region, as well as in Southwest Asia, with 39 species growing wild in Iran [15,16]. Extracts, tinctures, salves, and teas derived from *Anthemis* are commonly used for their anti-spasmodic and anti-bacterial properties [17]. *Thymus vulgaris* has a history of traditional use for inflammation-related conditions such as muscle swelling, pains, and cold [18,19]. *Oliveria decumbens* Vent. is an endemic herb found in the southern and western regions of Iran. It is traditionally used in Persian medicine to treat infections, gastrointestinal issues, abdominal pains, and fever [20, 21]. While scientific information on *O. decumbens* is limited, recent studies have confirmed its anti-microbial and antioxidant activities [20, 22].

In this study, we aimed to explore the potential anti-RA effects of a combination of three medicinal plants. We established an RA model in Sprague Dawley rats using Complete Freund's adjuvant (CFA) via intradermal injection. We then injected a combination of *T. vulgaris*, *O. decumbens*, and *A. hyaline* hydroethanolic extract directly into the joints to investigate their potent anti-arthritic effects in the animal model. Additionally, we examined the protective effects of this combination extract against extra-articular manifestations of RA in the kidney and liver.

MATERIALS AND METHODS

Plant Extract

The aerial parts of *T. vulgaris*, *O. decumbens*, and *A. hyaline* were collected from the Faculty of Agriculture at Razi University in the early spring. The taxonomy of the plants was confirmed by Dr. Mohammad Masoumi, a plant taxonomist at Razi University in Kermanshah, Iran. The plants were dried at room temperature away from light, then coarsely powdered with a grinder. Five grams of each plant (15 grams total) were extracted by soaking them in a 70:30 ratio of ethanol and water for 48 h in a dark condition. During this time, the container was shaken several times to ensure the material was in contact with the solvent. The extract obtained was filtered three times using Whatman® filter paper No. 2. The extract was then concentrated using a rotary evaporator at 40 °C. Next, the concentrated extract was poured into laboratory tubes and centrifuged at 4500 rpm for 10 min to separate suspended particles. Since the joint pH reaches 6 in RA, the pH of the final combined extract was adjusted to this level using 1 M sodium hydroxide [23]. At the time of injection, 10 mg of the extract was dissolved in 90 µl of distilled water for the intra-articular injection.

Total Phenolic Content (TPC)

The TPC of hydroethanolic extracts of the combination and each of the plants was assessed using the Folin-Ciocalteu assay. To do this, 50 µl of diluted extract and 450µl of distilled water were added to 2.5 ml of Folin-Ciocalteu reagent (diluted 1:10), followed by the addition of 2 ml of 7% w/v sodium carbonate. The mixture was then incubated in the dark for 1 h. The absorbance of the solutions was measured using a spectrophotometer at 760 nm. Results were calculated using a standard curve of gallic acid and expressed as mg gallic acid equivalent (GAE) per 100 g dry matter (DM) [24].

Animals, Establishment of RA Model, and Injection of Combination Extract

The study was approved by the Kermanshah University of Medical Sciences Ethics Committee (No: IR.KUMS.AEC.1401.028). Twenty-one female inbred Sprague Dawley rats (220-240 g) were housed in polycarbonate cages in an animal laboratory with 12 h of light/dark cycles, at 30-32 °C. The rats were randomly divided into three groups (n = 7):

- I: Healthy control group without RA and any treatment intervention
- II: RA animals treated with the combination extract injection
- III: RA animals without intervention

After one day of adaptation to the environmental conditions, the model was created according to a previous study [25]. The rats were euthanized by intraperitoneal injection of 50 mg/kg ketamine hydrochloride and 2 mg/kg xylazine hydrochloride (both from Alfasan, Woerden, Holland). The 100 µl of CFA solution was injected into the tail, 1-2 cm from the body, towards the body for consistent drainage to the inguinal lymph nodes, and at two sites on the back. After 7 days, the rats were administered 0.1 ml of CFA as a booster via subcutaneous injection on the back. These rats were then assigned to the RA. The healthy control group did not receive any compound. The rats were checked three times a week to assess the establishment of arthritis. Between days 11 and 13 after the initial induction of CFA and disease onset, the symptoms of arthritis became more pronounced as the disease progressed. At this point, rats that had successfully developed RA were randomly assigned to either Group II or Group III.

On day 14 after the primary immunization, the combination extract suspended in 300 µl of normal saline containing 5% human serum albumin (HSA) was injected into both knee joints. Control rats received an infusion of 300 µl normal saline containing 5% HSA. After the injections, animals were closely monitored for 2 h for any issues such as profound paralysis that inhibited their ability to eat or drink, squinted eyes, ataxia, or labored breathing. Subsequently, monitoring and evaluations were conducted every three days until the animals were sacrificed on day 25. Rats were sacrificed under ketamine anesthesia by cervical dislocation as described in the "AVMA Guidelines for the Euthanasia of Animals", and tissue and blood samples were obtained.

