Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Anti-inflammatory effect of thyme on rheumatoid arthritis in animal model

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Article Info

Article history:

Received: March 18, 2025
Accepted: June 22, 2025
Published: July 31, 2025

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Abstract

Thyme (Thymus vulgaris) is a Mediterranean herb known for its culinary, cosmetic, and medicinal applications as it has been discovered that the plant has many clinical properties such as anti-inflammatory, antimicrobial, and antioxidant properties. Sandwich Elisa technique was used to determine the concentration of cytokines. Phenolic contents and other active compounds of thyme were analyzed by Gas Chromatography-Mass Spectrometry (GC-Mass). In this study 15 male adult albino rats were divided into 3 groups (n=5), group one (G1) was the control group which fed on basal diet. Group two (G2) was the Rheumatoid arthritis (RA) group in which the rats were inoculated with 0.1 ml of CFA (Complete Freund's Adjuvant) yet fed on basal diet. Group three (G3) was the treatment group in which rats were inoculated with CFA along with the administration of thyme extract orally for 22 days. The results show that treatment with thyme extract significantly reduced proinflammatory cytokines IL-1 (interleukin-1), IL-6 (interleukin-6) and TNF- α (tumor-necrosis factor-alpha). Anti-inflammatory IL-10 (interleukin-10) showed a significant increase in the thyme-treated group. CD4 T (cluster of differentiation-4) cell levels showed a significant difference, while sCD14 (soluble cluster of differentiation-14) levels were non-significant in the thyme group compared to the RA group. Inflammatory markers (C-reactive proteins) CRP and (anti-cyclic citrullinated peptide) Anti-CCP antibodies were both significantly elevated in RA and significantly reduced by thyme treatment. Although body weight changes were statistically non-significant, they were visibly prominent. Paw edema was significantly decreased in the thyme-treated group. (Matrix-metalloproteinase) MMP-1 levels and neutrophil counts were both elevated in RA and significantly reduced following thyme extract treatment.

Keywords: Anti-inflammatory, Rheumatoid arthritis, Animal model, Cytokine, Thyme.

1. Introduction

Rheumatoid arthritis (RA) is one of the most common immune-mediated diseases, typically manifests as synovial joint pain, swelling and stiffness [1]. Cartilage and bone deterioration is a hallmark of RA, often leading to joint pain and physical weakness in affected patients [2]. The pathophysiology of RA involves a complex network of immune cells and cytokines, which contribute to cartilage and bone damage increasing the proliferation of synoviocytes. The pathogenesis of RA is mainly driven by cytokines, primarily interleukin-6 and TNF-alpha. However, recent studies have shown that other cytokines, including interleukin-1, interleukin-17, and interleukin-23 also play a crucial role in RA pathogenesis [3].

Patients with RA are typically prescribed medications like painkillers, non-steroidal anti-inflammatory drugs (NSAIDs), and disease-modifying anti-rheumatic drugs (DMARDs), which help reduce inflammation, alleviate pain and prevent further disease progression, even though the effect of these drugs has not been quite satisfactory [4]. Several therapeutic strategies have been developed to reduce synovial joint inflammation in rheumatoid arthritis, based on an understanding of its underlying pathophysiology [5]. Significant attention and research efforts have been directed toward biological drugs commonly known as biologics for treatment of RA [6]. These therapies include monoclonal antibodies or soluble receptors targeting IL-1, IL-6 and TNF alpha [7]. Although these drugs can reduce inflammation and joint destruction, their long-term benefits and risks remain unclear [8].

Herbal remedies have also been used for thousands of years as alternative treatments for various diseases including RA, due to their perceived safety and efficacy [9]. Thyme (Thymus vulgaris) is an aromatic plant native to the Mediterranean region [10] which has anti-inflammatory properties and is commonly used in herbal medicinal products [11]. Thyme exhibits an excellent safety profile and is generally well-tolerated. It represents valuable potential for a range of therapeutic, preventive, and commercial applications [12]. Essential oil and Extracts derived from thyme are generally recognized as safe, with no toxicological symptoms reported [13].

Thyme (Thymus vulgaris) is well documented for its anti-inflammatory properties primarily through its essential oil (TEO) and active compounds like thymol [14]. Studies have examined the effect of thyme in various diseases [15] including autoimmune diseases [16]. Although thyme has been reported as one of the most frequently used her-

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Doi: http://dx.doi.org/10.14715/cmb/2025.71.7.8

bal remedies among RA patients [17], the specific anti-inflammatory effect of thyme extract in the context of RA has not been experimentally evaluated in either human clinical studies or animal models. This represents a clear gap in the literature regarding the use of extract thyme to deliver a broader range of bioactive compounds for evaluating anti-inflammatory effect in rheumatoid arthritis using animal model.

