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

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Anti-inflammatory and immunomodulatory effect of purslane and turmeric in rheumatoid arthritis rat models

Dween Faqi Rasool[1*], Hiwa Ramadhan Fatah[1], Emad Khelil Mohammed Zangana[2]

[1] Department of Biology, Faculty of Science and Health, Koya University, Koya, KOY45, Kurdistan, Iraq
[2] Department of Chemistry, Faculty of Science and Health, Koya University, Koya, KOY45, Kurdistan Region, Iraq

Article Info

Article history:

Received: March 28, 2025
Accepted: June 23, 2025
Published: August 31, 2025

Use your device to scan and read the article online

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by joint inflammation. Given the side effects of conventional treatments, this study focuses on the anti-inflammatory effects of purslane (Portulaca oleracea) and turmeric (Curcuma longa). The research is driven by the growing demand for plant based-treatment for safer therapeutic options for RA management. Five groups were formed; the control group included only healthy rats and was used for baseline comparison. RA was experimentally induced in male rats using Complete Freund's Adjuvant (CFA). Treated groups received extracts of purslane, turmeric and combination of both and one group was left untreated (RA group). Bioactive compounds in plant extracts were identified by GC-MS analysis. Paw edema and body weight were monitored thrice weekly for statistical analysis, and neutrophil counts were assessed microscopically. Enzyme-linked immunosorbent assay (ELISA) was used to quantify the inflammatory biomarkers including IL-1, TNF-α, IL-6, IL10, CD14, CD4, MMP-1, alongside measuring cyclic citrullinated peptide (anti-CCP) levels. CFA-induced RA significantly increased paw edema, neutrophil counts ($P<0.0001$), and elevated levels of anti-CCP, CD4, IL-1, IL-6, and TNF-α compared to the control group ($P<0.001$). Treatments with purslane, turmeric and combination reduced paw swelling and these inflammatory markers in RA induced rats significantly ($P< 0.01$). Despite the increasing serum level of MMP-1, CD14 and IL-10 the reduction by plant extract did not show significant results. It is concluded that the bioactive compounds in the purslane and turmeric have anti-inflammatory effects through reducing inflammatory markers in RA induced rats.

Keywords: Anti-inflammatory, Immunomodulatory, Purslane, Rheumatoid arthritis, Turmeric.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with inflammatory process and progressive joint involvement [1]. Rheumatoid arthritis begins gradually, often presenting with swelling in one or more joints]. Recent finding suggests that the development of RA is triggered by both genetic and epigenetic factors, although the environment could also play critical role in disease onset [2]. A hallmark feature of RA is aberrant activation of the immune system, particularly B cells, T cells, and macrophages[3, 4] .Which leads to the overproduction of cytokines such as tumor necrosis factor-alpha (TNF-a), interleukin-1beta(IL-1), and interleukin-10 (IL-10). These inflammatory cytokines serve important roles in the key processes in the joints, which usually cause inflammation, articular damage [5]. Matrix Metalloproteinases (MMPs) particularly MMP-1 are enzymes produced by synovial fibroblast and chondrocytes. They degrade extracellular matrix components [6]. Also in RA the synovial tissue is infiltrated by CD4 helper T cells and CD14 monocyte derived macrophage, while the presence of anti-CCP antibody indicates a strong autoimmune response [7]. Conventional treatment commonly involves disease-modifying antirheumatic drugs (DMARDs) including methotrexate, which can help reduce disease activity and prevent joint damage [8]. DMARDs become less effective with prolonged use or advanced disease duration, unwanted side effects especially in combination therapies [9]. Natural compounds have been used as a source for therapeutic agents for mankind. Medicinal plants produce bioactive compounds with pharmacological attributes with anti-inflammatory, anti-ageing, immune modulator, antioxidant and others [10]. Such medicinal plants with these properties may help in reducing RA progression, purslane (*Portulaca oleracea*) and turmeric (*Curcuma longa*) with notable therapeutic effects. Purslane recognized for its rich nutritional profile, notably its high including high omega-3 fatty acids content, and is recognized for its anti-inflammatory and antioxidant properties [11]. Purslane is rich in a wide variety of bioactive compounds including polysaccharides, fatty acids, proteins, alkaloids, sterols, phenols, flavonoids, and vitamins [12]. The rhizome of turmeric is rich in bioactive constituents, especially polyphenolic compounds known as curcuminoids, which include cur-

* Corresponding author.
E-mail address: dwenfaqe@gmail.com (D. Faqi Rasool).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.8.4

cumin [diferuloylmethane; 1,7-bis-(4-hydroxy-3-methoxy prenyl)-1,6-heptadiene-3,5-dione)], demethoxycurcumin and bis-demethoxycurcumin [13]. Curcumin therapeutic application include anti-inflammatory, antioxidant, anti-diabetic, hepatoprotective, antibacterial, and anticancer effects against various cancers [14-17]. The aim of this study is to show the effect of turmeric and purslane individually and their combination on rheumatoid arthritis using rat models, by assessing their impact on inflammation through immunological markers, also to provide their insights into their potential impact as RA therapy.

2. Materials and methods

2.1. Preparation of turmeric and purslane extract

2.1.1. Turmeric extraction

Dried turmeric (*Curcuma longa*) rhizomes were obtained from a farm in Halabja, Kurdistan region, Iraq. The rhizomes were finely ground and subjected to extraction using 99% ethanol at a 1:10 (w/v) for 24 hours. Ultrasonic-assisted extraction (UAE) was performed using a sonicator for 60 minutes, following the method outlined [18]] to enhance Curcumin yield. The mixture was passed through Whatman filter paper, and the solvent was removed using a rotary evaporator set at 40°C. To ensure complete removal of ethanol, the concentrate was incubated at 35°C for 24 hours. The final extract was transferred into glass container, sealed tightly, and stored at 4°C in a refrigerator to protect it from light, moisture, and thermal degradation until further use.