Body Weight, Organ Weight, and Hind Paw Thickness

Body weight was assessed using a digital scale with an accuracy of 0.01 g. After completing the experiment, the liver and kidneys were removed from the animals, washed, and weighed. Inflammation levels were assessed using a Plethysmometer (Ugo Basile, USA). Paw swelling was measured using dial calipers to determine the average thickness of the ankle joints in both hind paws.

Arthritis Score

Signs of arthritis were assessed by two independent observers, and the severity of arthritis was scored individually using a scoring system [26]. In this system, the points are as follows: no erythema and swelling: 0, erythema and mild swelling confined to the tarsals or metatarsals: 1; erythema and moderate swelling of the tarsal and metatarsal or tarsal and ankle joints: 2; erythema and severe swelling extending from the ankle to metatarsal joints: 3; erythema and severe swelling encompassing the ankle, foot, and digits: 4. The severity and progression of the disease were determined by scoring all four paws of each rat.

Sample Collection

Animals were humanely euthanized on day 25, and their hind paws, kidneys, and livers were collected. Tissues were fixed in 10% formalin (pH 7.2) for histological assessments. Blood samples (3 ml for each animal) were obtained via cardiac puncture, centrifuged at 3000 rpm for 10 min, and the harvested sera were stored at -80 °C until analysis.

Inflammation and Tissue Injury Markers

Levels of two enzymes, AST and ALT, were measured to assess liver damage. Creatinine and urea were determined to evaluate kidney function. Inflammation was investigated through RF and CRP levels. All kits were purchased from Pars Peyvand (Iran), and experiments were conducted according to the manufacturer's instructions.

Antioxidant/prooxidant Activity

SOD, CAT, and GSH levels were measured using kits from Navand Salamt Co. (Iran) according to the manufacturer's protocols.

Enzyme-linked Immunosorbent Assay (ELISA) Assay

The serum levels of proinflammatory cytokines: IL-1β (Cat No: RLB00), TNF-α (Cat No: RTA00), IL-6 (Cat No: R6000B), and IL-17 (Cat No: M170), as well as the anti-inflammatory IL-10 (Cat No: R1000, R&D, USA), were detected using ELISA assay kits from R&D Systems, MN, USA. Protein levels were measured following the manufacturer's protocols.

Histological Evaluations

The paws that had been formalin-fixed were decalcified using 10% ethylenediaminetetraacetic acid (EDTA) for 14 days at room temperature, then processed and embedded in paraffin. The kidney and liver tissues were fixed for 3 days. Sections were stained with hematoxylin and eosin (H&E). The histological slides were examined using light microscopy (Olympus BX51; Olympus, Japan) by two operators in a blind manner. Any changes in the experimental histopathologic parameters were recorded for each group.

Statistical Analysis

Analyses were performed using IBM SPSS Statistics (v. 19). One-way ANOVA was used to assess differences between groups. Data is expressed as mean ± standard deviation (SD). A P-value of < 0.05 was considered statistically significant.

RESULTS

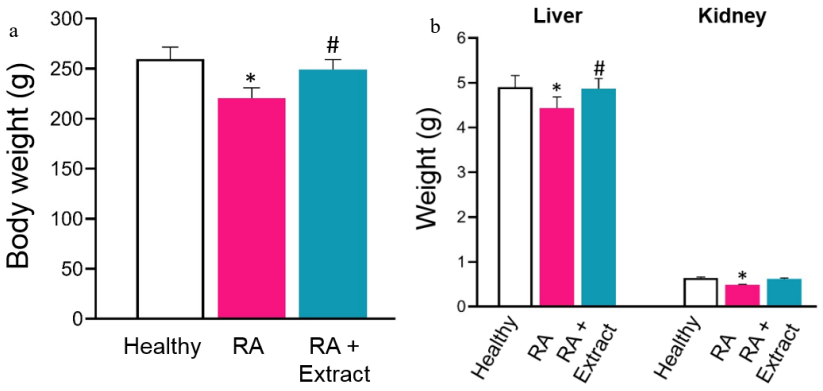
Total Phenolic Content of Combination Extract

TPC (based on mg GAE/g DM) of hydroethanolic extracts of thyme, *O. decumbens,* and *A. hyaline* was 51 ± 4, 42 ± 8, and 87 ± 6, respectively. The TPC value for the combination extract was 114 ± 2.

