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Phytochemical screening and determination of treatment dose of *Capparis spinosa* L. roots extract in adjuvant induced arthritis in mice

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Abstract--This study was aimed to investigate phytochemical properties of *Capparis spinosa* roots extract and to evaluate anti-arthritic activity of this extract in adjuvant-induced arthritis in mice which initiated using the complete Freund's adjuvant. For these purposes, the roots of *C. spinosa* were collected from different rural areas in Wasit province, and the extract was prepared and analyzed phytochemically. Also, a total of 42 male mice were prepared, divided equally to 7 groups involved 2 controls [negative (NC) and positive (PC)] as well as 5 treated (TG1, TG2, TG3, TG4 and TG5) received respectively 100, 150, 200, 250 and 300 mg/kg B.W of the extract for 21 days. Phytochemical analysis of *C. spinosa* L. roots extract showed a significant elevation in alkaloids and lacking of steroid, with existence of tannins, saponins, polysaccharides carbohydrates and glycosides, proteins and amino acid, coumarins, flavonoids, and polyphenolic compounds. The clinical findings of the right paw thickness for mice of different study groups were revealed a significant variation in their values. Comparing to NC, values of PC were increased significantly; while, the findings of treated groups with different concentrations of *C. spinosa* L. roots extract were variable significantly. However, the overall results of the TG4 were showed the potent protective effect against arthritis when compared to those of other treated groups; TG1, TG2, TG3 and TG5. Concerning histology, the findings of TG4 were revealed a significant amelioration when compared to PC. In conclusion, alkaloids seemed to be the major constituent of *C. spinosa* L. roots extract. Histological examination of tissue section revealed that *C. spinosa* L. roots extract can be treated

effectively the injured tissues caused by arthritis. Furthermore studies on other systemic or local diseases are needed, and therapeutic effects of other *C. spinosa* L. parts such as leaves and seeds should be aimed.

Keywords---*C. spinosa*, complete Freund's adjuvant, histopathology, alkaloid, Iraq.

Introduction

Joint inflammation and related issues including arthritis, are normal illnesses influencing many humans and animals (Burmester and Pope, 2017). In spite of the fact that a number of medications utilized as a part of the treatment of arthritis have been developed over the previous two decades, there is as yet a requirement for more effective drugs with lower side effects (Curtis *et al.*, 2016). Arthritis is characterized usually by painful inflammatory reaction that confined movement, in addition to bony disfigurements and joint inability occurred by dynamic disintegration of ligament (Nazaal *et al.*, 2005; Tong *et al.*, 2012; Dhakad *et al.*, 2018). The cardinal pathological alterations incorporate hyperplasia of synovial membrane, and eventually prompt ligament disintegration with articular destruction (Agarwal *et al.*, 2008; Gibofsky, 2014).

The main classes of drugs used to treat arthritis are analgesics (Solomon *et al.*, 2010), corticosteroids (Ruyssen-Witrand *et al.*, 2011), immunosuppressants (Roubille, 2015) and a disease modulator anti-rheumatoid drug (DMARD), (Sambamoorthi *et al.*, 2017). All these drugs associate with harmful symptoms such as gastric ulcer and kidney disease (Amalraj *et al.*, 2017). As a result, alternative medicine for the treatment and management of diseases is currently attracting the attention of researchers around the world (Schicchi *et al.*, 2017; Husain *et al.*, 2019). Humans have relied on plant-based remedies to treat disease from almost the beginning of our existence. The oldest evidence of using plants as medicine comes from a 5,000-year-old Sumerian clay tablet containing recipes made with plants like the poppy, henbane and mandrake. For treating arthritis, several herbal remedies are promoted today including turmeric, ginger, *Capparis spinosa*, *Boswellia serrata*, devil's claw, willow bark and feverfew (Storl, 2012). *Capparis spinosa* belongs to *Capparidaceae* family, is described as tough, open, branched, bushy, leafy or leafless small plant that native to the arid desert regions of the world (Rajesh *et al.* 2009). This plant is used as a laxative, antidote, aphrodisiac, enhances stamina, and against rheumatism, cough, back pain and asthma (Arrar *et al.*, 2013). *Capparis spinosa* L. extract contains a high concentration of flavonoids, phenolic compounds, steroids, alkaloids, vitamins, quaternary ammonium compounds, terpenoids, and many other botanical ingredients related to their medicinal properties (Ao *et al.*, 2007). According to recent data, the root of this plant contains diterpenes, cappariditerpenyl esters, β -limonesterols, saponins, tannins, diterpenal alcohols and β -carotene (Zhang and Ma, 2018; Saleem *et al.*, 2021). This study was aimed to investigate phytochemical properties of *C. spinosa* roots extract and to evaluate anti-arthritic activity of this extract in adjuvant-induced arthritis in mice which initiated using the complete Freund's adjuvant (CFA).