2.1.2. Purslane extraction

Fresh Purslane leaves and stems were thoroughly washed and air-dried in a shaded environment at room temperature. Once completely dried, the plant material was finely ground and subjected to ethanol-based extraction using 99% ethanol in a 1:10 (w/v) ratio for 24 hours. UAE was applied for 60minutes, subsequently solvent evaporation at 40°C using rotary evaporator. The concentrated extract was transferred into glass container, sealed tightly, and stored at 4°C in a refrigerator to protect it from light, moisture, and thermal degradation until further use. Extracts were prepared fresh as needed, with doses adjusted based on animal body weight.

2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The gas chromatography-mass spectrometry (GC-MS) analysis of Turmeric (*Curcuma longa*) and Purslane (*Portulaca oleracea*) was performed using (GCMS-QP2010 Plus), A 40µL of the samples were diluted to a final volume of 5 mL with ethanol. The analysis was conducted using an interCap capillary column (30m, 100% dimethylpolysiloxane), anon-polar stationary phase. Two µL was set as the injection volume, with a split ratio of 2:1 Helium was used as the carrier gas at a constant flow rate of 30mL/min. The oven temperature program was initialized at 70°C and held for 3 min, followed by a temperature ramp of 25°C/min to the final temperature 200°C, which was remained for an additional 3minutes the run took 30 min for each analysis. Mass spectra have been recorded for both samples over an m/z range of 900–50 with an energy of ev72. The chemical compounds extracted from the samples were identified by comparing the obtained mass spectra with those available in the device's spectral libraries.

2.3. Study design and sample collection

2.3.1. Animal procedure and preparation for bioassays

Thirty male Wister albino rats (10 weeks old, 250-300 g) were obtained from the house of Cihan University animal facility, Erbil, Iraq. Animals were housed in standard plastic cages at a controlled temperature of 25±2°C, under and maintained under 12-hour light/dark cycle, with unrestricted access to food and water. Before experimentation, animals underwent a 7-days acclimatization period. All the procedures were followed ethical guidelines for animal research.

2.3.2. Complete Freund's Adjuvant (CFA)

Complete Freund's adjuvant (catalog no. F5881) was obtained from Sigma-Aldrich (USA). It contained 1mg of heat-killed Mycobacterium tuberculosis (strain H37Ra, ATCC 25177), blended with 0.85ml of paraffin oil and 0.15 ml mannide monooleate. In contrast, incomplete Freund's adjuvant (IFA) (F5506) contained only the oil mixture 0.85ml of paraffin oil and 0.15ml of mannide monooleate.

2.3.3. Establishment of CFA-induced rheumatoid arthritis in animal models (rats) and plant extract administration

Rats were randomly divided to five experimental groups (n=5 per group). The control group was injected with normal saline and left untreated. Rheumatoid arthritis (RA) group arthritis was induced using CFA but left untreated. Turmeric (Tur) group CFA-induced arthritis rats treated with turmeric extract (150mg/kg body weight) this dose have been applied for both curcumin and turmeric extract as in studies[19-21]. Purslane (Pur) group CFA-induced arthritis rats were treated with purslane extract (300mg/kg body weight)[22]. The combination (Pur+Tur) group CFA-induced arthritis rats treated with turmeric (150 mg/kg) and purslane (300 mg/kg) extracts, following previously established protocols. Treatments were administered orally and daily for 28 days, starting 3 days after induction.

Arthritis was induced by subcutaneous injection of 0.1mL CFA into the plantar fascia of the right hind paw, as described in the previous study [23]. The control group received an equivalent volume of saline. Visible swelling and redness were observed within 72 hours, marking successful arthritis induction, after which treatment was initiated.

2.3.4. Anesthesia and blood collection

At the end of the experiment period, rats were anesthetized using a combination of xylazine-ketamine as this combination has been used in previous study [24] approximately at ratio 1:10. Blood was collected via cardiac puncture from all groups for hematological and immunological assessment. Blood samples were centrifuged at 3000 rpm for 15 minutes to isolate the serum, which was aliquoted and stored at -80°C for later immunological determinations. For hematological parameters, samples were transferred into EDTA tubes for neutrophil count.

2.4. Assessed parameters

2.4.1. Measurement of body weight and paw swelling

Body weight was recorded three times per week to

monitor changes associated with arthritis progression and treatment effects of the plant extracts, by placing each animal in container and using sensitive balance. Paw edema (thickness) of right hind paw was measured using a standard manual caliper by gently placing the paw between the caliper jaws and recording the width at thickest point. Recording paw edema continued three times per week and started two days after CFA injection until the conclusion of the experiment.

2.2.4.2. Neutrophil count

2.4.2. Neutrophil count

Collected blood samples in EDTA tubes for neutrophil counting were processed according to previous study [25]. Neutrophils were counted by using Wright-Giemsa-stained blood smears at 40x magnification using light microscope.

2.4.3. ELISA for cytokines and biomarkers

Serum concentrations of CD4(ELISA kit, SL1201Ra, Sunlong, China), CD14(ELISA kit, SL0629Ra, Sunlong, China), TNF-a (ELISA kit , EL0013Ra, Sunlong, China), IL-6 (ELISA kit, SL0411Ra, Sunlong, China), IL-1 (ELISA kit, SL0392Ra, Sunlong, China), IL-10 (ELISA kit, SEA056Ra, Sunlong, China), Anti cyclic citrullinated peptide (anti-CCP) (ELISA kit, SL1624Ra, Sunlong, China), and matrix metalloproteinase-1 (MMP-1) (ELISA kit, SL0480Ra, Sunlong, China) were measured by using enzyme-linked immunosorbent assay (ELISA) kits (sandwich ELISA) following the manufacturer's instructions.