This study aimed to investigate and evaluate the anti-inflammatory effect of alcoholic extract of thymus vulgaris in animal models of rheumatoid arthritis, to assess its potential in reducing inflammation.

2. Materials and methods

2.1. Chemicals and reagents

Table 1 lists all chemicals and reagents used throughout the experimental procedures, including plant extraction, animal treatment, and biochemical analyses.

2.2. Plant material: collection and extraction

2.2.1. Plant collection and identification

Fresh leaves of thyme (Thymus vulgaris) were collected between the 15th to 20th of May 2024 in Qirzha and Qarasinj in Swlawk resort in Kurdistan then spread on a clean surface mat and left for about seven to ten days to dry indoors, after they grounded into a fine powder by using electrical grinder. The plant has been identified by Dr. Ikbal Muhammad Gharib Tahir (Ph.D. degree in Pre- and Post-harvest Physiology), department of biology, Koya University.

2.2.2. Preparation of thyme extract

The dried leaves of thyme plant were finely powdered using an electrical grinder, 50 grams of the dried powder weighed on sensitive balance and subjected to alcoholic extraction with 400 ml of absolute ethanol (99%), (solvent to fed ratio (8:1)) and left for 48 hours in a parafilm-covered flask. After 48 hours, the solution was filtered through Whatman No. 1 filter paper to remove particulate matter. The resulting filtrate was then concentrated and dried using a rotary vacuum evaporator (BUCHI Rotavapor RII, Daihan LabTech Co.), yielding a 4% extract. The thyme extract was administered to rats at a dose of 500 mg/kg body weight by incorporating it into the basal diet.

2.3. Animal model and arthritis induction

2.3.1. Experimental animals

Fifteen healthy adult male albino rats (8 weeks old), weighing between (200-300) grams were obtained from Animal House of Cihan University- Erbil. Rats were fed ad libitum and housed at room temperature (25 ^{0}C) with 12-hour light/dark cycle and acclimated for 7 days as they fed on basal diet for one week before the experiment. The basal diet given to rats composed of the following nutrients; yellow corn, soybean meal %46, feed wheat, wheat bran, mono-calcium, phosphate, table salt, limestone powder, vitamins, minerals, and toxin binder. With the specification (protein %20, energy 3000 kcal/kg, fiber %3, ash %5). All animal procedures were ethically approved by the faculty of Science and Health-Koya University (006 bio) on 18/11/2024

2.3.2. Arthritis induction in rats

To evaluate the therapeutic potential of thyme extract in arthritis, a Complete Freund's Adjuvant-induced arthritis model was used. Arthritis was induced by subcutaneously injecting 0.1 ml of CFA into the footpad of the left hind paw in both the RA and Thyme groups [18]. The CFA used contained heat-killed mycobacterium tuberculosis suspended in paraffin oil and mannide monooleate (Sigma-Germany), as a vehicle. In contrast, the control group received 0.1 ml of normal saline at the same site. The right hind paw served as non-injected control paw. A low concentration of CFA was used to minimize morbidity and mortality of animals.

2.4. Experimental design

After one one-week acclimatization period, fifteen healthy male albino rats were randomly divided into three groups with five rats housed per steel cage. Group one (n=5) was control group (C) and was injected with normal saline, group two (n=5) was the arthritis group (RA), rats were injected with CFA to induce arthritis, and both these groups control and RA were fed on basal diet for 25 days, and group three (n=5) was the treatment group by thyme (T) which the rats were injected with CFA and followed by administration of thyme extract orally (500 mg/kg orally once daily for 22 days).

Oral administration of thyme extract treatment of thyme group was started 3 days post-CFA injection. To prevent the influence of circadian rhythm, all dosages of thyme extract treatment were given at 04:00 pm.

The experiment lasted for 25 days, on day 26, animals were fasted overnight for 12 hours prior to blood collection. Rats were injected intraperitoneally (IP) with ketamine (100 mg/kg body weight) and xylazine (100 mg/kg body weight) [19] then blood samples were collected directly from the heart and put in EDTA test tubes and gel tubes for biochemical analysis.

