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Article

Evaluation of the anti-arthritic activity of *Capparis spinosa* L. roots extracts incomplete Freund's adjuvant-induced arthritis in mice.

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Abstract
This study aimed to estimate the ant-arthritic activity of *Capparis spinosa* L. roots extract in complete Freund's adjuvant (CFA)-induced arthritis mice by assessment of paw thickness, serological detection of inflammatory markers [rheumatoid factor (RF) and tumor necrosis factor-alpha (TNF-α)] using ELISA, and histopathology. In total, 30 male mice were selected, prepared, and divided equally into five groups, including 2 control (NC and PC)and 3 experimental: EG1 (arthritis mice treated total effective dose of extract), EG2(arthritis mice treated total effective dose of Diclofenac Sodium), and EG3 (arthritis mice treated a half effective dose of each the extract and Diclofenac). Clinically, the extract administration lowered the paw thickness from day 7th onwards, the 21st day of study, while the extract administration lowered the paw thickness in EG1 from day 7th onwards compared to EG2 and EG3. For hematology, significant decreases were reported in RBCs, HCT, Hb, WBCs and neutrophils due to arthritis, and significant amelioration was seen obviously in mice of EG1 as a result of therapy. Significant increases in platelets, lymphocytes and monocytes were observed in PC and experimentally groups, significantly improving the values of treated groups due to therapy, in particular, EG1. All study groups ' values of MCV, MCH, MCHC, basophils and eosinophils have differed insignificantly. Concerning RF, the highest significant value was reported in PC while the lowest in NC, but without significant differences between EG1, EG2 and EG3 values. For TNF-α, elevation had been recorded in PC mice, whereas reduction in NC, EG1 and EG3. Also, the value of EG2 was significantly higher than recorded in mice of EG1 and EG3. Concerning histology, the findings of EG1 revealed a significant amelioration compared to other groups. In conclusion, *C. spinosa* L. root extract demonstrated anti-inflammatory and anti-arthritic activity. Furthermore, studies to detect the therapeutic effects of the root extract on other systemic or local diseases are needed.

Keywords: Paw thickness; Rheumatoid factor; Inflammatory markers; Diclofenac Sodium; Iraq

Introduction

Arthritis is derived from the Greek word for "joint disease" and is defined as acute or chronic inflammation of the joints, often with pain and structural damage [1]. The disease can range from autoimmune processes such as rheumatoid arthritis (RA) to inflammations and infections [2, 3]. In addition, the disease can relate to other dis-

eases such as scleroderma, myositis, tuberculosis and celiac disease and risk factors such as immune suppression, aging, diabetes, and artificial joints [4,5,6]. RA is an inflammatory disease manifested by the infiltration of inflammatory cells to synovial tissue with the initiation of inflammatory processes that have an essential role in joint deformity that result in severe joint pain and inflammation, causing joint dysfunction [7,8]. Severe fatigue is an almost pronounced sign of RA compared to normal fatigue seen in people not diagnosed with RA [9]. Factors associated with increased anxiety in RA patients, such as medical problems, psychological and social drawbacks, lack of lifelong activities, and loss of control of prognosis, are the main financial burdens of RA [10, 11]. The environment can produce anti-citrullinated protein antigens (ACPA) in RA, and epigenetic regulation links the environment with genes [12]. Gene-environment interactions affect auto-antibodies responsiveness to the citrullinated antigen of RA [13].

Many studies have attempted to define RA diagnostic methods in clinical practice to identify the actual onset of the disease better. However, there is no single diagnostic test for patients in the early stages of RA, and evaluation requires a combination of clinical features and laboratory tests [14,15,16]. When looking at these patients, an important question is to determine what the illness is- starting with patients with self-restrictive illness, not those at risk of developing chronic inflammation or arthritis. Therefore, it referred to integrating the clinical features, laboratory tests, and methods for assessing the risk of chronic or irreversible RA patients [17,18,19]. Biopsy of synovial tissue joint lesions should be a powerful tool for identifying early RA synovium histopathological features and quantitatively comparing the different types of RA synovitis [20]. Despite significant advances in therapeutic technology, disease-modifying anti-rheumatoid drugs (DMARDs), a group of medications, are commonly used in people with RA [21]. Corticosteroids and non-steroid anti-inflammatory drugs (NSAIDs) can directly target the symptoms and provide rapid relief but with long-term risk to the kidney, liver, and heart [22, 23]. *Capparis spinosa* L, belonging to the family Capparidaceae, is a tough, branched, bushy, leafy and small plant native to the arid desert regions of the world [24]. Worldwide, *C. spinosa* is used as a laxative, amenorrhea, antidote and aphrodisiac in addition to its ability to enhance stamina and activity against rheumatism, cough, back pain and asthma as it contains many botanical ingredients related to their medicinal properties [25]. Hence, the current study aimed to estimate the ant-arthritic activity of *C. spinosa* L. roots extract in adjuvant-induced arthritic mice by measurement of paw thickness, serological detection of inflammatory markers using the sandwich enzyme-linked immunosorbent assays (ELISAs) and investigation histopathological changes of ankle joint.

Materials and Methods

Ethical approval

This study was licensed by the Scientific Committee of the Department of Physiology, Biochemistry and Pharmacology in the College of Veterinary Medicine (University of Baghdad, Baghdad, Iraq).

Experimental animals

In total, 30 male mice aged ≤4 months and weighing 25-40 gm were purchased from the local market and transported to the Lab Animal House (College of Veterinary Medicine, University of Wasit). Initially, all study animals were subjected to a preparation period of 1 week, during which they were fed a pellet, presented to tap water and exposed to 12 / 12 hours of light/dark.

Preparation of C. spinosa L. roots extract

The roots of *C. spinosa* were collected from different rural areas in Wasit province (Figure 1a), washed, dried and ground by a grinder. The obtained dry powder (about 100 grams) was solved in 70% ethanol and set for extraction by the Soxhlet apparatus at a temperature of 45ºC [26]. The extract was then filtered and dried under the vacuum (150 rpm / 4 hours) at 40ºC. Later, the crude extract of *C. spinosa* was concentrated in a glass petri dish by placing it in an incubator at 40ºC until the semi-solid, thick and dark brown crude extract appeared (Figure 1b). All dried extract was collected, stored in aseptic containers, and frozen at 4ºC.

(a) (b)

Figure 1. (a) Fresh collection of *Capparis spinosa* L.; (b) Concentrated *C. spinosa* L. roots extract.