2.4.4. Statistical analysis

All statistical analyses were carried out using GraphPad (version: 9). Results are presented as the mean ± standard error mean (SEM). Group differences were evaluated using One-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Identification of anti-inflammatory compounds in purslane and turmeric by GC-MS

Gas chromatography was performed to identify the bioactive compounds in purslane and turmeric. The obtained chromatograms revealed the presence of several phytochemicals that known for their anti-inflammatory properties. Purslane and turmeric extract analysis results are shown in (Table1 and Table2) along with retention time (RT), peak area %, and molecular weight of the compounds.

3.2. Biological effects of purslane and turmeric extract on RA

3.2.1. Paw edema and body weight gain in rats with induced RA

The effect of purslane and turmeric on body weight gain and paw edema in RA induced rats are shown in Figure 1. The severity of paw edema peaked during the first week and progressively decreased in the treatment groups, the RA group. While the body weight gain was non-significantly increased in control, purslane, turmeric and combination groups compared to the RA group as shown in

Table 1. Identification and quantification of compound in turmeric via GC-MS .

SN	R. Time	Peak Area%	Library/ID	Molecular weight(g/mol)
1	20.614	59.32	1,6-Heptadiene-3,5-dione, 1,7-bis(4-hydroxy-3-methoxyphenyl)-, (1E,6E)-(Curcumin)	368
2	13.84	4.30	Metronidazole	171
3	20.377	4.57	Flavoxate	391
4	13.614	6.30	Xanthine, 1,3-diallyl-8-[4-carboxymethoxypheny]-	382
	20.510	6.50	1-Pyrroline, 2-phenyl-	145

Table 2. Identification and quantification of compound in purslane via GC-MS .

SN	R. Time	Peak Area%	Library/ID	Molecular weight(g/mol)
1	5.274	12.49	8,11,14 Eicosatrienoic acid, methyl ester	320
2	7.187	22.53	Linolenic acid, trimethylsilyl ester	350
3	29.864	5.70	9-Octadecenoic acid, ethyl ester	306
4	7.693	4.37	cis-Aconitic anhydride	156
5	29.269	2.43	Bupropion	239
6	24.224	3.16	Phenol, 4-butoxy-2-[(dimethylamino)methyl]phenol	223
7	8.224	12.88	2,6,10,14,19-Eicosapentaenoic acid, 2,6,11,15,19-pentamethyl-, ethyl ester	400
8	7.310	12.70	9H-Pyrido[3,4-b]indole	212
9	5.135	9.11	1H-Pyrido[2,3-b]indole	168
10	29.893	12.06	Pyrido[3,4-b]indole, 1,9-dimethyl-4-(1-methyl-2-pyrrolidinyl)	279
11	29.269	7.63	beta.-Carboline, 2-methyl-	182
12	7.010	2.6	4H-1-Benzopyran-4-one, 8-methoxy-2-phenyl-	252
13	19.425	2.79	2H-1-Benzopyran-4-ol, 3,4-dihydro-2-phenyl-	226
14	19.90	2.47	2H-1-Benzopyran-7-ol, 3,4-dihydro-5-methoxy-2-phenyl-	256

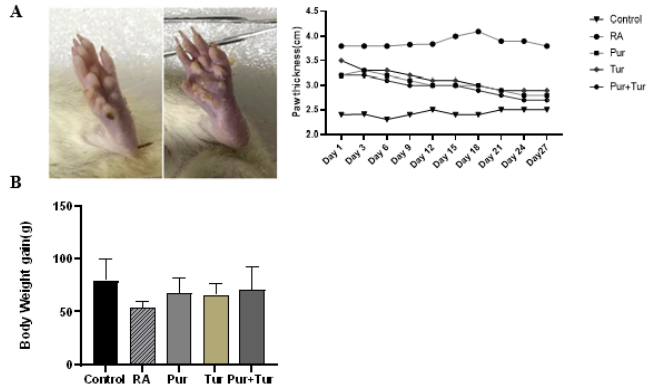

Fig. 1. The effect of purslane and turmeric extract on body weight gain and paw edema. A: Reduction of paw edema P= 0.0001, n=5. The data are expressed in mean ± SEM. B: Body weight gain was not significant .

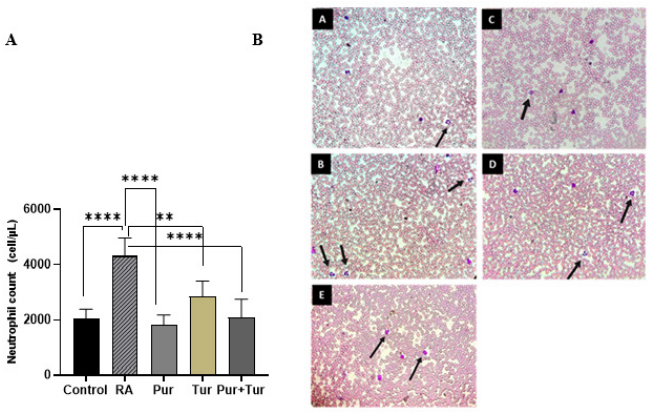

Fig. 2. Neutrophil count in RA induced rats and purslane, turmeric, and combination treatedgroups. A: Significant increase of neutrophil count in RA induced rats was reduced significantly by purslane P<0.0001, turmeric P=0.0026, and combination of purslane and turmeric P<0.0001 in rats. B: Shows neutrophils in the blood of rats in all groups (A) control, (B) RA induced, (C) Purslane, (D) Turmeric, and (E) Purslane + Turmeric. Arrows indicate neutrophil in the blood. Magnification 400X.

(Figure.1).

3.2.2. Determination of inflammatory markers in RA induced rats treated with purslane and turmeric and combination of both

3.2.2.1. The anti-inflammatory effect of plant extracts on Neutrophil count

Neutrophil counts in all groups are.The RA group exhibited a significant increase in neutrophil count (3879 ± 123.7 cell/ µL) compared to the control group (2035.76 ± 147.9 cell/µL). Treatment with purslane and turmeric significantly reduced neutrophil count (2068 ± 189.3 cell/ µL and 2285.2 ± 194.4 cell/ µL) respectively. A similar reduction was observed in the combination treatment group (2480± 162.2 cell/ µL) shown in (Figure 2).