The serum was obtained by centrifugation of blood in the gel tubes at 4000 rpm. The cytokines such as interleukin-10, interleukin-1, interleukin-6, TNF-alpha, anti-CCP, MMP-1, CD4, and CD14 were examined from the serum using ELISA kit (Sunlong-China). The blood in EDTA test tubes was put on roller allowing the blood to mix properly to prevent blood coagulation which was used to examine complete blood count including five differential counts of white blood cells. The heart and spleen were removed from the rats and fixed in %10 formalin for histological analysis.

Table 1. List of chemicals and reagents used in the study.

Reagents	Company	Details
Complete Freund's Adjuvants (CFA)	Sigma-Aldrich (St. Louis, MO, USA)	Used for arthritis induction
ELISA kits	SunLong Biotech Co., LT (China)	Kits for IL-1, IL-6, IL-10, TNF-α, Anti-CCP, MMP-1, CD4, sCD14
Ethanol	————	Absolute ethanol (99%) for extraction method

2.5. Assessment and evaluation of arthritis

To evaluate the progression of rheumatoid arthritis and the potential therapeutic effects of Thymus vulgaris, various physiological and immunological parameters were assessed throughout the experiment:

2.5.1. Assessment of body weight change

The body weight change of each rat of all the groups was recorded and measured by sensitive balance on days 0, 1, 3, 5, 8, 11, 15, 19, 23, and 26 after injection to monitor changes associated with disease progression and treatment effect.

2.5.2. Assessment of paw edema and knee edema

Immunological changes in the left and right hind paws, as well as the left and right knees, were recorded. Paw edema was measured using a manual measuring strip marked with numerical increments to assess swelling on days 0, 1, 3, 5, 9, 12, 15, 19, 23, and 26 after injection.

2.5.3. Assessment of cytokines and markers

For immunological analysis, pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and the anti-inflammatory cytokine IL-10, along with immune cell markers CD4 and CD14, as well as MMP-1 and Anti-CCP antibodies, were quantified in serum using the Sandwich ELISA technique. Additionally, C-reactive protein (CRP) levels were measured as a systemic inflammation marker by an immunoturbidimetric method using the Cobas c501 analyzer.

2.6. Statistical analysis

All data were expressed as mean and standard error of mean (Mean ± SEM), (n=5) and the statistical analysis was performed using (GraphPad Prism 9. Ink). Data was statistically analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test.

3. Results

3.1. Effect of thyme extract on rat body weight

A notable reduction in body weight was observed in the rats with induced RA compared to the control group, indicating a systemic effect of inflammation. On the other hand, a relative increase in body weight was observed in rats treated with thyme extract compared to those with rheumatoid arthritis, indicating a potential protective effect of thyme against RA-associated weight loss through reduced inflammation and improved metabolic function. The results showed that rats injected with CFA developed arthritis, which negatively affected their total body weight gain. During the 25-day experimental period, the rheumatoid arthritis group gained an average of 65.4 grams in weight, compared to 84.4 grams in the control group and 76.2 grams in the thyme-treated group as shown in Table 2 and Figure 1. This also clarifies that food intake was relatively lower in the RA group, compared to both the control and thyme groups.

A noticeable reduction in body weight gain was observed in the RA group compared to the control group (65.4±2.977, and 84.38±9.496) respectively. In contrast, rats treated with thyme extract showed improved weight gain compared to the RA group (76.2±3.821, and 65.4±2.977) respectively. Even though the weight change is not significant but its prominent and noticeable. Normality test confirmed using Shapiro-Wilk test.

3.2. Effect of thyme extract on paw edema and knee edema

The edema in the injected paw peaked between 3-6 days post-CFA injection, with a measured thickness of 3.8 cm on day 3. This was significantly greater than the baseline paw diameter of 2.5 cm observed in the control group. The control group exhibited no paw edema, as they were

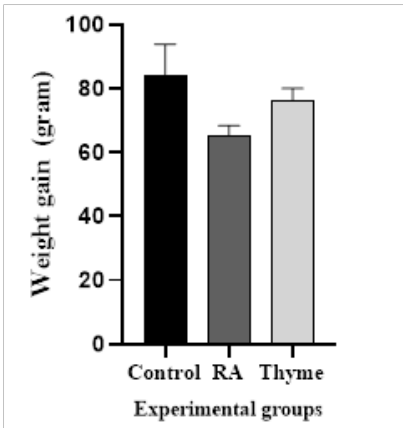

Fig. 1. Effect of Thyme Extract on Body Weight Gain in Rats. Body weight changes were monitored in all groups from day 0 (baseline, prior to CFA injection) to day 26. The RA group showed reduced weight gain compared to controls, while thyme-treated rats exhibited improved weight gain relative to the RA group.