Study design

The study mice were divided equally into 5 groups as follows:

1. Negative control (NC): Mice received only distilled water.
2. Positive control (PC): Mice injected only complete Freund's adjuvant (CFA)
3. EG1: Mice were injected with a single dose of CFA and then, daily, treated with an effective dose of *C. spinosa* L. roots extract (250 mg/kg BW).
4. EG2: Mice were injected with a single dose of CFA and then, daily, treated with Diclofenac Sodium (0.71 mg/kg BW).
5. EG3: Mice were injected with a single dose of CFA and treated daily with the half-effective dose of each *C. spinosa* L. roots extract (125 mg/kg BW) and Diclofenac Sodium (0.355 mg/kg BW).

Measurement of paw thickness

The study mice of all groups were subjected to measurement thickness of injected paw at 0, 3rd, 7th, 14th and 21st days throughout the study experiment period that continued for 21 days.

Samples collection

After the end of this period, the study mice were euthanized with chloroform and subjected to direct sampling of blood and tissue sectioning of the ankle joint. The blood samples were collected into an EDTA glass tube to measure hematological parameters and inflammatory markers. In contrast, the tissue specimens were saved into plastic containers containing 10% neutral buffered formalin (NBF).

Hematology

Hematological parameters were evaluated using the automated Mythic 18 Vet Blood Analyser System (Orphèe / Switzerland).

Inflammatory markers

According to manufacturers' instructions for the rheumatoid factor (RF) ELISA kit (Solarbio Life Science / China) and tumor necrosis factor-alpha (TNF-α) ELISA kit (Solarbio Life Science / China), the sera and ELISA solutions were prepared and processed. After adding the Stop solution, the kits' optical density (OD) was measured at 450 nm, and the concentrations of inflammatory markers were determined based on ODs and concentrations of the Standards of each kit.

Histopathology

According to Gharban et al. (2019), the fixed tissue in 10%NBF was dehydrated using increased ethanol concentrations, cleared by xylene, infiltrated and embedded in xylene and then paraffin, sectioned by microtome (LEICA / Germany), and mounted while staining using the Haematoxylin and Eosin was carried out following the manufacturer's instruction(SYRBIO / Syria) [27].

Statistical analysis

The results were analyzed using the GraphPad Prism version 6.0.1 (GraphPad Software Inc; USA). ANOVA test was applied to detect significant variation between values of different study groups at P<0.05. In each table, capital horizontal and small vertical letters referred to significant differences, and values were represented as mean ± standard errors (M±SE) [28, 29].

Results

Clinical measurement of paw thickness

The mice of PC showed an elevation in values of paw thickness when compared to those of NC from the 3rd day onwards to the 21st day of study, while the extract administration lowered the paw thickness in EG1 from day 7th onwards as compared to EG2 and EG3 (Table 1).

Group	Day				
	0	3	7	14	21
PC	1.68 ± 0.01 Bb	5.52 ± 0.11 Aa	6.51 ± 0.08 Aa	6.2 ± 0.17 Aa	5.78 ± 0.18 Aa
NC	1.70± 0.02 Bb	1.71± 0.04 Ac	1.70± 0.03 Ad	1.70± 0.04 Ad	1.70± 0.06 Ad
EG1	1.66 ± 0.02 Cb	4.49 ± 0.11 Ab	4.81 ± 0.11 Ac	4.43 ± 0.08 Ac	3.8 ± 0.1 Bc
EG2	1.65 ± 0.03 Cb	4.96 ± 0.1 Ab	5.4 ± 0.07 Ab	5.19 ± 0.11 Ab	4.82 ± 0.1 Bb
EG3	1.66 ± 0.01 Cb	4.88 ± 0.1 Ab	5.01 ± 0.14 Ab	4.85 ± 0.06 Ab	4.57 ± 0.14 Bb

Table 1. Results of paw thickness values (cm) among the study groups.
Represent mean ± standard error, Number of group animals = 6, Different capital letters at same groups mean significant difference (p<0.05) between groups, Different small letters at same row mean significant difference (p<0.05) between groups.

Hematology

The finding of blood parameters varied significantly (P<0.05), with significant decreases in values of RBCs, HCT, Hb, WBCs and neutrophils in PC, EG1, EG2 and EG3 due to arthritis, and ameliorating of these values significantly as a result of therapy, in particular, in mice of EG1. , there was a significant increase in platelets, lymphocytes and monocyte values of PC and experimental groups, with significant improvement in values of treated groups due to therapy. In particular, EG1 received the total dose of *C. spinosa* L. roots extract. However, insignificant

variation (P>0.05) was seen in values MCV, MCH, MCHC, basophils and eosinophils between the study groups (Table 2).

Parameter	Unit	Group				
		NC	PC	EG1	EG2	EG3
RBCs	'10^6	9.54 ± 0.15 A	6.29 ± 0.45 D	8.66 ± 0.33 B	7.31 ± 0.17 C	7.85 ± 0.32 C
HCT	%	40.92 ± 1 A	27.14 ± 0.56 C	34 ± 0.7 B	28.46 ± 1.72 C	31.74 ± 1.17 B
Hb	g/dl	12.04 ± 0.34 A	7.86 ± 0.2 D	9.86 ± 0.23 B	8.08 ± 0.5 C	9.24 ± 0.34 B
MCV	fl	43 ± 1.6 B	44.15 ± 3.65 B	48.77 ± 2.1 B	43.04 ± 4.25 B	43.44 ± 1.26 B
MCH	pg	12.65 ± 0.53 B	12.78 ± 1.06 B	14.11 ± 0.59 B	12.37 ± 1.26 B	12.62 ± 0.37 B
MCHC	g/dl	29.41 ± 0.15 B	28.95 ± 0.2 B	29 ± 0.1 B	28.71 ± 0.17 B	29.11 ± 0.1 B
PLT	'10^3	145.33 ± 19.1 E	736 ±62.56 A	216.33 ± 57.46 D	538.33 ± 44.1 B	439 ± 50.35 C
WBCs	'10^3	10.46 ± 0.23 A	8.83 ± 0.22 C	10.03 ± 0.11 A	9.41 ± 0.15 B	9.59 ± 0.05 B
Lymphocytes	%	64.25 ± 2.21 C	79.75 ± 1.25 A	62.5 ± 3.28 C	71.5 ± 2.72 B	70 ± 2.27 B
Monocytes	%	2 ± 0.41 C	4.25 ± 0.48 A	4 ± 0.24 A	3.5 ± 0.67 B	2.5 ± 0.25 C
Neutrophils	%	29 ± 1.87 A	13.25 ± 1.38 D	25.75 ± 1.6 B	22 ± 3.14 C	24.5 ± 1.85 C
Basophils	%	0.25 ± 0.25 B	0.25 ± 0.25 B	0.5 ± 0.29 B	0.5 ± 0.29 B	0.5 ± 0.29 B