3.2.2.2. The anti-inflammatory effect of plant extracts on serum concentration of anti-CCP

(Figure 3) shows the serum concentration of anti-CCP in all groups. Serum anti-CCP level was significantly higher in the RA group (126.4 ± 5.547 U/mL) than in the control group (90.64 ± 6.982 U/mL). Both Purslane and turmeric treatment were significantly reduced anti-CCP levels (67.32 ± 9.48U/mL and 65.53 ± 9.03U/mL respectively) compared to the RA group (126.4 ± 5.547 U/mL). On the other hand, combination treatment shows a non-significant reduction in Anti-CCP level (102.8 ± 5.6 U/mL). This may be due to potential antagonistic interactions, overlapping mechanisms reaching a saturation point, or pharmacokinetic interference reducing bioavailability. Alternatively greater variability in combination group may have limited statistical detection of differences.

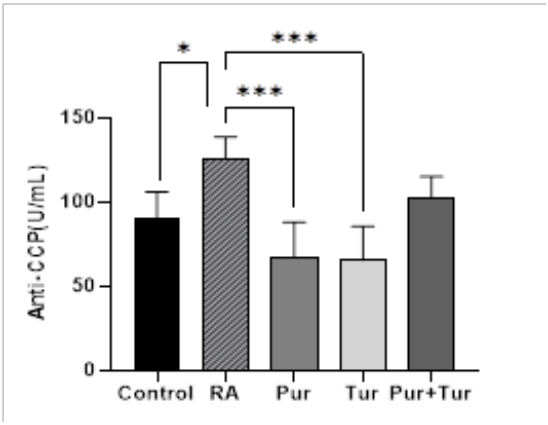

Fig. 3. Purslane and curcumin reduced the increased concentration of anti-CCP in RA induced rats significantly P=0.0002 and P=0.0001 respectively. Ant-CCP was determined using ELISA as described in materials and methods.

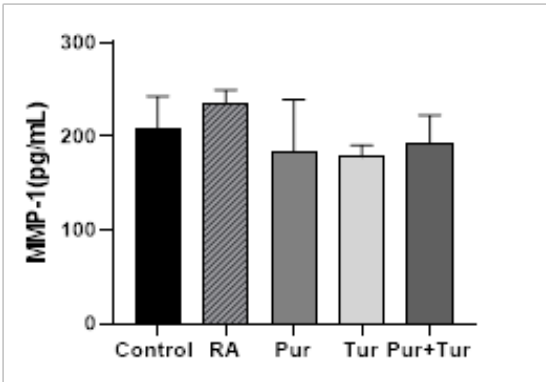

Fig. 4. Serum concentration MMP-1 among different groups. Although the MMP-1 level were elevated in RA rats and appeared reduced in treated groups, differences were statistically non-significant.

3.2.2.3. the anti-inflammatory effect of plant extracts on MMP-1

The serum concentration of MMP-1 in RA group was (234.6 ± 6.829pg/mL) recorded the higher level compare to control (209.5 ± 15pg/mL) and treatment groups including purslane, turmeric and combination (184.4 ± 24.66pg/mL, 178.3 ± 5.49pg/mL, 192.8 ± 13.42pg/mL, respectively). Although the results were not significant P>0.05 as shown in (Figure.4).

3.2.3. Effect of plant extracts on pro-inflammatory and anti-inflammatory cytokines

The impact of plant extract treatments on cytokine levels was evaluated using ELISA (Figure 3). Pro-inflammatory cytokines (IL-1, IL-6, TNF-a) were significantly ele-

vated in RA (166.8 ± 4.597, 111.4 ± 1.28, 184.4 ±6.720pg/mL, P<0.05, respectively) compared to the control group (142.8 ± 5.47, 83.64 ± 4.93, 170.0 ± 6.40pg/mL, respectively). Treatment with purslane (134.9 ± 9.049, 92.5 ± 3.03, 135.5 ± 11.25pg/mL), turmeric (136.5 ± 2.08, 90.68 ± 5.186, 147.6 ±1.48pg/mL), and their combination (110.8 ± 9.46, 81.03 ± 4.63, 150.4 ± 6.61pg/mL) significantly reduced these pro-inflammatory cytokines. The level of anti-inflammatory cytokine IL-10 was significantly elevated in RA (108.7 ±8.69pg/mL) compared to the control group (27.78 ±3.399pg/mL). Treatment with plant extracts resulted in a non-significant reduction of IL-10 concentration compared to the RA group.

3.2.4. Evaluation of CD markers in RA and treated groups

CD4 and CD14 expression were analyzed to assess cell involvement in inflammation and treatment effects Figure 6. The RA group demonstrated a significant increase in CD4 level (1195 ± 7.93pg/mL, P<0.05) compared to the control group (823 ± 57.6pg/mL), Treatment with turmeric and the combination treatment (840.5 ± 88.6pg/mL, 907.6 ± 53.27pg/mL) significantly reduced CD4 level P<0.05, whereas the reduction of CD4 level in the Purslane treatment was not show significant (977.6 ± 53.27pg/ mL, P<0.05).

Although serum level CD14 in the RA group was elevated (5.032 ± 0.178ng/mL) compared to the control group (4.438 ± 0.1578ng/mL), these differences were not statistically significant. Similarly, CD14 levels in treatment groups, purslane, turmeric, and combination (4.122 ± 0.3395ng/mL, 4.419± 0.1616ng/mL, 4.461± 0.250ng/mL, respectively) did not show statistically significant reduction.