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

Totally, 42 male mice aged ≤ 4 months and weight 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 for 1 week, during which, they 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 1), washed, well-dried and grinded 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 45°C (26). The extract was then filtered and dried under the vacuum (150 rpm / 4 hours) at 40°C. Later, the crude extract of *C. spinosa* was concentrated in a glass petri-dish by placing it in an incubator at 40°C until the semi-solid, thick and dark brown crude extract was appeared (Figure 2). All dried extract was collected and stored in aseptic containers and kept frozen at 4°C.



Figure (1): A sample of collected roots of *C. spinosa* L. from rural areas in Wasit province

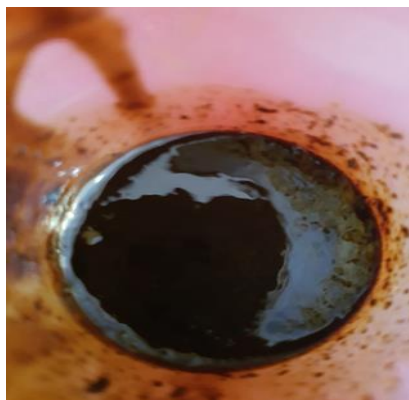


Figure (2): Concentrated extract of *C. spinosa* L. roots

Phytochemical screening of *Capparis spinosa* L. roots extract

As described by other studies; three methods were used to estimate alkaloids concentrations in *C. spinosa* roots extract [Dragendroff's reagent (Mondal et al., 2015), Mayer reagent (Chimenti et al., 2008) and Hager's reagent (Harborne, 2005)], 3 tests for flavonoids [Shinoda test (Peach and Tracey, 1956), ferric chloride 7.5% test (Harborne, 2005), and alkaline test (Roopashree et al., 2008)], ferric chloride test for polyphenols (Awoyinka et al., 2007), 2 for tannins [lead acetate (Evans, 2002), and ferric chloride 5% (Cannell, 1998)], mercuric chloride test for saponin (Shihata and Mrak, 1951), 4 for carbohydrates and glycosides [Anthrone test (Hedge and Hofreiter, 1962), Molisch's test (Evans, 2002), Benedict's test (Harborne, 2005), and Keller-Kiliani test (Harborne, 2005)], Sallowski's test for steroid, 2 for proteins and amino acids [Ninhydrine reagent (Harborne, 2005), and Biuret test (Harborne, 2005)], and 10% NaOH for coumarins (Cannell, 1998).

Preparation of different concentrations

The suspensions of different concentrations of *C. spinosa* roots extract were prepared for different doses by dissolving of concentrated solvent in the normal saline (Rawri *et al.*, 2013).

Study design

The study mice were divided equally into 7 groups; 2 controls [negative (NC) and positive (PC)] in addition to 5 treated groups (TG) as following:

1. NC: Healthy mice received only distilled water.
2. PC: Arthritis mice did not receive any treatment.
3. TG1: Adjuvant arthritis mice treated daily with the extract of *C. spinosa* L. roots at a dose of 100 mg/kg B.W.
4. TG2: Adjuvant arthritis mice treated daily with the extract of *C. spinosa* L. roots at a dose of 150 mg/kg B.W.
5. TG3: Adjuvant arthritis mice treated daily with the extract of *C. spinosa* L. roots at a dose of 200 mg/kg B.W.

6. TG4: Adjuvant arthritis mice treated daily with the extract of *C. spinosa* L. roots at a dose of 150 mg/kg B.W.
7. TG5: Adjuvant arthritis mice treated daily with the extract of *C. spinosa* L. roots at a dose of 300 mg/kg B.W.

Measurement of paw thickness and samples collection

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. After ending of this period, the study mice were euthanized with chloroform and subjected for sectioning of ankle joint that saved into plastic containers contain 10% neutral buffered formalin (NBF).

Histopathology

According to Gharban *et al.* (2019), the fixed tissue in 10% NBF were 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*).

Statistical analysis

The obtained 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, large horizontal and small vertical letters referred to significant differences, and values were represented as mean \pm standard errors ($M \pm SE$).

Results and Discussion

Phytochemical screening

The findings for phytochemical analyzing of *C. spinosa* L. roots extract were varied significantly ($P < 0.05$), (Table 1). However, value of alkaloids (20%) was elevated; while value of steroid (0%) decreased when compared to values of tannins (13.33%), saponins (13.33%), polysaccharides carbohydrates and glycosides (13.33%), proteins and amino acid (13.33%), coumarins (13.33%), flavonoids (6.67%), and polyphenolic compounds (6.67%).