Effect of Combination Extract on body Weight, Weight of Organs, Arthritis Score, and Paw Thickness of RA-induced Rats

The treatment started on the 14th day after the initial immunization and continued until the 25th day. As shown in Fig. 1b, animals with RA had significantly decreased body weight, as well as lower weights of their kidneys and liver compared to healthy rats (Fig. 1a). The loss of body weight followed a time-dependent trend during the model establishment. Administration of the combination extract led to a significant improvement in body weight compared to the RA group. The weights of the kidneys and liver were decreased in the RA-induced group but improved in the combination extract-treated animals (Fig. 1b). These improvements in weight gain in the combination extract group were accompanied by a 42.7% increase in food intake (data not shown).

In the CFA vehicle group, swelling and erythema in the hind paw reached maximum intensity on day 4 (first swelling phase). From day 14 to 21 after primary immunization, erythema and swelling in the toes, ankles, and tarsus were evident compared to the healthy control (second swelling phase). A noticeable increase in hind paw thickness and arthritis severity score was observed in RA animals (Fig. 1 c, d). The combination extract resulted in a decrease in paw thickness and arthritis score in animals with RA (Fig. 1 c, d). These results indicate that the extract injection significantly suppressed the second swelling phase.



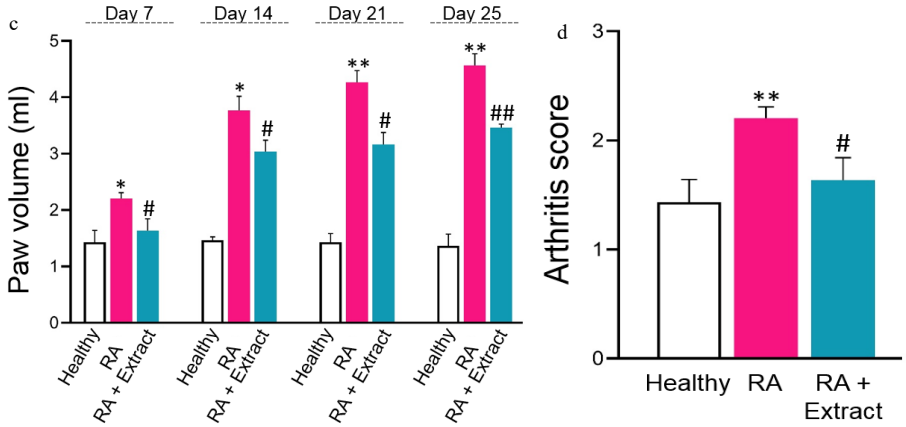


Fig. 1 Effect of combination extract on body weight (a), weights of kidney and liver (b), hind paw thickness (c), and arthritis score (d) of rats. Healthy: Healthy control animals; RA: RA-induced animals; RA + Extract: RA-induced rats received the combination extract. Data is presented as the mean ± SD (n = 7). '#' indicates statistical significance compared to the control, and '*' represents statistical significance compared to the RA-induced group. *p < 0.05, **p < 0.01; # p < 0.05, ## p < 0.01

Effect of Combination Extract on Tissue Function/Injury and Inflammatory Markers in RA-Induced Rats

The serum levels of CRP and RF, inflammatory markers, were remarkably increased in the CFA-induced group compared to the control. Injection of the combination extract decreased their levels (Fig. 2a). Arthritic rats induced with CFA showed a significant increase in the levels of hepatic damage indicators AST and ALT compared to the healthy control group. A noticeable decrease in these enzymes was observed in arthritic rats treated with the combination extract compared to RA animals (Fig. 2b). To assess the alterations in kidney function, urea and creatinine levels were investigated. The urea and creatinine levels showed increased values in the RA group and were decreased in combination extract-treated animals (Fig. 2c).