Table 2. Effect of Thyme Extract on Body Weight Gain in Rats. Total body weight was measured intermittently from day 0 (baseline, prior to CFA injection) until day 26 (end of experiment, at blood collection).

Day post-injection	Control rats' weight(g)	RA-rats weight (g)	Thyme rats' weight (g)
Day 0	239.9	269.5	245.44
Day 1	244.5	271.42	247.4
Day 3	252.46	278.28	258.46
Day 5	258.9	276.8	260.7
Day 8	270.56	288.32	268.84
Day 11	282.88	295.92	281.68
Day 15	286.24	315.9	292.96
Day 19	300.92	335.9	297
Day 23	306.86	324.34	308.98
Day 26	324.36	334.92	321.64
Range	**84.4**	**65.4**	**76.2**

Table 3. Effect of thyme extract on paw edema.

Groups	Day (0)	Day (1)	Day (3)	Day (5)	Day (9)	Day (12)	Day (15)	Day (19)	Day (23)	Day (26)	Inhibition value (cm)
Control	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0
RA	2.5	3.6	3.8	3.4	3.5	3.5	3.6	3.6	3.5	3.5	0.04
Thyme	2.5	3.5	3.7	3.3	3.2	3.1	3.2	3.2	3.1	3	0.5

injected with normal saline instead of CFA (Figure 2. B). Edema in the left injected paw was considered the primary lesion, while immunological changes, such as inflammation in the non-injected site (left knee), were recorded as secondary lesions. Notable swelling was recorded in the left knee on day 3 post-injection, measuring 3 cm, while the right knee (control knee) measured 2 cm. No secondary lesion appeared on the right paw or right knee. The administration of thyme extract resulted in a significant reduction in paw edema, with a percentage inhibition of (0.5 cm) (Figure 2A and Table 3).

3.3. Effect of thyme extract on inflammatory markers (Anti-CCP and CRP)

The concentration of Anti-CCP was elevated in the RA group in comparison to the control group. However, treatment with thyme extract led to a reduction in anti-CCP levels in the thyme group compared to the RA group (Figure 3).

Likewise, CRP levels were increased in the RA group compared to the control group. A decrease in CRP concentration was observed in the thyme-treated group following administration of thyme extract as compared to the RA group (Figure 4).

3.4. Effect of thyme extract on MMP-1 and neutrophil count

MMP-1 concentration was higher in the RA group compared to the control group, treatment with thyme extract led to a reduction in MMP-1 concentration in the thyme group compared to the RA group (Fig.5).

Likewise, neutrophil count was higher in the RA group (25%) compared to the control group (16%). However, following thyme extract administration, the thyme group exhibited a reduced neutrophil count (21%) compared to the RA group (25%) (Figure 6. A). Furthermore, blood film slides of neutrophils for each group under 40X magni-

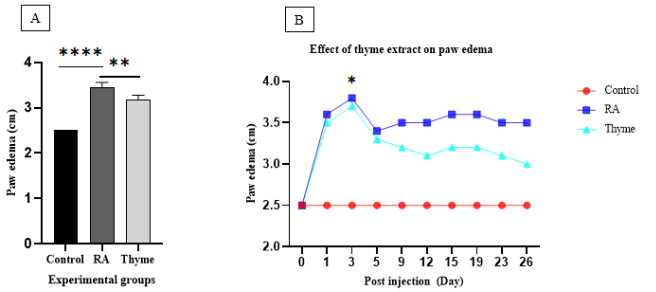

Fig. 2. Effect of thyme extract on paw edema. A. A highly significant increase in paw edema was observed in the RA group compared to the control group ($p<0.0001$) (3.45 ± 0.11 and 2.5 ± 0.000) respectively. In contrast, administration of thyme extract resulted in a significant reduction in the paw edema formation compared to the RA group ($p=0.0024$) (3.18 ± 0.099, and 3.45 ± 0.11) respectively. **B.** Comparisons of paw edema formation between control, RA and thyme-treated group.