Table (1): Result of phytochemical analysis of *C. spinosa* L. roots extract

No.	Test	Reagent	Indication	Result
1	Alkaloids	Dragangroff	Orange-brown precipitate	+++
		Mayer	Yellow precipitate	+++
		Hager	Yellow precipitate	+++
	Flavonoids	Shinoda	Orange color	+
		FeCl ₃ (7.5%) solution	Dark color	+

2		Alkaline reagent (NaOH)	Yellow color	+	
3	Polyphenolic compounds	FeCl ₃ (5%) solution	Brown color	+	6.67% C
4	Tannins	Lead acetate (1%) solution	Creamy precipitate	++	13.33% B
		FeCl ₃ (1%) solution	Dark color	++	
5	Saponins	Foam formation	Foam	++	13.33% B
		HgCl ₂ (1%) test	White ppt	++	
6	Polysaccharides carbohydrates and glycosides	Anthrone test	Green color	++	13.33% B
		Molisch's test	Purple violet color	++	
		Benedict test	Reddish-brown color	++	
		Keller-Kiliani test	Reddish-brown ring	++	
7	Steroid	Sallowski's test	No Red color	-	0% D
8	Proteins and amino acid	Ninhydrine	Blue color	++	13.33% B
		Biuret test	Purpule color	++	
9	Coumarins	NaOH (10%) solution	Yellow color	++	13.33% B

Satyanarayana et al. (2018) reported that *C. spinosa* contains many phytochemicals from different parts of the plant. For example, the roots contain 0.91% alkaloids and the seeds contain 0.86% alkaloids. In other studies, Sadykov et al. (2008) showed that the root alkaloid content was very high in particular the stachydrine that accounting for 87.43% of the total alkaloids. A new alkaloid, cadabicine, has been isolated from other subspecies of *C. spinosa* (Khanfar et al., 2003). Three new alkaloids have been isolated from *C. spinosa* root including the capparispine, caparesin, capparispine 26-O- β -d-glucoside and cadabicine 26-O- β -d-glucoside hydrochloride (Fu et al., 2008). In addition to alkaloids, *C. spinosa* roots are known for their antioxidant properties (Tlili et al., 2010; Nijveldt et al., 2021) which attributed to existence a large amount of flavonoids has improved health status of patients (Nijveldt et al., 2021). Polyphenols are secondary metabolites commonly found in various parts of plants such as flowers, fruits, fruits, roots, leaves and stems. Currently, polyphenols are of interest to modern civilization because of their health benefits and their ability to prevent cardiovascular disease and diabetes, hypertriglyceridemia and hyperglycemia (Jagdale, 2021; Luo, 2021; Wang and Zheng, 2021).

Tannins, saponins, carbohydrates, proteins and coumarins consider other important compounds found greatly in *C. spinosa* roots as observed in the present study. These results are in agreement with those reported in other studies (Rahnavard and Razavi, 2017; Vahid et al., 2017; Mahmoudvand et al., 2021). Rajhi et al. (2021) found that the hydroethanol extract of *C. spinosa* is rich in many highly tannin bioactive compounds. This is explained by the difference in solubility of the two molecules extracted from the plant and the choice of solvent to use since aqueous extraction can reduce or increase the content of other compounds in the extract (Gull et al., 2015; Dhingra et al., 2017). Galib and Algfri (2016) discovered significant amounts of flavonoids (5.1%) and saponins (1.8%) in *C. spinosa* extract, and this may explain their important medicinal properties. Inocencio et al. (2020) discovered that several glycoside derivatives such as quercetin, kaempferol 3-rutin, and kaempferol 3-O-ramnortin produce different

levels of activity. In a study conducted in Tunisia, Tlili et al. (2010) showed that caper protein content ranges from 23.5% to 30.12%, at an average of 27.52%. These results are slightly higher than the data reported by Akgül and Özcan (1999) for the protein content of *C. spinosa* seeds alone. However, the protein content in plants depends on the species, soil and local climate of the plant, so these differences may be due to their geographical distribution. Coumarin is another important class of botanical compounds that contribute to the odor of *C. spinosa*, which having many biological estrogenic, decoagulant, epidermal photosensitizing, antibacterial, vasodilator, parasiticidal, analgesic and hypothermic activities (Anwar, 2016; Stringlis et al, 2019).

Clinical effect of anti-arthritic activity using different concentrations of *C. spinosa*

The clinical findings of the right paw thickness for mice of different study groups were revealed a significant variation in their values ($P < 0.05$). Comparing to NC, values of PC were increased significantly ($P < 0.05$); while, the findings of treated groups with different concentrations of *C. spinosa* L. roots extract were variable significantly ($P < 0.05$). However, the overall results of the TG4 were showed the potent protective effect against arthritis when compared to those of other treated groups; TG1, TG2, TG3 and TG5 (Table 2).