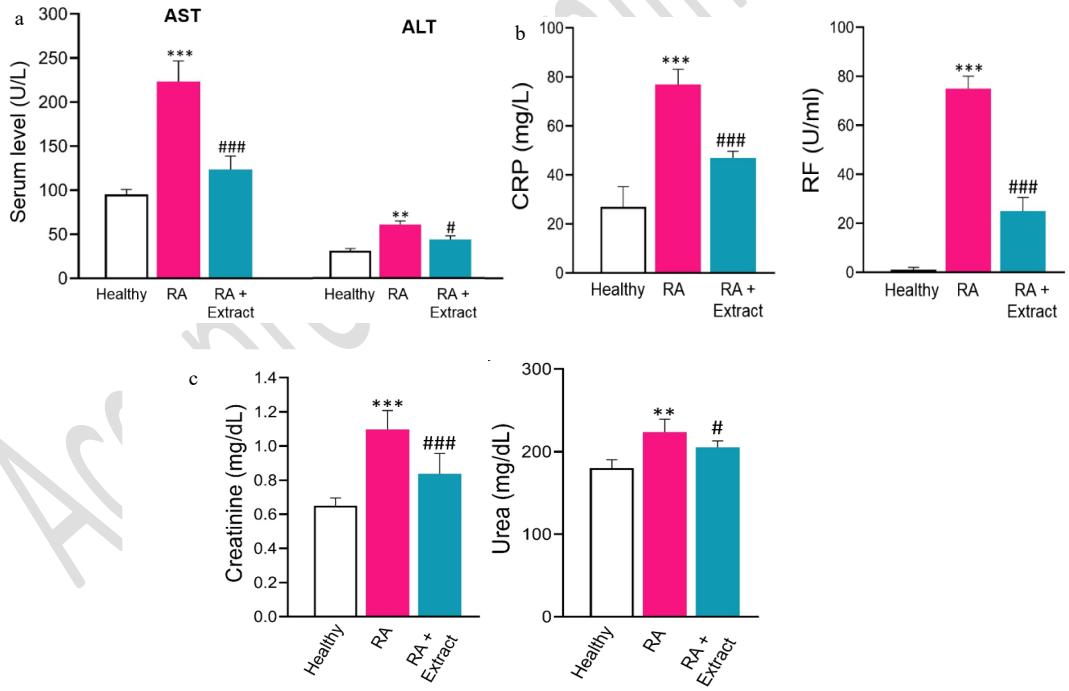


Fig. 2 Effects of combination extract on inflammatory markers C-reactive protein (CRP) and Rheumatoid factor (RF) (a), liver function markers aspartate transaminase (AST) and alanine transaminase (ALT) (b), renal injury markers urea and creatinine (c) in rats. Healthy: Healthy control animals; RA: RA-induced animals; RA + Extract: RA-induced rats treated with the combination extract. The data indicate the mean ± SD (n = 3). '#' represents statistical significance compared to the control, while '*' indicates statistical significance compared to the RA-induced group. **p < 0.01, ***p < 0.001; #p < 0.05, ### p < 0.001

Effect of Combination Extract on Oxidative Stress in RA-induced Rats

The activities of antioxidant enzymes SOD, CAT, and GSH declined in animals with RA. All of these changes were significantly modulated in the RA group that received the combination extract (Fig. 3).

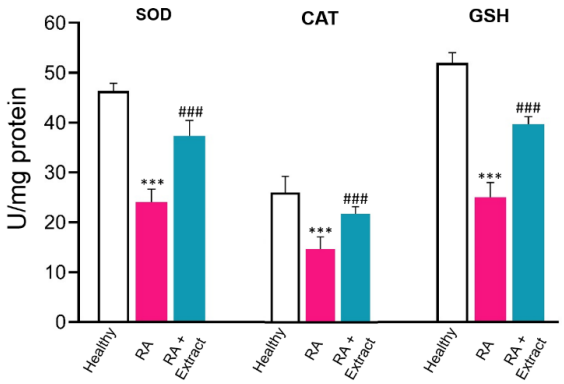


Fig. 3 Effect of combination extract on oxidative stress markers in CFA-induced arthritic rats. Healthy: Healthy control animals; RA: RA-induced animals; RA + Extract: RA-induced rats treated with the combination extract. The data indicate the mean ± SD (n = 4). '#' represents statistical significance compared to control, and '*' represents statistical significance compared to RA-induced group. ***p < 0.001; ### p < 0.001

Effects of Combination Extract on Levels of Inflammation in Rats with RA

To examine the impact of RA induction and subsequent improvements in disease severity and progression after receiving combination extract injection, the serum levels of proteins involved in the inflammatory response were measured. The levels of TNF-α, IL-1β, IL-6, and IL-17 were found to be elevated in the arthritis-induced group, but the combination extract effectively suppressed their expression (Fig. 4). Conversely, CFA effectively inhibited the expression of IL-10 (Fig. 4), and its value became closer to the level of the healthy control following the combination extract injection (Fig. 4).