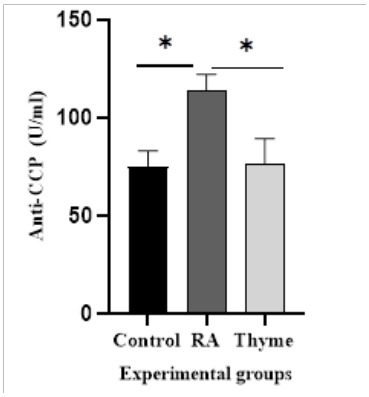

Fig. 3. Effect of Thyme extract on Anti-CCP. A significant increase in Anti-CCP levels was observed in the RA group compared to the control group ($p<0.05$) (114.3 ± 8.04, and 75.06 ± 8.17) respectively. Anti-CCP levels were significantly reduced in the thyme group compared to the RA group (76.51 ± 12.92, and 114.3 ± 8.04) respectively.

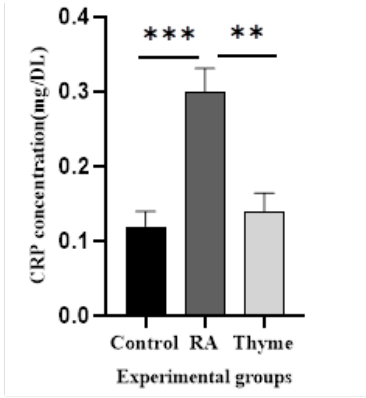

Fig. 4. Effect of Thyme extract on CRP. C-reactive protein levels were significantly elevated in the RA group compared to the control group ($p=0.0009$) (0.3±0.03 and 0.12±0.02) respectively. A significant reduction in the CRP levels was observed in the thyme group compared to the RA group ($p=0.024$) (0.14±0.02 and 0.3±0.03) respectively.

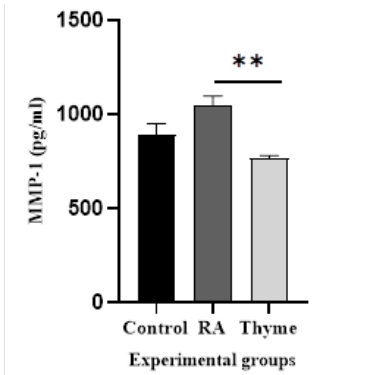

Fig. 5. Effect of thyme extract on MMP-1. A non-significant increase ($p=0.08$) in MMP=1 levels was observed in the RA group compared to the control group (894.6±55.64). A highly significant ($p=0.0026$) decrease in MMP-1 level was observed in the thyme group (768.6 ± 13.86) compared to RA group (1045 ± 53.45).

Table 4. Comparison between control, RA and thyme group in relation to IL-1, IL-6, and TNF-alpha as mean ± SEM (n=5).

Group parameter	Control	RA	Thyme
Interleukin-1	180.6±7.241	218±7.741	188±6.931
Interleukin-6	80.21±0.7359	90.62±2.312	76.73±3.512
TNF-alpha	182.8±5.667	205.7±4.63	173.7±5.737

fication of light microscope. The result indicates that the neutrophil count was higher in the RA (B) group compared to the control group (A). However, the neutrophil count in the thyme group (C) was almost similar to the control group and reduced compared to the RA group (Figure 6B).

3.5. Effect of thyme extract on pro-inflammatory cytokines

The concentration of pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha) was higher in the RA group compared to the control group. However, these concentrations were reduced in the thyme group compared to RA group, indicating potential anti-inflammatory and protective effect of thyme as shown in (Figure 7) and (Table 4).

3.6. The effect of thyme extract on immune cell markers (CD4 and sCD14)

The concentration of CD4 was higher in the RA group compared to the control group, while a reduction in CD4 concentration was observed in the thyme group following thyme extract administration compared to the RA group (Figure 8).

The sCD14 concentration was elevated in the RA group in comparison to the control group, whereas the thyme group exhibited a decrease in sCD14 levels in the thyme group compared to the RA group (Figure 9)

3.7. Effect of thyme extract on regulatory cytokine (interleukin-10)

The concentration of IL-10 was lower in RA group

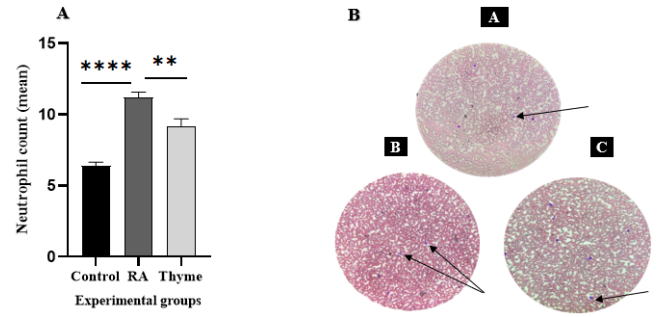

Fig. 6. Effect of thyme extract on neutrophil. A highly significant ($p<0.0001$) increase in neutrophil count was observed in the RA group (11.2 ± 0.37) compared to the control group (6.4 ± 0.24). The percentage of neutrophils significantly ($p=0.008$) decreased in the thyme group (9.2 ± 0.48) after administration of thyme extract compared to the RA group (11.2 ± 0.37). The black arrows show neutrophil(s)