Table 2. Results of hematology among the study groups.
Values represent mean ± standard error, Number of group animals = 6, Different capital letters at same groups meaning a significant difference (p<0.05) between groups

Inflammatory markers

The inflammatory markers (RF and TNF-α) findings demonstrated a significant variation in their values (Table 3). Concerning RF, the highest significant value was reported in PC mice, while the lowest was seen in mice of NC. However, insignificance was reported among EG1, EG2 and EG3. For TNF-α, elevation was recorded in PC mice, whereas the reduction was observed in NC, EG1 and EG3. However, the value of EG2 was significantly higher than recorded in mice of EG1 and EG3.

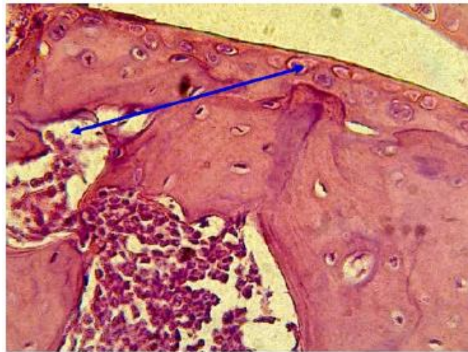
Group	Marker	
	RF	TNF-α
NC	67.78 ± 5.92 c	64.24 ± 15.72 c
PC	215.58 ± 10.67 a	182.95 ± 20.74 a
EG1	109.32 ± 20.51 b	66.78 ± 7.4 c
EG2	113.29 ± 7.83 b	85.71 ± 10.49 b
EG3	90.89 ± 14.16 b	78.27 ± 5.27 c

Table 3. Results of inflammatory markers among the study groups.
Values represent mean ± standard error, Number of group animals = 6, Different small letters at same column meaning significant difference (p<0.05) between groups.

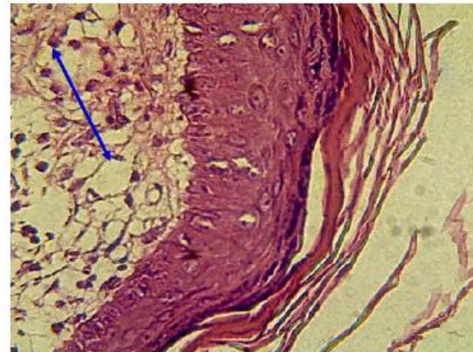
Histopathology

The findings observed in mice of EG1 included the proliferation of chondrocytes of articular cartilage with moderate cellular and subchondral marrow space (Figure 2a), infiltration of few inflammatory cells in the dermis (Figure 2b), the proliferation of chondrocytes in articular cartilage with the absence of inflammatory reaction in synovial cavity (Figure 2c), regenerative articular cartilage and regular tidemark line with presence of very narrow distance between articular cartilage and marrow bone (Figure 2d). In mice of EG2, there was a moderate distance between articular cartilage and bone marrow (Figure 2e), the thickness of bone trabeculae and infiltration of inflammatory cells in bone marrow (Figure 2f), the proliferation of chondrocytes with regular tidemark (Figure 2g), and dead fragments of bone and sclerosis in the subchondral bone (Figure 2h). In mice of EG3, there was an infiltration of few inflammatory cells in the papillary

dermis and subcutaneous tissue (Figure 2i), marked proliferation of osteoclast that was lining the trabeculae (Figure 2j), marked proliferation of regular tidemark line with moderate thickness of subchondral bone and absence of fibrosis in bone marrow (Figure 2k), the narrowing distance between cartilage and bone (Figure 2 l).



(**a**)



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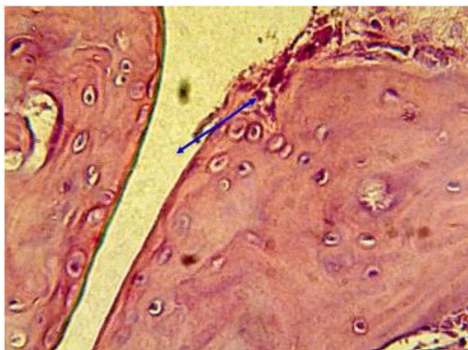

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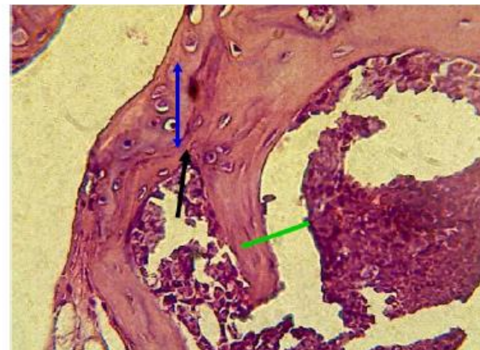

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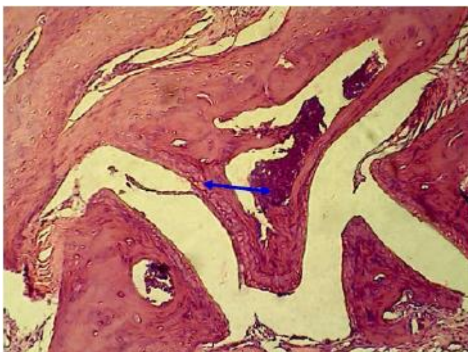

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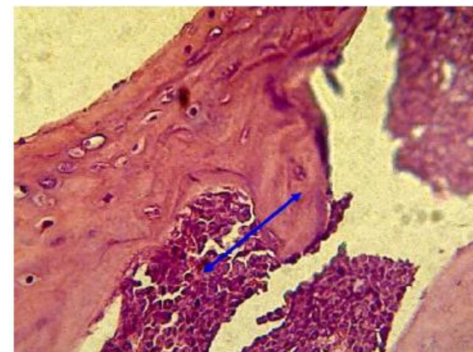