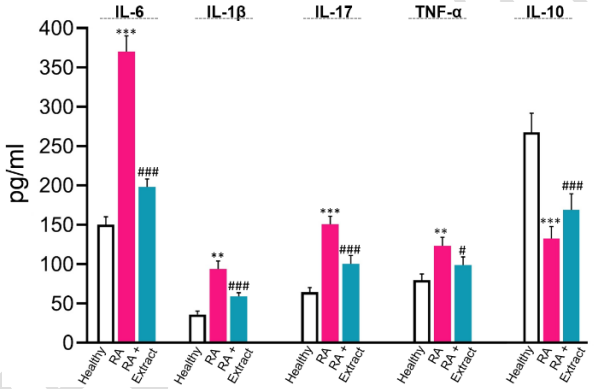


Fig. 4 Effect of combination extract on secretion of proteins involved in inflammation in CFA-induced arthritic rats. Healthy: Healthy control animals; RA: RA-induced animals; RA + Extract: RA-induced rats received the combination extract. The data indicate the mean ± standard deviation (n = 4). '#' indicates statistical significance compared to the control, and '*' represents statistical significance compared to the RA-induced group. **p < 0.01, ***p < 0.001; # p < 0.05, ###p < 0.001

Effect of Combination Extract on Histopathological Features of Hind Paw, Kidney and Liver in Rats

H&E staining of the hind paws of healthy rats showed normal articular cartilage with an intact joint space, and the synovial tissue did not exhibit any architectural changes (Fig. 5a). In the CFA-induced group, synovial hyperplasia was detected (Fig. 5b). Additionally, the height of the articular cartilage layer was decreased, and point destruction was observed. In the combination extract-treated group, the articular cartilage layer developed, and the number of point destructions decreased. In RA animals treated with the combination extract injection, a significant decrease in bone/cartilage destruction and improvements in blood supply were observed compared to the healthy control group. Moreover, animals treated with the combination extract showed lower inflammatory cell infiltration compared to the CFA-induced control (Fig. 5c). In CFA-induced rats, histopathological scores indicated significant increases in inflammation, angiogenesis, chondrocyte hyperplasia, cartilage destruction, bone resorption, and sclerosis (Fig. 5b). However, all of these scores showed improvement following treatment with the combination extract (Fig. 5c). The histopathological scoring of cartilage tissue is summarized in Figure 6d.

In the RA-untreated group, epithelial cell necrosis and moderate tubular dilatation were observed in some proximal and distal tubules (Fig. 6b). Protein casts were formed in the lumen of kidney tubules, Bowman's space was narrowed, and tubular cells became vacuolated (Fig. 6a). In RA animals treated with combination extract, the severity of necrotic changes in the glomeruli and tubules of the renal cortex was less than those in the untreated group (Fig. 6c). In addition, the number of red blood cells in the glomerulus, the amount of protein cast in the renal tubule lumen, and the vacuolation of tubular cells were all decreased (Fig. 6c). The results of histopathological scoring of kidney tissue are shown in Figure 6d.