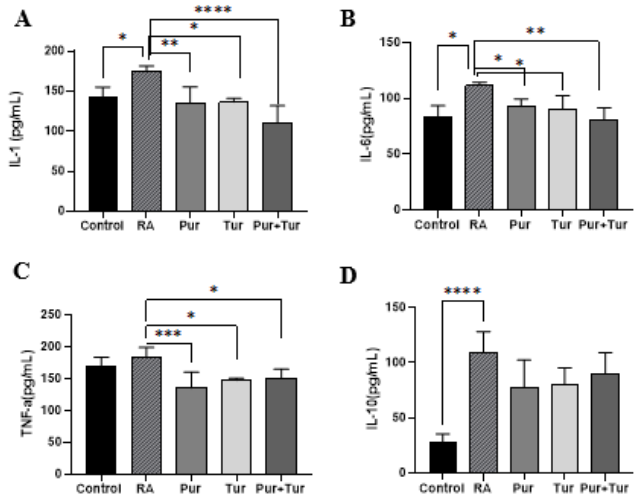

Fig. 5. Proinflammatory and anti-inflammatory cytokines, (A) Plant extract reduced IL-1 level significantly including purslane, turmeric and combination P<0.0001. (B) Similarly serum IL-6 concentrations. P=0.0046, (C) as were TNF-a, p=0.0009. (D) The reduction of IL-10was not significant. The data are expressed in mean ± SEM. p < 0.05 is considered as significant.

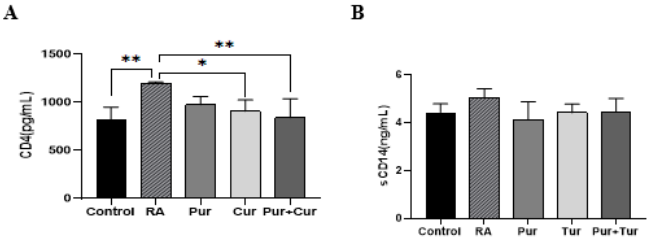

Fig. 6. The effect of purslane and turmeric extract on CD marker expression. (A) CD4 RA group compare to control increased p=0.001, reduction in purslane p>0.05, Turmeric p= 0.012 and combination p=0.008, as for CD14 the results were not significant p>0.05. The data are expressed in mean ± SEM. p < 0.05 is considered as significant.

4. Discussion

This study investigated the therapeutic effect of purslane, turmeric and their combination in a rat model of rheumatoid arthritis. The aim was to determine the anti-inflammatory effect of these plant extracts. Purslane is the rich source of omega-3 fatty acid, particularly alpha-linolenic acid (ALA) as found in previous study [26], while the main components of turmeric including curcumin and other anti-inflammatory active compounds [27]. In our investigation, the GC-Ms analysis of (*Portulaca oleracea*) purslane identified several bioactive compounds, among which linolenic acid and trimethylsilyl, a derivatized form of α-linolenic acid (ALA) omega-3 fatty acid known for its anti-inflammatory effects. The presence of ALA in purslane align with previous phytochemical reports and supports its traditional use in managing inflammatory conditions such as [28]. Other detected fatty acid derivatives, including 8,11,14-eicosatrienoic acid methyl ester further support therapeutic potential of purslane[29, 30]. The GC-MS analysis of (*curcuma longa*) identified curcumin (1,6-heptadiene-3,5-dione derivative) as it has been analyzed in previous study[31], the principal bioactive compound known for its potent anti-inflammatory, antioxidant, and immunomodulatory effects. The detection of curcumin confirm the efficacy of the extraction method and supports therapeutic role of turmeric in managing RA[32].

Phenotypically the animal model used in this study closely displays RA pathology, as evidenced by significantly increasing of paw edema which was found to be the highest during the first week after CFA injection as indicated in the previous studies [33-35]. However swelling progressively decreased with initiation of giving treatments, indicating that the compounds found in purslane and turmeric extracts have role in reduction of inflammatory reaction as shown in previous studies [36, 37]. Body weight gain measured to assess the overall health status of rats with rheumatoid arthritis and to evaluate the effects of treatments. The RA group exhibited less weight gain compared to the control and treated groups, which may be attributed to reduced food intake as consequences of systemic inflammation and disease-related discomfort. To support this, food consumption was monitored over 16-hour period. Healthy rats in the control group as well as treated groups, were able to consume approximately 160g of food within this period. In contrast the RA group consistently consumed noticeably less food under the same conditions. Due to reduced food intake in the RA group showed less weight gain compared to the control and treatment group. Although the body weight gain was not significantly increased in treatment group, increased movement and food

intake evidenced the reduction of joint pain in treatment groups.