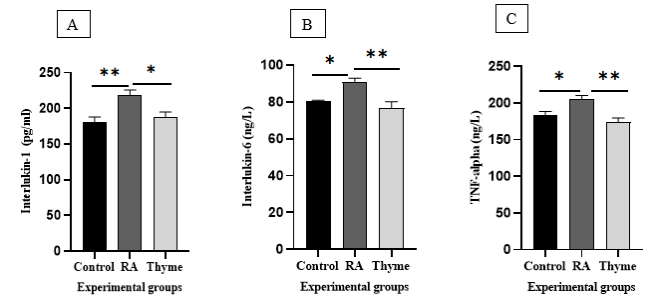

Fig. 7. Effect of thyme extract on pro-inflammatory cytokines A. A highly significant ($p=0.0092$) increase in IL-1 concentration was observed in the RA group compared to the control group, while IL-1 level was significantly reduced ($p=0.03$) in the thyme group compared to the RA group. **B**. IL-6 concentration was significantly ($p=0.028$) higher in the RA group compared to the control group, whereas, a highly significant reduction ($p=0.0048$) in IL-6 level was observed in the thyme group compared to the RA group. **C**. TNF-alpha concentration was significantly elevated in the RA group compared to the control group ($p=0.027$), While a significant reduction in TNF-alpha was observed in the thyme group compared to the RA group ($p=0.0032$) (Table 4).

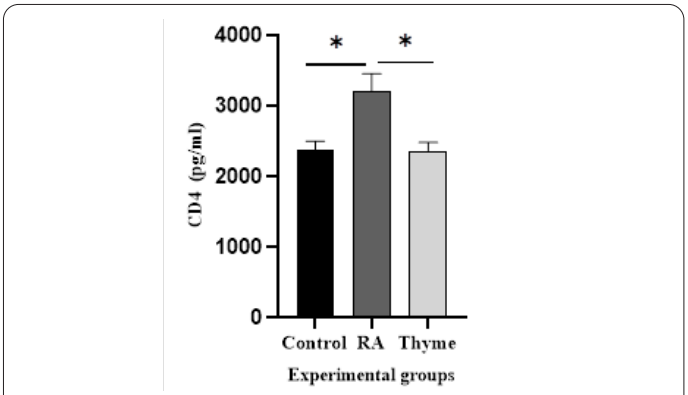

Fig. 8. The effect of thyme extract on immune cell markers CD4. A significant increase ($p=0.0146$) in CD4 concentration was observed in the RA group compared to the control group (3207 ± 249.2, and 2367 ± 135.3) respectively. A significant reduction ($p=0.0144$) in CD4 concentration was observed in the thyme group compared to the RA group (2365 ± 116.2, and 3207± 249.2) respectively.

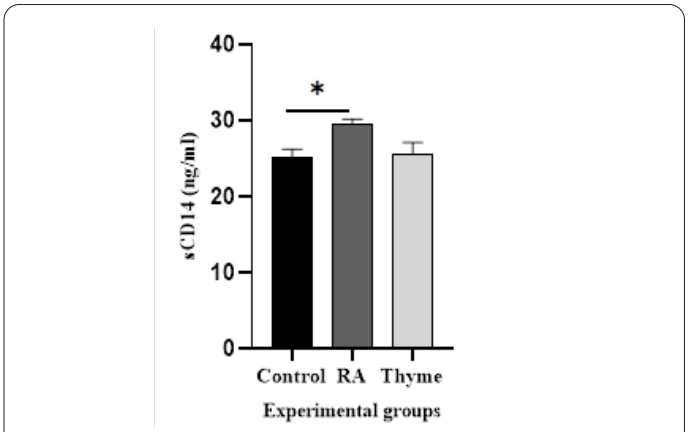

Fig. 9. The effect of thyme extract on immune cell markers sCD14. The concentration of sCD14 was significantly higher in the RA group (29.53± 0.637) compared to control group (25.16 ± 1.087) ($p=0.04$), while the concentration of sCD14 was reduced non-significantly ($p=0.07$) in the thyme group (25.58± 1.556) compared to the RA group (29.53± 0.637).

compared to control group; however, thyme extract exhibits a potential effect on thyme group as IL-10 was increased in thyme group compared to RA group as shown in (Figure 10).