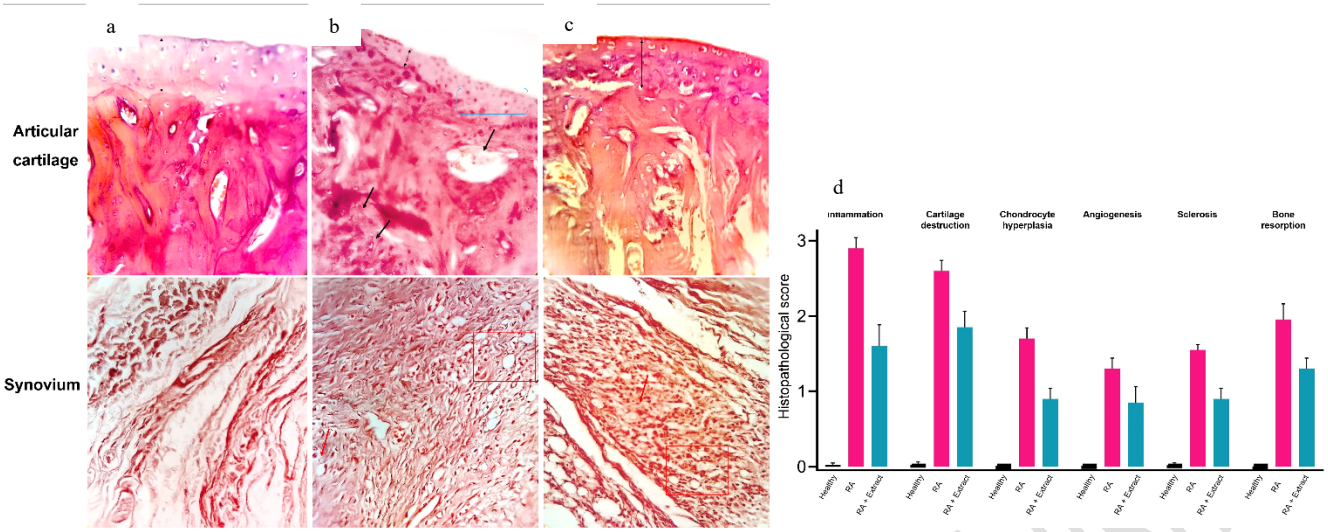


Fig. 5 Histological evaluation of the hind ankle joints. H&E staining (a-c): The normal control group (a) displayed intact articular cartilage with regular synovial tissue. Infiltration of inflammatory cells was not observed in these animals. The CIA-induced group (b) had hypertrophy, mononuclear cell infiltration of the synovial tissue, and hypervascularity. Inflammatory tissue, along with increased blood vessels and signs of bone destruction, was shown in CIA animals. In histological scores (d), inflammation, chondrocyte proliferation, angiogenesis, sclerosis, and bone resorption were compared between groups. Each of the shapes indicates: Double-sided arrows, width of the lining layer; Black arrows, bone resorption; Blue rectangle, cartilage destruction, chondrocyte hyperplasia, and changes in architecture; Red arrows, inflammation; Red rectangle, increased blood vessels.

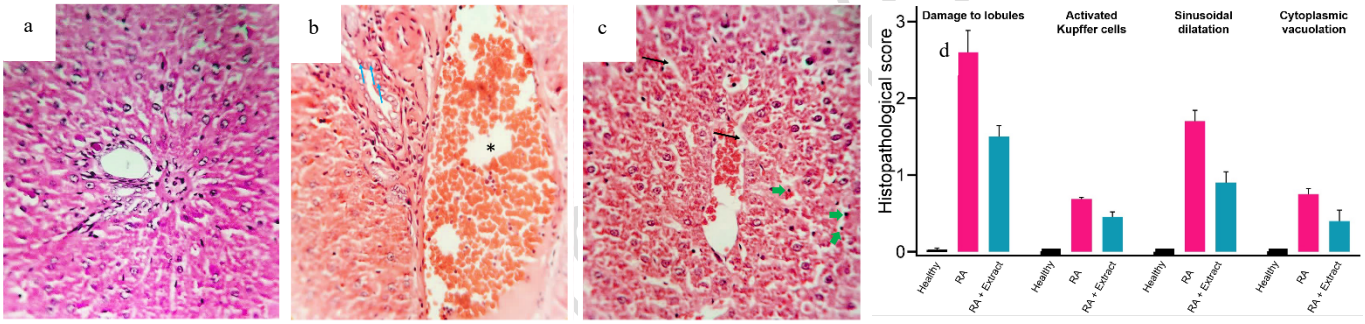


Fig. 6 Histological evaluation of the kidney tissue. H&E staining: (a) Normal tissue, (b) CIA-induced group, (c) Rats with RA received combination extract, d) Histological scores. Each shape indicates: Blue stars, protein casts; Black arrows, dilatation of the Bowmen's capsule; Green arrows, dilatation of the distal and proximal tubules. Black star, dilatation of the intertubular space, along with necrosis.

No damage was observed in the liver lobules, portal space, bile ducts, and sinusoids in the healthy control group. Histological examination of the liver samples revealed some abnormal morphological appearances in the RA-induced group. Inflammation of lobules was increased, sinusoidal dilatation and cytoplasmic vacuolation were observed (Fig. 7b). Deposition of amyloid masses along the capillaries was observed between the stellate endothelial cells (Fig. 7b). In the combination extract-treated group, lobular inflammation, portal space, and vacuolation of hepatocytes were decreased. No change was observed in the proliferation of bile ducts (Fig. 7c). The changes in the experimental histopathologic parameters for the liver are shown in Figure 7d.

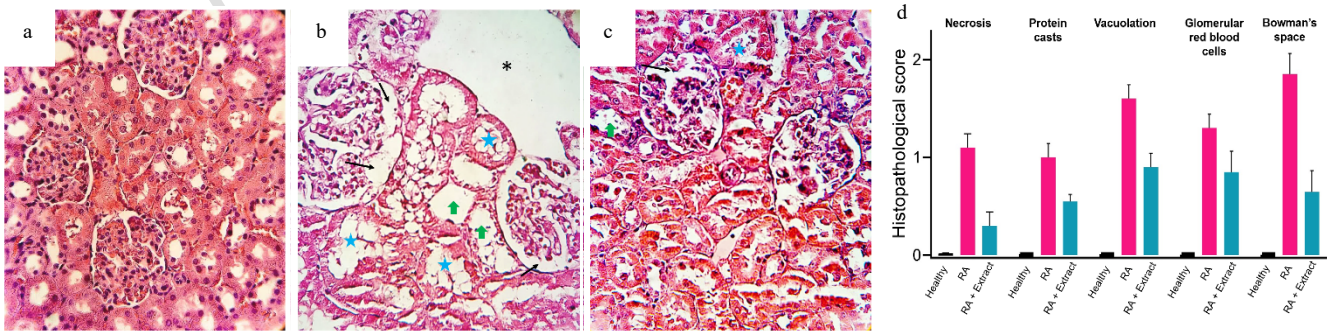


Fig. 7 Histological evaluation of the liver tissue. H&E staining: (a) Normal hepatic tissue, (b) CIA-induced group, (c) Rats with RA received combination extract, d) Histological scores. Each shape indicates: Black arrows, sinusoidal dilatation; Star, a congested central vein; Green arrows, activated Kupffer cells