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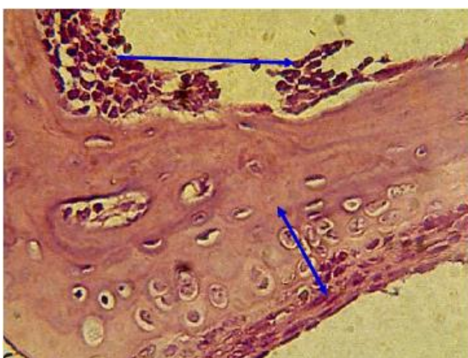
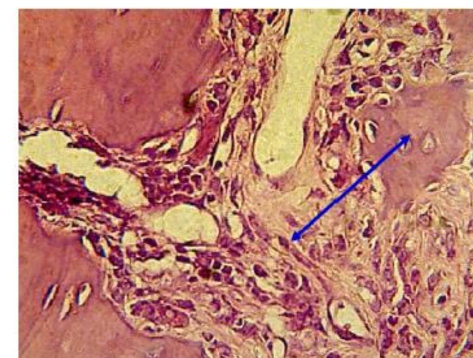


(**g**)



(**h**)

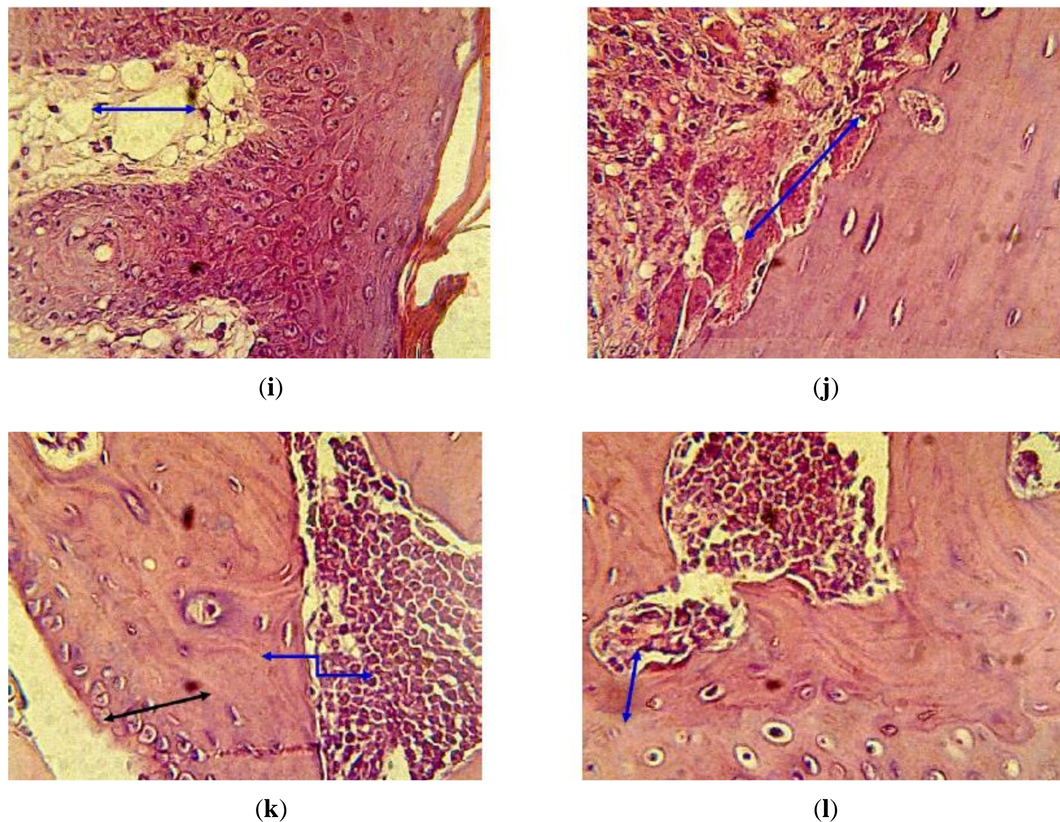


Figure 2. Histopathological section of: (a) EG1 shows proliferation of chondrocytes of articular cartilage (Blue arrow), (H & E stain 400X); (b) EG1 shows infiltration of few inflammatorycells in dermis (Blue arrow), (H & E stain 400X); (c) EG1 shows proliferation of chondrocytes in articular cartilage with absence of inflammatory reactionn in synovial cavity (Blue arrow), (H &E stain 400X); (d) EG1 shows regenerative articular cartilage (Black & Blue arrow) with presence of very narrow distance between articular cartilage and marrowbone (Green arrow), (H & E stain 400X); (e) EG2 shows moderate distance between articular cartilage and bone marrow (Blue arrow), (H & E stain 100X); (f) EG2 shows thickness bone trabeculae with inflammatory cells in bone marrow (Blue arrow), (H & E stain 400X); (g) EG2 shows proliferation of chondrocytes with regular tidemark (Blue arrow), (H & E stain 400X); (h) EG2 shows dead fragment bone and sclerosis in subchondral bone (Blue arrow), (H & E stain 400X); (i) EG3 shows few inflammatory cells in papillary dermis (Blue arrow), (H & E stain 400X); (j) EG3 shows marked proliferation of osteoclast linining the trabeculae (Blue arrow), (H & E stain 400X); (k) EG3 mice shows marked proliferation of regular tidemark line (Black arrow) with moderate thickness of subchondral bone (Blue arrow), (H & E stain 400X); (l) EG3 mice shows narrowing distance between cartilage and bone (Blue arrow), (H & E stain 400X).

Discussion

Clinical data

As detected in this study, the anti-inflammatory effect of *C. spinosa* L. roots extract was found by other studies in root [30, 31], fruits [32], buds [30] and leaves [33]. Other studies mentioned that the root extract could be an analgesic and antibacterial due to various traditional medicines' excellent economic and medicinal properties in many Asian and European countries such as Iran, China, Greece and Arabia [34, 35]. According to Syrian tradition, only powdered roots or "pastes" obtained by mixing powder with water are widely used for oral/topical application on painful areas [36]. In traditional Jordanian medicine, the root bark is rubbed against the painful area during burns for 15-25 minutes to relieve inflammation and muscle aches [37]. This ability to reduce human pain is of particular interest, as few natural compounds have so far been shown to be effective in controlling cytokine gene expression through blood mononuclear cells [38, 39]. Some alkaloids were also found to increase blood clotting and to reduce bleeding time and blood loss [36]. Various secondary metabolites such as phenol, free sterols and glycosylated sterols have been isolated, and their properties have been determined from dried roots [40, 41].