3.8. Chemical composition of alcoholic thyme extract

Compounds of thyme extract were identified by analysis of the GC mass technique (Table 5). The main component found in thyme extract was thymol with a concentration of 17%.

4. Discussion

Inflammatory mediators such as interleukin-1, interleukin-6, and TNF-alpha are elevated in RA disease [20], which if left untreated can lead to invasion and infiltration of macrophages into the site of inflammation and produce excessive amounts of auto-antibodies [21]. The present study shows that thyme extract has positive effect on RA, by reducing the number of pro-inflammatory cytokines such as TNF-alpha, IL-1, and IL-6, with increasing concentration of anti-inflammatory cytokines such as interleukin-10 compared to RA group rats which is due to protective role of thyme against RA progression, this result is in line with previous study that highlighted pharmacological properties of thyme [22]. Another mechanism that contributes to RA pathogenesis is neutrophil cascade which can lead to inflammation by influencing cytokines and chemokines [23]. Pathophysiology of RA is mainly influenced by Tumor Necrosis Factor (TNF), which is expressed at greater concentration in RA patients [24]. Furthermore, TNF contributes to variety of functions in development of RA, especially endothelial-cell activation and recruitment of pro-inflammatory cells such as macrophages and synovial fibroblast, which in turn release pro-inflammatory cytokines such as TNF-alpha, IL-1, IL-6 [25].

In the present study, the RA group showed notable decrease in body weight compared to the control group, especially during the first days following RA induction, same result conducted in [26], although weight loss is a common symptom of many auto-immune disorders including RA, which is probably due to the increased release of cytokines related with inflammation, and loss of appetite, which in turn enhance protein catabolism [27]. However, rats treated with thyme extract showed prominent increment in body weight which could be due to the phenolic compounds of thyme which exhibit anti-oxidant effect and can improve body weight gain by reducing oxidative stress related to RA as natural phenolic compounds can modulate both the pro-oxidant and anti-inflammatory mechanism and reduce the onset of the RA progression [28].

In the current study, rats injected with CFA showed highly significant swelling in paw of the rats which is the common characteristic of RA, as patients with RA suffer from soft tissue swelling [29]. The severity of RA peaked between 3 and 6 days after CFA injection, which is consistent with previous studies using the CFA-induced arthritis model, where paw edema typically reaches its maximum between days 3 and 5 post-injection [26]. On the other hand, rats treated with thyme extract exhibited a significant reduction in paw edema which is possibly due to thymol and carvacrol compounds found in thyme which are effective anti-inflammatory compounds that inhibit inflammatory edema [30].

It has been demonstrated by Castro-Sánchez (2017) that immune cells (T helper cells) particularly CD4 T cell markers have a great role in mediating inflammation related to auto-immune disorders such as RA, which supports the present study that CD4 has been increased significantly in RA group compared to the control group [31]. Many studies are consistent with the hypothesis that CD4 plays a prominent role in RA pathogenesis, however, CD4 when activated can be found in the inflammation site of rheumatoid synovium in joints, as aligned with the results of the current study that CD4 concentration was higher in RA group and contributed to inflammation [32].

Another marker that is associated with inflammation is sCD14 [33]. The data of the present study shows significant increase in sCD14 level in the RA group compared to the control group as sCD14 is linked to inflammatory disorders such as RA and is involved in innate immune response [34].

C-reactive protein (CRP) is an acute phase protein [35] primarily produced by liver hepatocytes and in smaller amounts by some other cells like macrophages and vascular smooth muscle cells [36]. Even though CRP is a non-specific marker in inflammation and RA [37], it reflects systemic inflammation which is common in RA and plays key roles in modulation of cytokine release particularly IL-6 and TNF-alpha [38] both of which are critical mediators in pathogenesis of RA [3]. The results of the current study show that the CRP concentration is significantly higher in the RA group compared to the control group, and

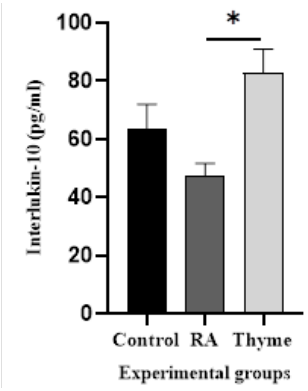

Fig. 10. Effect of thyme extract on regulatory cytokine interleukin-10. A non-significant (p>0.05) decrease in concentration of IL-10 was observed in the RA group compared to the control group (47.24±4.49, and 63.41±8.623) respectively. Following the administration of thyme extract, a significant increase (p=0.0123) in IL-10 concentration was observed in the thyme group compared to the RA group (82.76±8.231, and 47.24±4.49) respectively.