Neutrophils are first cells that attracted to the site of injury and inflammation. Counting neutrophils indicated that absolute neutrophil count was significantly elevated in the RA group, reflecting inflammatory response. However treatment with purslane, turmeric, and their combination resulted in significant reduction in neutrophil count. Dai et al., 2018 [38], were observed that these plants have a direct effect on the neutrophil infiltration and pro-inflammatory cytokines. Curcumin, in particular, is known for its potential to modulate neutrophil-mediated inflammation in RA. Matrix metalloproteinase-1 (MMP-1), an enzyme which is involved in joint degradation, was higher in the RA group compared to the control and treatment groups. MMP-1 is a protease enzyme was studied to have a role in the destruction and degradation of bone and cartilage[39]. Anti-cyclic citrullinated peptide (CCP) detection is one of the newer markers that help diagnosing RA. The sensitivity of the assay is similar to RF, but it has better specificity [40]. Treatment with purslane and turmeric significantly reduced the level of anti-CCP, consistent with previous findings [41, 42]. However the combination treatment did not result in a significant reduction of anti-CCP level. The results of the current study revealed that the pro-inflammatory cytokines (IL-1, IL-6, TNF-a) were significantly elevated in the RA, and elevated those pro-inflammatory cytokines cause remarkable effects on the IGF-1/Akt and NF-κB pathways, IGF-1/Akt is crucial for protein synthesis [43]. They also involved in muscle atrophy by acting on nuclear factor-κB, p38MAPK, and JAK/STAT pathways through the corresponding receptors [44]. Cytokines, including (IL-1, IL-6, IL-10, TNF-a) play crucial roles in inflammatory response [45, 46]. On the other hand, treatment of RA induced rats with purslane and turmeric were significantly reduced these cytokines, except for IL-10 which was increased, suggesting that these plant extracts exert anti-inflammatory effects by modulating cytokine production and release. The anti-inflammatory potential of purslane has been well established in the previous studies [28, 47], and curcumin has been reported in inhibit the activation of PI3K/AKT signaling pathway and inhibiting expression of pro-inflammatory cytokine [48, 49]. Interestingly, the anti-inflammatory cytokine IL-10 was significantly elevated in both the RA and treatment groups, compared to the control group. Similar findings have been reported in previous studies, where higher IL-10 level were associated with increased seropositivity for rheumatoid factor (FR) and anti-CCP antibodies [50]. The present study shows a significant increase in CD4 level in the RA group compared to the control group, supporting the role of T-cell activation in RA pathogenesis. Treatment with turmeric and their combination significantly reduced CD4 levels, suggesting that turmeric extract, particularly curcumin, plays a more effective role in immune modulation. A study by Rahimi et al., 2019 [51] has documented curcumin's potential to target T-cell as it acts as an immunomodulator. Additionally, curcumin has been shown to inhibit cell proliferation, differentiation, and cytokine production, and resulting in CD2/CD3/CD28-initiated CD4 T cell activation suppression [52]. CD14 monocyte/macrophage marker involved in innate immune signaling and bacterial recognition compounds, contribute to synovial inflammation in rheumatoid arthritis. However, CD14 levels did not show a statistically significant reduction in the treatment groups. Further studies are necessary to determine the factors influencing CD14 expression in RA and the potential effects of plant-based treatments.

In conclusion the administration of purslane (*Portulaca oleracea*) and turmeric (*curcuma longa*) extract resulted in significant effect on key biomarkers of rheumatoid arthritis in rat model induced by Complete Freund's Adjuvant. The results demonstrated that bioactive compounds in these plants are able to reduce pro-inflammatory cytokines, paw edema, Anti-cyclic citrullinated peptide (CCP) and CD4, suggesting the potential role of purslane and turmeric in managing RA.

References

1. Radu A-F, Bungau SG (2021) Management of rheumatoid arthritis: an overview. Cells 10 (11): 2857. doi: 10.3390/cells10112857
2. Scherer HU, Häupl T, Burmester GR (2020) The etiology of rheumatoid arthritis. J Autoimmun 110: 102400. doi: 10.1016/j.jaut.2019.102400
3. Wu F, Gao J, Kang J, Wang X, Niu Q, Liu J, Zhang L (2021) B Cells in Rheumatoid Arthritis: Pathogenic Mechanisms and Treatment Prospects. Front Immunol 12: 750753. doi: 10.3389/fimmu.2021.750753
4. Tran CN, Lundy SK, Fox DA (2005) Synovial biology and T cells in rheumatoid arthritis. Pathophysiology 12 (3): 183-189. doi: 10.1016/j.pathophys.2005.07.005
5. Zhang C (2020) Flare-up of cytokines in rheumatoid arthritis and their role in triggering depression: shared common function and their possible applications in treatment (Review). Biomed Rep 14 (1): 16. doi: 10.3892/br.2020.1392
6. Yap H-Y, Tee SZ-Y, Wong MM-T, Chow S-K, Peh S-C, Teow S-Y (2018) Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. Cells 7 (10): 161. doi: 10.3390/cells7100161
7. Roberts CA, Dickinson AK, Taams LS (2015) The interplay between monocytes/macrophages and CD4+ T cell subsets in rheumatoid arthritis. Front Immunol 6: 571. doi: 10.3389/fimmu.2015.00571
8. Smith MH, Berman JR (2022) What is rheumatoid arthritis? JAMA 327 (12): 1194-1194. doi: 10.1001/jama.2022.0786
9. Han R, Ren HC, Zhou S, Gu S, Gu Y-Y, Sze DM-y, Chen M-H (2022) Conventional disease-modifying anti-rheumatic drugs combined with Chinese herbal medicines for rheumatoid arthritis: a systematic review and meta-analysis. J Tradit Complement Med 12 (5): 437-446. doi: 10.1016/j.jtcme.2022.01.005
10. Siddiqui AJ, Jahan S, Singh R, Saxena J, Ashraf SA, Khan A, Choudhary RK, Balakrishnan S, Badraoui R, Bardakci F (2022) Plants in anticancer drug discovery: from molecular mechanism to chemoprevention. Biomed Res Int 2022: 5425485. doi: 10.1155/2022/5425485
11. Makangali K, Tultabayeva T, Zamaratskaia G, Konysbayeva D, Tokysheva G, Shoman A, Kiyan V, Ospankulova G, Amirkhan A (2024) Evaluation of Antioxidant Properties and Nutritional Composition of Portulaca Grown in the Arid Region of Kyzylorda, Republic of Kazakhstan. doi: 10.20944/preprints202411.0657.v1
12. Montoya-García CO, García-Mateos R, Becerra-Martínez E, Toledo-Aguilar R, Volke-Haller VH, Magdaleno-Villar JJ (2023) Bioactive compounds of purslane (Portulaca oleracea L.) according to the production system: A review. Sci Hortic 308: 111584. doi: 10.1016/j.scienta.2022.111584
13. Wojtyłko M, Kunstman P, Bartylak H, Raszewski Ł, Osmałek T, Froelich A (2023) A Well-Known Plant and New Therapeu-