Hematology

The complete blood count is one of the most sensitive parameters for assessing the efficacy and toxicity of human and animal drugs since it provides crucial information about the body's response to injury and stress [42]. The findings of this study were similar to those obtained by Majeed and Esmaeel (2019) [43]. We suggested that arthritis may cause hemolysis, decrease hemoglobin production, and increase immunomodulatory activity that affects the immune response. Daily intakes of the extract actively restored normal blood levels, especially RBCs, HCT, Hb, PLT, WBC, lymphocytes, and neutrophils. Subsequently, increased RBCs, Hb, and PCV levels in treated groups indicated that the extract had hematopoietic activity. These extract effects may be associated with secondary metabolites such as tannins, phenols, flavonoids and alkaloids. Flavonoids reduce inflammatory mediators, and the malondialdehyde (MDA) level is closely related to oxidative stress and oxidative degradation [44]. In addition, flavonoids contain potent antioxidant polyphenol compounds that prevent the peroxidation of polyunsaturated fats in cell membranes and the formation of superoxide ions, peroxides, and hydroxyl radicals [45, 46]. Significant increases in platelet (PLT) levels have been observed by Cuenca-Zamora et al. (2020), suggesting that PLT can bind to a variety of inflammatory molecules present in arthritis patients [47]. Many studies have demonstrated the role of PLT as a critical cytokine in circulation and in transporting and expressing inflammatory mediators that play a regulatory role between hemostasis and immunity [48, 49]. Activated platelets can also produce micro-particles and act as a receptor for activation, ligand binding, and dysfunctional signaling pathways in arthritis patients [50-52].

Inflammatory markers

Concerning RF, our findings were similar to those reported by other studies [53, 54]. Early studies of the etiology of arthritis were focused on the role of RF and immune complexes associated with vasculitis and synovitis [55, 56]. Subsequent studies identified the role of RF in increasing T cell responses and cytokines and its role in the severity of arthritis [57, 58]. Croatia (2019) showed that significant activity in reducing arthritis was evident by the significant reduction in serum RF levels [59]. The role of *C. spinosa* root extract in decreasing the level of RF may be related to the critical role of alkaloids and flavonoids in the production, synthesis and degradation of proteins such as RF. Furthermore, Lam et al. (2009) isolated a protein from *C. spinosa,* which has an inhibitory effect on RF [60]. There is strong evidence that TNF-α released by peripheral blood mononuclear cells (PBMCs) contribute to the development of the immune system against infections. In arthritis, the high levels of TNF-α observed in patients are active and widespread and are involved in mechanisms that regulate T cell inhibitory activity [61]. Our findings showed that the TNF-α level was increased in arthritis mice, which agrees with that observed by others [62-64]. As observed in EG1 of this study, various studies have reported the effect of *C. spinosa* extract in decreasing TNF-α levels in arthritis patients, and this effect may be attributed to the fact that roots of *C. spinosa* are rich in flavonoids that have an intense anti-inflammatory activity [40, 65, 66].

Histopathology

Stimulation and destruction of immune cells, pro-inflammatory cytokines and other inflammatory mediators are associated with the pathogenesis of RA [67]. Therefore, changes in the immune response/inflammation are essential mechanisms explaining the true incidence of RA lesions, as shown in various publications and experimental studies of co-arthritis models [65, 68, 69]. Frasnelli et al. (2005) have assessed the severity of histological signs of arthritis, reporting that infiltration of inflammatory cells at day 8 indicates cartilage deterioration [70].

El-Tanbouly and Abdelrahman (2022) found that arthritis and related symptoms are associated with the signs of synovial hyperplasia and cartilage, bone destruction, inflammation, and increased vascularity in the synovial membrane [54]. In this study, tissue sections of treated mice, especially EG1 mice, revealed a significant improvement in their structure, demonstrating the effect of *C. spinosa* root extract. This could be explained by the fact that this extract is rich in glucosinolates (glucosinolates, glucosides, myrosins, glucosinolates), flavonoids, phenolic acids and alkaloids which provide health benefits from a variety of biological properties that have different biological activities including antioxidant, anti-inflammatory, anti-cancer, antibacterial, anti-mutagenic, and antidiabetic properties [71-74]. Interestingly, *C. spinosa* is safe and has no scientific evidence of side effects or toxicity. Based on the composition of the active ingredient of *C. spinosa*, it is believed that it may have potential protective effects against oxidative stress, genotoxicity and cytotoxicity in animal models [34, 75, 76].

Conclusions

This study demonstrated the anti-arthritic effects of *C. spinosa* L. roots extract in vivo and justified using this extract as an anti-inflammatory and anti-arthritic crude drug. This is the first study that detected the association of *C. spinosa* L. root extract and RF. Histological examination of the tissue section revealed that *C. spinosa* L. roots extract could effectively treat the injured tissues caused by arthritis, suggesting its activity in medication for other health problems and injuries. Therefore, further studies are needed to detect the therapeutic effects of *C. spinosa* L. root extract on other systemic or local diseases. Also, therapeutic effects of other *C. spinosa* L. parts, such as leaves and seeds, should be aimed.

Author Contributions: Conceptualization, RJAJ and AHC; methodology, RJAJ; software, RJAJ; validation, RJAJ; and AHC; formal analysis, RJAJ; investigation, RJAJ; writing-original draft preparation RJAJ; writing-review and editing RJAJ; and AHC; visualization, AHC; supervision, AHC; project administration, RJAJ All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Scientific Committee of the Department of Physiology, Biochemistry and Pharmacology in the College of Veterinary Medicine [University of Baghdad, Baghdad, Iraq].

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Manoto, S.L; Maepa, M.J; Motaung, S.K. Medical ozone therapy as a potential treatment modality for regenerating damaged articular cartilage in osteoarthritis. *Saudi J Bio Sci* **2018**, 25 (4), 672-679.
2. Nazaal, A.R; Al-Judi, A.M.H; Abdulla, F.A. Bacterial, cytological and biochemical study of arthritis in sheep and goats. *Iraqi J Vet Med* **2005**, 29 (2), 93-100.
3. Picchianti-Diamanti, A.; Rosado, M.M; D'Amelio, R. Infectious agents and inflammation, the role of microbiota in autoimmune arthritis. *Frontiers Micro* **2018**, 8, 1-14.
4. Al-Awadi, A.Q; Alwan, M.J. The influence of whole sonicated Pseudomonas aeruginosa antigens on experimental p. aeruginosa arthritis in rabbits. *Iraqi J Vet Med* **2014**, 38 (1), 1-10.