Table 5. Main Compounds Identified in Thyme Extract by GC-MS Analysis. The table lists the major compounds detected in the thyme extract along with their corresponding retention times, as determined by gas chromatography-mass spectrometry (GC-MS).

Compounds	Retention time
Thymol	15.784
Carvacrol	15.701
p-cymene	16.198
Chlorothymol	29.445
Linalool	11.500
Gallic acid	28.301
Catechin	16.550

this result is consistent with previous study, as it is a biomarker for chronic RA [39].

A significantly high concentration of anti-CCP was observed in the RA group compared to the control group, as anti-CCP is a specific marker for early progression of RA [40] and is a great predictor of RA, as auto-antibodies can be detected early in RA pathogenesis. It is also more effective in disease predictability especially RA [41].

Results in the current study recorded significantly higher neutrophil count in the RA group compared to the control group, as invasive tissue composed of activated synovial fibroblasts and neutrophils which secrete pro-inflammatory mediators such as cytokines and chemokines, while fibroblast-like synoviocytes can eliminate MMP-1 to the plasma membrane [42]. It's in line with the results of the present study that a higher MMP-1 concentration was observed in RA group compared to both the control and thyme group.

Rats were inoculated with 0.1 ml of CFA, containing 10 mg/ml of heat-killed Mycobacterium tuberculosis. This dosage has been widely recognized as the most effective concentration for inducing polyarthritis in rats, closely mimicking the pathophysiological features of human rheumatoid arthritis [18]. The results of present study showed that thyme (Thymus Vulgaris) has anti-inflammatory and protective effects on RA which is mostly due to thymol the main active compound of the plant [43].

In the current study, secondary lesions did not appear neither on the right hind paw, or the right knee this is possibly due to the route and site of inoculation in which this study administered CFA subcutaneously, in contrast to the study by Gomes et al., (2013) applied two different routes and sites of inoculation (a single intradermal CFA injection, followed by an intra-articular injection at a separate site), and this resulted in secondary arthritic lesions at non-injected paw [44].

Ethanolic extract of thymus vulgaris was administered for the treatment of RA in the study except for the aqueous extract and this is because ethanolic extract of thyme is richer in total phenolic content and exhibits more prominent effect than aqueous extract as reported in result of study by Elbahnasawy et al (2019) [43].

4.1. Limitation of the study

While multiple pro-inflammatory and anti-inflammatory markers were measured, histological analysis results of joint tissue were not performed, which could have provided more definitive evidence of structural improvement. Hence, further studies are needed to determine the structural effect of the plant through histological evaluation and further validate the therapeutic potential of *Thymus vulgaris* extract.

This study demonstrates that thyme (*Thymus vulgaris*) extract exerts significant anti-inflammatory effects in a rat model of rheumatoid arthritis. Administration of thyme extract led to a marked reduction in paw and knee edema, decreased levels of pro-inflammatory cytokines (IL-1, IL-6, TNF-α), and lowered inflammatory markers (CRP, Anti-CCP, MMP-1) in treated rats. Additionally, an increase in the anti-inflammatory cytokine IL-10 was observed, indicating an immunomodulatory effect. These results suggest that thyme extract may offer a promising natural therapeutic approach for managing rheumatoid arthritis and its associated inflammation. Further studies, including clinical trials, are warranted to confirm these findings and explore the underlying mechanisms of action.

Acknowledgments

I would like to thank Koya University, the Faculty of Science and Health, Department of Biology and Koya Research Center. I would like to express my sincere gratitude to Mr. Shallaw Kamal, Mr. Hiwa Ahmad, and Mr. Shwan Salih for their invaluable support and guidance throughout my time at the research center.

Conflict of interest

The authors declare no conflicts of interest and take sole responsibility for the content and authorship of this article. The author did not receive any specific funding from public, commercial, or non-profit organizations.

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