tic Strategies: Turmeric and Its Components in Oral Inflammatory Diseases Treatment. Appl Sci 13 (13): 7809. doi: 10.3390/app13137809
14. Tagde P, Tagde P, Islam F, Tagde S, Shah M, Hussain ZD, Rahman MH, Najda A, Alanazi IS, Germoush MO (2021) The multifaceted role of curcumin in advanced nanocurcumin form in the treatment and management of chronic disorders. Molecules 26 (23): 7109. doi: 10.3390/molecules26237109
15. Razavi BM, Ghasemzadeh Rahbardar M, Hosseinzadeh H (2021) A review of therapeutic potentials of turmeric (Curcuma longa) and its active constituent, curcumin, on inflammatory disorders, pain, and their related patents. Phytother Res 35 (12): 6489-6513. doi: 10.1002/ptr.7224
16. Sumeet G, Rachna K, Samrat C, Ipshita C, Vikas J, Manu S (2018) Anti inflammatory and anti arthritic activity of different milk based formulation of curcumin in rat model. Curr Drug Deliv 15 (2): 205-214. doi: 10.2174/1567201814666170320142851
17. Chen L, Liu T, Wang Q, Liu J (2017) Anti-inflammatory effect of combined tetramethylpyrazine, resveratrol and curcumin in vivo. BMC Complement Altern Med 17: 1-10. doi: 10.1186/s12906-017-1739-7
18. Insuan W, Hansupalak N, Chahomchuen T (2022) Extraction of curcumin from turmeric by ultrasonic-assisted extraction, identification, and evaluation of the biological activity. J Herbmed Pharmacol 11 (2): 188-196. doi: 10.34172/jhp.2022.23
19. Bradūnaitė R, Leonavičienė L, Akramas L, Leonavičiūtė-Klimantavičienė M, Vasiliauskas A, Dumalakienė I, Jonauskienė I, Baleišis J, Mackiewicz Z (2020) Anti-inflammatory and antioxidant effects of turmeric extracts in rat adjuvant arthritis. Vet Arhiv 90 (4): 393-402. doi: 10.24099/vet.arhiv.0675
20. Escobedo-Martínez C, Guzmán-Gutiérrez SL, Carrillo-López MI, Deveze-Álvarez MA, Trujillo-Valdivia A, Meza-Morales W, Enríquez RG (2019) Diacetylcurcumin: Its Potential Antiarthritic Effect on a Freund's Complete Adjuvant-Induced Murine Model. Molecules 24 (14): 2643. doi: 10.3390/molecules24142643
21. Pundarikakshudu K (2019) Antiinflammatory and antiarthritic activities of some foods and spices. Bioact Food Diet Interv Arthritis Relat Inflam Dis: 51-68. doi: 10.1016/B978-0-12-813820-5.00004-0
22. Rao BM, Kavitha R, Subash K, Rao NJ (2012) Evaluation of anti arthritic activity of pet–ether extract of Portulaca oleracea (Linn.). doi: [missing]
23. Ismail SA, Mohamed WA Clinicopathological Studies on the Protective Effect of Phytoestrogens on Freund's Complete Adjuvant Induced Arthritis in Ovariectomized Rats. doi: [missing]
24. Irwin MR, Curay CM, Choi S, Kiyatkin EA (2023) Basic physiological effects of ketamine-xylazine mixture as a general anesthetic preparation for rodent surgeries. Brain Res 1804: 148251. doi: 10.1016/j.brainres.2023.148251
25. Messias JB, de Oliveira Messias IM, Farias IP, Ramos ALG, de Magalhães Caraciolo MC (2017) Automated method and not automated: the evaluation of hematological parameters of rats. Rev Bras Ci Vet 24 (2): 77-80. doi: 10.4322/rbcv.2017.016
26. Nemzer B, Al-Taher F, Abshiru N (2020) Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (Portulaca oleracea L.) genotypes. Food Chem 320: 126621. doi: 10.1016/j.foodchem.2020.126621
27. Zeng L, Yang T, Yang K, Yu G, Li J, Xiang W, Chen H (2022) Efficacy and safety of curcumin and Curcuma longa extract in the treatment of arthritis: a systematic review and meta-analysis of randomized controlled trial. Front Immunol 13: 891822. doi: 10.3389/fimmu.2022.891822
28. Karimi E, Aryaeian N, Akhlaghi M, Abolghasemi J, Fallah S, Heydarian A, Rostami R, Bayat MH, Mahmoudi M (2024) The effect of purslane supplementation on clinical outcomes, inflammatory and antioxidant markers in patients with rheumatoid arthritis: A parallel double-blinded randomized controlled clinical trial. Phytomedicine 135: 156006. doi: 10.1016/j.phymed.2024.156006
29. Saxena S, Rao PB (2021) Comparative GC-MS and FT-IR analysis of Portulaca oleracea L. and Portulaca quadrifida L. leaf extracts. Pharma Innov J 10 (12): 40-48. doi: [unavailable]
30. Durgawale TP, Khanwelkar CC, Pratik PD (2018) Phytochemical analysis of Portulaca oleracea and Portulaca quadrifida extracts using gas chromatography and mass spectrophotometry. Asian J Pharm Clin Res 11 (9). doi: 10.22159/ajpcr.2018.v11i9.26662
31. Masih R, Iqbal MS (2022) Thermal degradation kinetics and pyrolysis GC–MS study of curcumin. Food Chem 385: 132638. doi: 10.1016/j.foodchem.2022.132638
32. Pourhabibi-Zarandi F, Shojaei-Zarghani S, Rafraf M (2021) Curcumin and rheumatoid arthritis: A systematic review of literature. Int J Clin Pract 75 (10): e14280. doi: 10.1111/ijcp.14280
33. Zhu L, Zhang Z, Xia N, Zhang W, Wei Y, Huang J, Ren Z, Meng F, Yang L (2020) Anti-arthritic activity of ferulic acid in complete Freund's adjuvant (CFA)-induced arthritis in rats: JAK2 inhibition. Inflammopharmacology 28: 463-473. doi: 10.1007/s10787-019-00642-0
34. Weng W, Wang F, He X, Zhou K, Wu X, Wu X (2021) Protective effect of corynoline on the CFA induced rheumatoid arthritis via attenuation of oxidative and inflammatory mediators. Mol Cell Biochem 476: 831-839. doi: 10.1007/s11010-020-03948-8
35. Noh ASM, Chuan TD, Khir NAM, Zin AAM, Ghazali AK, Long I, Ab Aziz CB, Ismail CAN (2021) Effects of different doses of complete Freund's adjuvant on nociceptive behaviour and inflammatory parameters in polyarthritic rat model mimicking rheumatoid arthritis. PLoS One 16 (12): e0260423. doi: 10.1371/journal.pone.0260423
36. Anandakumar S, Joseph JA, Bethapudi B, Agarwal A, Jung E-B (2014) Anti-inflammatory effects of turmeric (Curcuma longa L.) extract on acute and chronic inflammation models. J Korean Soc Food Sci Nutr 43 (4): 612-617. doi: 10.3746/jkfn.2014.43.4.612
37. Fahd A, Saliem A (2024) Comparative analgesic effect of Portulaca oleracea L. extracted apigenin and diclofenac in mice. Adv Anim Vet Sci 12 (4): 629-636. doi: 10.17582/journal.aavs/2024/12.4.629.636
38. Dai Q, Zhou D, Xu L, Song X (2018) Curcumin alleviates rheumatoid arthritis-induced inflammation and synovial hyperplasia by targeting mTOR pathway in rats. Drug Des Devel Ther 12: 4095-4105. doi: 10.2147/DDDT.S175763
39. Rafraf M, Haghighian MK, Molani-Gol R, Hemmati S, Asghari Jafarabadi M (2024) Effects of Pomegranate (Punica granatum L.) Peel Extract Supplementation on Markers of Inflammation and Serum Matrix Metalloproteinase 1 in Women With Knee Osteoarthritis: A Randomized Double-Blind Placebo-Controlled Study. Nutr Metab Insights 17: 11786388241243266. doi: 10.1177/11786388241243266
40. Wahab AA, Mohammad M, Rahman M, Said MSM (2013) Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of rheumatoid arthritis. Pak J Med Sci 29 (3): 773. doi: 10.12669/pjms.293.2924
41. Emad ME-K, Sahar O, Fatma M (2018) Evaluation of Anti-Rheumatoid Arthritis Activity of White, Black Mulberry and Turmeric in Complete Freund's Adjuvant Induced Rheumatoid Arthritis Rats. Med J Cairo Univ 86 (December): 3715-3722. doi: 10.21608/mjcu.2018.61486
42. Elharrif MG, Maksoud HA, Abdullah M, Abd Elmohsen AS (2024) Biochemical evaluation of possible protective effect of purslane extract in experimentally induced arthritis associated with obesity. Prostaglandins Other Lipid Mediat 172: 106823.