5. Spyridakis, E; Gerber, J.; Schriver, E; Grundmeier, R.W; Porsche, E.A; Geme, J.; Downes, K.J. Clinical features and outcomes of children with culture-negative septic arthritis. *J Pediat Infect Dis Society* **2019**, 8(3), 228-234.
6. Hong, Y.H; Song, C; Shin, K.K; Choi, E; Hwang, S.H; Jang, Y.J; Cho, J.Y. Tunisian Olea europaea L. leaf extract suppresses Freund's complete adjuvant-induced rheumatoid arthritis and lipopolysaccharide-induced inflammatory responses. *J Ethnopharmacol Gulati* **2018**, 46 (4), 211-215.
7. Cai, W; Li, H; Zhang, Y; Han, G. Identification of key biomarkers and immune infiltration in the synovial tissue of osteoarthritis by bioinformatics analysis. *Peer J* **2020**, 8,1-20.
8. Katz, P. Fatigue in rheumatoid arthritis. *Current Rheumatol Rep* **2017**, 19 (5), 1-10.
9. Matcham, F; Ali, S; Irving, K; Hotopf, M; Chalder, T. Are depression and anxiety associated with disease activity in rheumatoid arthritis? A prospective study. *BMC Musculoskeletal Disord* **2016**, 17(1), 1-9.
10. Machin, A.R; Babatunde, O; Haththotuwa, R; Scott, I; Blagojevic-Bucknall, M; Chew-Graham, C.A; Hider, S.L. The association between anxiety and disease activity and quality of life in rheumatoid arthritis, a systematic review and meta-analysis. *Clinic Rheumatol* **2020**, 39(5), 1471-1482.
11. Kurowska, W; Kuca-Warnawin, E.H; Radzikowska, A; Maśliński, W. The role of anti-citrullinated protein antibodies (ACPA) in the pathogenesis of rheumatoid arthritis. *Central Europ J Immunol* **2017**, 42(4), 390-398.
12. Catrina, A.I; Deane, K.D; Scher, J.U. Gene, environment, microbiome and mucosal immune tolerance in rheumatoid arthritis. *Rheumatol* **2016**, 55 (3), 391-402.
13. Moots, R.J; Xavier, R.M; Mok, C.C; Rahman, M.U; Tsai, W.C; Al-Maini, M.H; Vlahos, B. The impact of anti-drug antibodies on drug concentrations and clinical outcomes in rheumatoid arthritis patients treated with adalimumab, etanercept, or infliximab, Results from a multinational, real-world clinical practice, non-interventional study. *PloS One* **2017**, 12(4), 1-7.
14. Nikiphorou, E; Nurmohamed, M.T; Szekanecz, Z. Comorbidity burden in rheumatic diseases. *Frontiers Med* **2018**, 5, 197-206.
15. Aletaha, D. Precision medicine and management of rheumatoid arthritis. *J Autoimmune* **2020**, 110, 1-12.
16. Orange, DE; Agius, P; DiCarlo, EF; Robine, N; Geiger, H; Szymonifka, J; Donlin, L.T. Identification of three rheumatoid arthritis disease subtypes by machine learning integration of synovial histologic features and RNA sequencing data. *Arthritis Rheumatol* **2018**, 70 (5), 690-701.
17. Genovese, M.C; Smolen, J.; Takeuchi, T.; Burmester, G.; Brinker, D.; Rooney, T.; Winthrop, K.L. Safety profile of baricitinib for the treatment of rheumatoid arthritis over a median of 3 years of treatment, an updated integrated safety analysis. *Lancet Rheumatol* **2020**, 2 (6), e347-e357.
18. Kolkhir, P; Church, MK; Weller, K; Metz, M; Schmetzer, O; Maurer, M. Autoimmune chronic spontaneous urticaria, what we know and what we do not know. *J Allergy Clinic Immunol* **2017**, 139(6), 1772-1781.
19. Coiffier, G; Ferreyra, M; Albert, J.D; Stock, N; Jolivet-Gougeon, A; Perdriger, A; Guggenbuhl, P. Ultrasound-guided synovial biopsy improves diagnosis of septic arthritis in acute arthritis without enough analyzable synovial fluid, a retrospective analysis of 176 arthritis from a French rheumatology department. *Clinic Rheumatol* **2018**, 37(8), 2241-2249.
20. Lin, Y.J; Anzaghe, M; Schülke, S. Update on the pathomechanism, diagnosis, and treatment options for rheumatoid arthritis. *Cells* **2020**, 9 (4), 880-897.
21. Thakur, S; Riyaz, B; Patil, A; Kaur, A; Kapoor, B; Mishra, V. Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis, An overview. *Biomed Pharmacother* **2018**,106, 1011-1023.
22. Thorne, J.; Woreta, F.A; Dunn, J.; Jabs, D.A. Risk of cataract development among children with juvenile idiopathic arthritis-related uveitis treated with topical corticosteroids. *Ophthalmol* **2020**, 127(4), S21-S26.
23. Rahnavard, R; Razavi, N. A review on the medical effects of *Capparis spinosa* L. *Adv Herbal Med* **2017**, 3(1), 44-53.
24. Zhang, H; Ma, ZF Phytochemical and pharmacological properties of *Capparis spinosa* as a medicinal plant. *Nutrients* **2018**,10 (2), 116-123.
25. Harborne, JB Phytochemical Methods, A guide to modern techniques of plant Analysis.2nd Edition, London New York **1984**, Pp, 194-238.
26. Gharban, H.A; Al-Shaeli, S.J; Al-Fattli, H.H; Altaee, M.N. Molecular and histopathological confirmation of clinically diagnosed lumpy skin disease in cattle, Baghdad Province of Iraq. *Vet World* **2019**, 12 (11), 1827-1832.