DISCUSSION

The present study found that a combination of three plant extracts, including *Thymus vulgaris*, *Oliveria decumbens,* and *Anthemis hyaline,* was effective in alleviating both articular and hepatorenal manifestations in CFA-induced rats. Currently, DMARDs, NSAIDs, and corticosteroids are used to relieve articular symptoms of RA [27], but they can cause side effects such as cardiovascular problems, gastrointestinal complications, renal injuries, and hepatic damage [28].

As mentioned, in addition to joint complications, extra-articular manifestations are common in patients with RA. Autoimmune liver disease and chronic kidney disease are two well-known extra-articular manifestations of RA, leading to increased morbidity and a lower quality of life in patients. Studies have shown that herbal medicine has ameliorative and protective effects on renal injuries and hepatic dysfunctions. The anti-inflammatory and anti-rheumatoid effects of many medicinal plants, as well as bioactive compounds like alkaloids, carotenoids, and phenolics, have been researched [29].

Phenolic molecules have recently garnered a lot of interest due to their antioxidant, anti-inflammatory, and immunomodulatory properties [30]. A study showed a strong correlation between the antibacterial, radical scavenging capacity, and the phenolic components in the extract of *Scrophularia striata* [31]. All three plants selected for this study are rich in phenolic compounds. *T. vulgaris* (*Lamiaceae* family) has been used for many years for various diseases [32]. More than 90% of the bioactive compounds in *T. vulgaris* are terpenoids, with the majority being carvacrol, thymol, γ-terpinene, ρ-cymene, and linalool [33]. *A. hyaline* is a species of annual herb from the *Asteraceae family* native to Iran. The most constituent components of this plant are cis-chrysanthenyl acetate, terpinen-4-ol, camphor, myrcene, α-pinene, and β-caryophyllene [34]. *O. decumbens* Vent. (*Apiaceae* family) is an annual herb growing wild in restricted regions of Iran. The main content of its essential oil is carvacrol, thymol, γ-terpinene, p-cymene, and myristicin [34]. This plant can be used in the food, cosmetic, and pharmaceutical industries [35].

Improvements in body weight and a decrease in arthritic score are considered simple indicators to assess disease recovery during the treatment period. The progression and severity of the disease led to a decrease in the body weight of animals with RA. Administration of herbal remedies was found to improve the body weight of animals [36, 37]. In our study, the reduction in the weights of the whole body, as well as the liver and kidney, observed in RA animals was reversed following treatment. Joint swelling observed in the hind paw following intradermal injection of CFA results from chronic inflammation and progression of RA in animals. This is due to increased production of synovial fluids, vascular permeability, and infiltration of inflammatory cells [6]. In the present study, we observed that edema of the hind paw and arthritis score in CFA-induced rats were decreased in combination extract-treated CFA-challenged animals.

Cytokines play crucial roles in the pathogenesis of RA. In the CFA-induced model, the protein levels of IL-1β, IL-6, and TNF-α were increased while the anti-inflammatory cytokine IL-10 was downregulated [37]. IL-1β and TNF-α contribute to the proliferation of synovial membrane cells in the joint, leading to thickening and reduced joint fluid volume [38]. Another key cytokine in RA is IL-6, which significantly impacts the proliferation and differentiation of macrophages, B and T cells, endothelial cells, chondrocytes, and osteoclasts [39-42]. IL-17 stimulates the production of IL-1β, TNF-α, IL-6, and IL-23 by synovial fibroblasts, monocytes, and macrophages [43]. On the other hand, IL-1β, IL-6, and TNF-α regulate Th17 cells [44]. IL-17 can exacerbate inflammation and contribute to bone and cartilage damage, resulting in pain and disability for RA patients [43]. We observed a significant increase in proinflammatory IL-1β, IL-6, IL-17, and TNF-α levels in the CFA group, while IL-10 decreased. These levels were improved by the combination of extract treatment.