doi: 10.1016/j.prostaglandins.2024.106823

43. Martín AI, Priego T, Moreno-Ruperez Á, González-Hedström D, Granado M, López-Calderón A (2021) IGF-1 and IGFBP-3 in Inflammatory Cachexia. Int J Mol Sci 22 (17): 9469. doi: 10.3390/ijms22179469
44. Ji Y, Li M, Chang M, Liu R, Qiu J, Wang K, Deng C, Shen Y, Zhu J, Wang W (2022) Inflammation: roles in skeletal muscle atrophy. Antioxidants 11 (9): 1686. doi: 10.3390/antiox11091686
45. Nooruldeen GM, Fatah HR (2023) Inflammatory Response and The Effect of Coronavirus on Some Body Organs in Hospitalized Patients. Qalaai Zanist J 8 (4): 1145-1164. doi: [missing]
46. Asaad HR, Aziz FM (2012) Protective role of omega-3 fish oil against the toxicity of ifosfamide in male rats. Jordan J Biol Sci 5 (1): 37-346. doi: [unavailable]
47. Allahmoradi E, Taghiloo S, Omrani-Nava V, Shobeir SS, Tehrani M, Ebrahimzadeh MA, Asgarian-Omran H (2018) Anti-inflammatory effects of the Portulaca oleracea hydroalcholic extract on human peripheral blood mononuclear cells. Med J Islam Repub Iran 32: 80. doi: 10.14196/mjiri.32.80
48. Xu Z, Shang W, Zhao Z, Zhang B, Liu C, Cai H (2022) Curcumin alleviates rheumatoid arthritis progression through the phosphatidylinositol 3-kinase/protein kinase B pathway: An in vitro and in vivo study. Bioeng 13 (5): 12899-12911. doi: 10.1080/21655979.2022.2078942
49. Zhang C, Weng Y, Wang H, Zhan S, Li C, Zheng D, Lin Q (2024) A synergistic effect of triptolide and curcumin on rheumatoid arthritis by improving cell proliferation and inducing cell apoptosis via inhibition of the IL-17/NF-κB signaling pathway. Int Immunopharmacol 142: 112953. doi: 10.1016/j.intimp.2024.112953
50. Hernández-Bello J, Oregón-Romero E, Vázquez-Villamar M, García-Arellano S, Valle Y, Padilla-Gutiérrez J, Román-Fernández I, Palafox-Sánchez C, Martínez-Bonilla G, Muñoz-Valle J (2017) Aberrant expression of interleukin-10 in rheumatoid arthritis: Relationship with IL10 haplotypes and autoantibodies. Cytokine 95: 88-96. doi: 10.1016/j.cyto.2017.02.022
51. Rahimi K, Ahmadi A, Hassanzadeh K, Soleimani Z, Sathyapalan T, Mohammadi A, Sahebkar A (2019) Targeting the balance of T helper cell responses by curcumin in inflammatory and autoimmune states. Autoimmun Rev 18 (7): 738-748. doi: 10.1016/j.autrev.2019.05.012
52. Kim G, Jang MS, Son YM, Seo MJ, Ji SY, Han SH, Jung ID, Park Y-M, Jung HJ, Yun C-H (2013) Curcumin inhibits CD4+ T cell activation, but augments CD69 expression and TGF-β1-mediated generation of regulatory T cells at late phase. PLoS One 8 (4): e62300. doi: 10.1371/journal.pone.0062300