27. Gharban, H.A; Yousif, A.A. Serological, Clinical and Hematological prevalence of Coxiella burnetii in Adult Cows, Iraq. *Biochem Cell Arch* **2020**, 20 (1), 67-74.
28. Gharban, H.A. Cumulative Effect of Subclinical Mastitis on Immunological and Biochemical Parameters in Cow Milk. *Arch Razi Inst* **2021**, 76(6), 1599-1608.
29. Arena, A.; Bisignano, G.; Pavone, B.; Tomaino, A.; Bonina, FP; Saija, A.; Trombetta, D. Antiviral and immunomodulatory effect of a lyophilized extract of *Capparis spinosa* L. buds. *Int J Pharmacol Der* **2008**, 22(3), 313-317.
30. Aichour, R; Benzidane, N; Arrar, L; Charef, N; Baghiani, A. Hepatoprotective and anti-inflammatory activities of Algerian Capparis spinosa. L. *Res Rev Biol* **2018**,7, 1-12.
31. Zhou, H; Jian, R; Kang, J; Huang, X; Li Y; Zhuang, C; Wu, X. Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J Agri Food Chem* **2010**, 58(24), 12717-12721.
32. Al-Tamimi, A; Khatib, M; Pieraccini, G; Mulinacci, N. Quaternary ammonium compounds in roots and leaves of *Capparis spinosa* L. from Saudi Arabia and Italy, investigation by HPLC-MS and 1H NMR. *Nat Prod Res* **2019**, 33(9), 1322-1328.
33. Hassan, S; Mohammad, N.A. Ethnobotanical and pharmaceutical evaluation of *Capparis spinosa* L, validity of local folk and Unani system of medicine. *J Med Plants Res* **2010**, 4 (17), 1751-1756.
34. Oudah, S.K; Al-Salih, R.M; Gusar, S.H; Roomi, A.B. Study of the role of polyphenolic extract of *Capparis spinosa* L. leaves as acute toxicity and antibacterial agent. *Plant Arch* **2019**, 19 (2), 3821-3829.
35. Khatib, M; Pieraccini, G; Innocenti, M; Melani, F; Mulinacci, N. An insight on the alkaloid content of *Capparis spinosa* L. root by HPLC-DAD-MS, MS/MS and 1H qNMR. *J Pharmaceut Biomed Analys* **2016**, 123, 53-62.
36. Hudaib, M; Mohammad, M; Bustanji, Y; Tayyem, R; Yousef, M; Abuirjeie, M; Aburjai, T. Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. *J Ethnopharmacol* **2008**,120(1), 63-71.
37. Feng, X; Lu, J; Xin, H; Zhang, L; Wang, Y; Tang, K. Anti-arthritic active fraction of *Capparis spinosa* L. fruits and its chemical constituents. *Yakugaku Zasshi* **2011**, 131(3), 423-429.
38. Moutia, M; El Azhary, K; Elouaddari, A; Al Jahid, A; Jamal Eddine, J; Seghrouchni, F; Badou, A. *Capparis spinosa* L. promotes anti-inflammatory response in vitro through the control of cytokine gene expression in human peripheral blood mononuclear cells. *BMC Immunol* **2016**,17(1), 1-12.
39. Saleem, H; Khurshid, U; Sarfraz, M; Ahmad, I; Alamri, A; Anwar, S; Ahemad, N. Investigation into the biological properties, secondary metabolites composition, and toxicity of aerial and root parts of *Capparis spinosa* L; An important medicinal food plant. *Food Chem Toxicol* **2021**, 155, 112-124.
40. AlMousa, L.A; AlFaris, N.A; Alshammari, G.M; ALTamimi, J.Z; Alsyadi, M.M; Alagal, R.I; Yahya, MA Antioxidant and antimicrobial potential of two extracts from *Capparis spinosa* L. and Rumex nervosus and molecular docking investigation of selected major compounds. *Saudi J Bio Sci* **2022**,9, 1-33.
41. Simanjuntak, N.J.P. Evaluation of acute toxicity of ethanol extract of pirdot leaf (saurauia vulcani korth.) in rats. *Indonesian J Pharm Clinic Res* **2020**, 3(2), 13-18.
42. Majeed, M.I; Esmaeel, B.A.M. Effect of *Capparis spinosa* L. Leaf bud Extract on The Hematological and Histological Changes Induced by Cyclophosphamide in Mice. *Res J Pharm Technol* **2019**, 12(7), 3245-3250.
43. Rakhshandeh, H; Asgharzade, S; Khorrami, M.B; Forouzanfar, F. Protective effect of *Capparis spinosa* extract against focal cerebral ischemia-reperfusion injury in rats. *Central Nerv Sys Agents Med Chem* **2021**, 21(2), 148-153.
44. Pal, D, Verma, P. Flavonoids, A powerful and abundant source of antioxidants. Internat J Pharmaceut Sci 2013,5(3), 95-98.
45. Cherrak, S.A; Mokhtari-Soulimane, N; Berroukeche, F; Bensenane, B; Cherbonnel, A; Merzouk, H; Elhabiri, M. *In vitro* antioxidant versus metal ion chelating properties of flavonoids, A structure-activity investigation. *PloS One* **2016**, 11(10), 1-16.
46. Cuenca-Zamora, E.J; Ferrer-Marín, F; Rivera, J; Teruel-Montoya, R. Tubulin in Platelets, When the Shape Matter. *Int J Molecul Sci* **2020**, 21 (10), 3577-3586.
47. Manne, B.K; Xiang, S.C; Rondina, M.T. Platelet secretion in inflammatory and infectious diseases. *Platelets* **2017**, 28(2), 155-164.