Oxidative stress has been proven to be involved in the progression of RA through damage to lipids, proteins, and DNA, resulting in synovial inflammation [43]. Essential oil of *T. vulgaris* and its carvacrol reduced neutrophils recruited during inflammation in zebrafish [46]. In carrageenan-induced paw edema, an experimental model of acute inflammation, a dose of 400 mg/kg of *T. vulgaris* essential oil could significantly reduce symptoms in mice through anti-inflammatory activities [47]. In another study, *T. vulgaris* extracts inhibited nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression induced by interferon-γ (IFN-γ) or lipopolysaccharide (LPS) in J774A.1 macrophage cell line [48]. In a study, thymol (100 mg/kg) and nicotine alone or in combination were orally administered to CFA-induced rats. These compounds exhibited a synergistic effect on the levels of RF, CRP, and NO. Thymol LOWERED IL-1β, IL-6, IL-17, TNF-α, and IFN-γ levels. However, the resulting synergistic effect could potentially have a greater inhibitory effect [49]. Carvacrol in tin oxide-chitosan-polyethylene glycol nanocomposites demonstrated anti-arthritic properties in rats with CFA-induced arthritis. The animals treated with nanocomposites displayed an increase in body weight, a decrease in hind paw thickness and arthritis score, and an increase in antioxidative status. Histopathological observations also indicated a reduction in bone destruction following treatment with the nanocomposites [37]. In a study, carvacrol was encapsulated in bovine serum albumin (BSA) nanoparticles, and its immunomodulatory effects were examined in adjuvant-induced arthritis (AIA). After inducing RA, the rats were treated intraperitoneally with nanoparticles for 28 days. This treatment significantly decreased the clinical severity score, erythrocyte sedimentation rate, NO production, and IL-17 expression compared to the untreated RA control group [50].

In a study, the administration of carvacrol (20 mg/kg) for seven consecutive days showed significant anti-inflammatory effects in a gouty arthritis rat model. It reduced oxidative stress and inflammation by significantly decreasing levels of uric acid, CRP, nuclear factor-kappaB (NF-κB), and reactive oxygen species (ROS) pathways, providing protective effects against joint degeneration [51]. Oil from *O. decumbens* showed anti-effects against glucose oxidation, lipid peroxidation, protein oxidation, and protein glycation. Therefore, it had an anti-diabetic effect due to its ability to scavenge free hydroxyl radicals [52]. A study confirmed the inhibitory effects of *A. hyaline*, *Citrus sinensis*, and *Nigella sativa* extracts on coronavirus replication and apoptosis. The extract showed a reduction in viral load and IL-8 production [53].

The administration of myrcene reduced blood flow and leukocyte trafficking in CFA-induced arthritic rats. These effects against inflammation and pain were mediated via the endocannabinoid system, similar to diclofenac [54]. α-Pinene exhibited an inhibitory effect on inflammatory

responses induced by LPS in mouse peritoneal macrophages by decreasing the expression/production of cyclooxygenase-2 (COX-2), IL-6, TNF-α, and NO [55].

Linalool markedly reduced spleen and thymus indices in CFA-induced arthritic animals. The overproduction of IL-1β, IL-6, IL-17, COX-2, TNF-α, and iNOS was significantly decreased in rats treated with linalool, while IL-10 level was elevated compared to arthritic animals. Disease parameters such as paw volume, arthritis score, mobility, and flexion pain in RA rats decreased. Additionally, improvements were observed in body weight, hematological parameters, and histological manifestations [56]. Linalool also inhibited the expression of iNOS, COX-2, IL-6, prostaglandin E2 (PGE2), and TNF-α in a mouse chondrocyte osteoarthritis model. In terms of the mechanism of action in osteoarthritis, an inhibitory effect of linalool on the signal transduction of NF-κB in chondrocytes was observed [57].

CONCLUSION

In recent years, patients have come to realize the beneficial role of medicinal plants in improving RA. In a cross-sectional case-control study, more than half of individuals expressed that they use herbal medicines such as thyme, chamomile, lavender, borage, cinnamon, and ginger, all of which have anti-inflammatory effects [58]. Our study suggests that injectable forms of extracts of thyme, *Oliveria decumbens*, and *Anthemis hyaline* have beneficial properties in alleviating RA symptoms as well as renal and hepatic damage caused by RA. This can be attributed to the large amounts of thymol, carvacrol, linalool, ρ-cymene, and myrcene present in these plants, which have antioxidant and anti-inflammatory properties. Further research aimed at identifying the effective plant components possessing anti-inflammatory and immune-regulatory properties can benefit in improving the condition of patients.

Author Contributions

B. Khan Ahmadi was responsible for conceiving the idea and carrying out the work; R. Tahvilian supervised the work and corrected the manuscript; S. Darakhshan scheduled the experiments and wrote the manuscript; D. Zarei-Bidsorkhy and M.J. Hoseini-Far cooperated in the experiments and edited the draft of the manuscript. M. Zhaleh contributed to histology experiments. All authors discussed the results and contributed to the final manuscript.

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