48. Roskoski, J.R. The role of small molecule platelet-derived growth factor receptor (PDGFR) inhibitors in the treatment of neoplastic disorders. *Pharmacol Res* **2018**, 129, 65-83.
49. Somodi, L; Debreceni, I.B; Kis, G; Cozzolino, M; Kappelmayer, J; Antal, M; Muszbek, L. Activation mechanism dependent surface exposure of cellular factor XIII on activated platelets and platelet microparticles. *J Thrombosis Haemostasis* **2022**, 6 (2), 1-12.
50. Olumuyiwa-Akeredolu, O.O.O; Pretorius, E. Platelet and red blood cell interactions and their role in rheumatoid arthritis. *Rheumatol Int* **2015**, 35(12), 1955-1964.
51. Pankratz, S; Bittner, S; Kehrel, B.E; Langer, H.F; Kleinschnitz, C; Meuth, S.G; Göbel, K. The inflammatory role of platelets, translational insights from experimental studies of autoimmune disorders. *Int J Molecul Sci* **2016**, 17(10), 1723-1738.
52. Buckley, C.D; Simón-Campos, J.A; Zhdan, V; Becker, B; Davy, K; Fisheleva, E; Tak, P.P. Efficacy, patient-reported outcomes, and safety of the anti-granulocyte macrophage colony-stimulating factor antibody otilimab (GSK3196165) in patients with rheumatoid arthritis, a randomised, phase 2b, dose-ranging study. *Lancet Rheumatol* **2020**, 2(11), e677-e688.
53. El-Tanbouly, G.S; Abdelrahman, R.S. Novel anti-arthritic mechanisms of trans-cinnamaldehyde against complete Freund’s adjuvant-induced arthritis in mice, involvement of NF-κB/TNF-α and IL-6/IL-23/IL-17 pathways in the immuno-inflammatory responses. *Inflammopharmacol* **2022**,11, 1-12.
54. Vollertsen, R.S; Conn, D.L. Vasculitis associated with rheumatoid arthritis. *Rheum Dis Clin North Am* **1990**,16 (2), 445-461.
55. Lotz, M; Moats, T; Villiger, P.M. Leukemia inhibitory factor is expressed in cartilage and synovium and can contribute to the pathogenesis of arthritis. *J Clinic Inves* **1992**,90 (3), 888-896.
56. Karami, J; Aslani, S; Jamshidi, A; Garshasbi, M; Mahmoudi, M. Genetic implications in the pathogenesis of rheumatoid arthritis, an updated review. *Gene* **2019**, 72, 8-16.
57. Giannini, D; Antonucci, M; Petrelli, F; Bilia, S; Alunno, A; Puxeddu, I. One year in review 2020, pathogenesis of rheumatoid arthritis. *Clinic Exp Rheumatol* **2020**,38(3), 387-97.
58. Croia, C; Bursi, R; Sutera, D; Petrelli, F; Alunno, A; Puxeddu, I. One year in review 2019, pathogenesis of rheumatoid arthritis. *Clinic Exp Rheumatol* **2019**, 37(3), 347-357.
59. Lam, S.K; Han, Q.F; Ng, TB Isolation and characterization of a lectin with potentially exploitable activities from caper (Capparis spinosa) seeds. *Bioscie Rep* **2009**, 29(5), 293-299.
60. Zhai, Z; Yang, F; Xu, W; Han, J; Luo, G; Li, Y; Sun, E. Attenuation of Rheumatoid Arthritis Through the Inhibition of Tumor Necrosis Factor–Induced Caspase 3/Gasdermin E–Mediated Pyroptosis. *Arthritis Rheumatol* **2022**, 74(3), 427-440.
61. Moelants, E.A; Mortier, A; Van Damme, J; Proost, P. Regulation of TNF-α with a focus on rheumatoid arthritis. *Immunol Cell Biol* **2013**, 91 (6), 393-401.
62. Thanapati, S; Ganu, M; Giri, P; Kulkarni, S; Sharma, M; Babar, P; Tripathy, AS Impaired NK cell functionality and increased TNF-α production as biomarkers of chronic chikungunya arthritis and rheumatoid arthritis. *Human Immunol* **2017**, 78(4), 370-374.
63. Arjumand, S; Shahzad, M; Shabbir, A; Yousaf, MZ. Thymoquinone attenuates rheumatoid arthritis by downregulating TLR2, TLR4, TNF-α, IL-1, and NFκB expression levels. *Biomed Pharmacother* **2019**, 111, 958-963.
64. Findeisen, K.E; Sewell, J; Ostor, A.J. Biological therapies for rheumatoid arthritis, An overview for the clinician. *Biol Targets Ther* **2021**, 15, 343-359.
65. Wang, Y; Chen, S; Du, K; Liang, C; Wang, S; Boadi, E.O; Chang, Y.X. Traditional herbal medicine, Therapeutic potential in rheumatoid arthritis. *J Ehnopharmacol* **2021**, 279, 1-14.
66. Sokolove, J; Johnson, D.S; Lahey, L.J; Wagner, C.A; Cheng, D; Thiele, G.M; Robinson, W.H. Rheumatoid factor as a potentiator of anti–citrullinated protein antibody–mediated inflammation in rheumatoid arthritis. *Arthritis Rheumatol* **2014**, 66 (4), 813-821.
67. Barsante, M.M; Roffê, E; Yokoro, C.M; Tafuri, W.L; Souza, D.G; Pinho, V; Teixeira, M.M. Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. *Europ J Pharmacol* **2005**,516(3), 282-289.
68. Yu, Y; He, J; Li, S; Song, L; Guo, X; Yao, W; Li, D. Fibroblast growth factor 21 (FGF21) inhibits macrophage-mediated inflammation by activating Nrf2 and suppressing the NF-κB signaling pathway. *Int Immunopharmacol* **2016**, 38, 144-152.

69. Frasnelli, M.E; Tarussio, D; Chobaz-Péclat, V; Busso, N; So, A. TLR2 modulates inflammation in zymosan-induced arthritis in mice. *Arthritis Res Ther* **2005**,7 (2), 1-10.
70. Nabavi, S.F; Maggi, F; Daglia, M; Habtemariam, S; Rastrelli, L; Nabavi, S.M. Pharmacological effects of *Capparis spinosa* L. *Phytother Res* **2016**, 30 (11), 1733-1744.
71. Tlili, N; Feriani, A; Saadoui, E; Nasri, N; Khaldi, A. *Capparis spinosa* leaves extract, Source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomed Pharmacother* **2017**, 87, 171-179.
72. Rad, M.K; Ghani, A; Ghani, E. *In vitro* effects of *Capparis spinosa* L. extract on human sperm function, DNA fragmentation, and oxidative stress. *J Ethnopharmacol* **2021**,269, 1-13.
73. Grimalt, M; Hernández, F; Legua, P; Amorós, A; Almansa, M.S. Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars. *Scientia Horticulturae* **2022**, 293, 1-10.
74. Vahid, H; Rakhshandeh, H; Ghorbani, A. Antidiabetic properties of *Capparis spinosa* L. and its components. *Biomed Pharmacother* **2017**, 92, 293-302.
75. Al-Anazi, K.M; Al-Mareed, A.A; Farah, M.A; Ali, M.A; Hailan, W.A; Al-Hemaid, F.M. Protective Effect of *Capparis spinosa* Extract against Potassium Bromate Induced Oxidative Stress and Genotoxicity in Mice. *Evid-Based Compl Alt Med* **2021**, 2021, 1-15.

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