Table (2): Results of hind paw thickness (cm) among mice of the study groups

Group	Day				
	0	3	7	14	21
NC	1.65 ± 0.01 Bb	1.79 ± 0.02 Bb	2.12 ± 0.01 Bc	2.25 ± 0.02 Ad	2.34 ± 0.15 Ae
PC	1.69 ± 0.03 Bb	4.07 ± 0.16 Aa	5.45 ± 0.1 Aa	6.2 ± 0.17 Aa	6.91 ± 0.02 Aa
TG1	1.65 ± 0.02 Cb	4.44 ± 0.08 Ba	5.37 ± 0.11 Aa	5.57 ± 0.07 Ab	5.47 ± 0.11 Ab
TG2	1.67 ± 0.01 Cb	4.36 ± 0.05 Ba	5.2 ± 0.13 Aa	5.48 ± 0.09 Ab	5.39 ± 0.09 Ab
TG3	1.66 ± 0.15 Cb	4.28 ± 0.09 Ba	5.02 ± 0.11 Aa	5.36 ± 0.06 Ab	5.3 ± 0.11 Ab
TG4	1.64 ± 0.01 Cb	4.56 ± 0.06 Aa	4.41 ± 0.09 Ab	4.23 ± 0.08 Ac	3.97 ± 0.2 Bd
TG5	1.68 ± 0.02 Cb	4.1 ± 0.01 Ba	4.87 ± 0.08 Ab	4.58 ± 0.1 Ac	4.5 ± 0.04 Bc

Medicinal plants have an important role in diet of people; but with increasing resistance due to overusing of chemical synthetic antibiotics, finding alternative medicines that have antibacterial properties and have the least side effects on human health appears to be necessary (Mansourabadi et al., 2016; Razavi et al., 2016). Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, bio pesticides and food additives (Rahnavard and Razavi, 2017). *Cappari spinosa* (*C. spinosa*) which was commonly used as a medicinal plant contained many

biologically active chemical groups including alkaloids, glycosides, tannins, phenolic, flavonoids, triterpenoids steroids, carbohydrates, saponins and a wide range of minerals and trace elements (Zhang and Ma, 2018). It exerted many pharmacological effects including antimicrobial, cytotoxic, anti-diabetic, anti-inflammatory, antioxidant effect and many others. The anti-inflammatory effects of the flavonoids from caper fruits were evaluated by secreted placental alkaline phosphatase (SPAP) reporter assay, which was designed to measure nuclear factor-kappa B (NF- κ B) activation (Zhou et al., 2011; Rahnavard and Razavi, 2017). The extracts of *C. spinose* were found to possess marked anti-inflammatory activity but devoid of analgesic activity in animal models, cappelrenol-13 isolated from *C. spinosa* showed significant anti-inflammatory activity (Al-Said et al., 2018). *Cappari spinosa* root extracts were extracted with solvents of varying polarity, and the findings exist that it contain the highest concentration of phenolic compounds and flavonoids followed by the chloroform extract of roots. The antioxidant activity of different extracts of *C. sspinosa* was evaluated by DPPH radical scavenging method. The antioxidant activity (IC₅₀ μ g/ml) of methanol and ethyl acetate extracts were 94.4 ± 4.5 and 57.75 ± 2.3 respectively (Alsabri et al., 2012). In accordance with the present work, AL-Asady et al. (2012) had demonstrated that a highly significant difference among all crude and secondary metabolites extracts and among concentrations. Also, we found that the using of high concentrations of *C. spinosa* L. roots extracts (300 mg/kg B.W) had revealed less activity against arthritis suggesting that the overdose extract concentration could be caused reverse negative effects in the body.

Histopathological effect

In NC mice, the findings of histopathology revealed that there were no obvious lesions in skin, articular cartilage and synovial cavity, and bone marrow (Figure 3). In mice of PC, histological findings showed that there were inflammatory cells filled the marrow spaces with osteoblasts lining the tubercule, degenerative changes in the articular cartilage with necrosis of subchondral bone tubercule, infiltration of inflammatory cells with the fibrosis of synovial membrane and degeneration of cartilage and destruction of bone trabeculae and inflammatory cells filled the marrow spaces, sclerosis of marrow spaces, irregular of Tidemark line and increase distance between bone marrow filled with inflammatory cells and cartilage, thickness of trabecular bone with severe inflammatory cells filled bone marrow, abscess formation in subcutaneous tissue of the skin, several trabecular bone formation, severe infiltration of inflammatory cells in dermis and subcutaneous tissue, destruction of subchondral trabeculae with intra trabecular cartilage, proliferation of chondrocytes as well as increasing of distance between bone marrow and cartilage which filled with the inflammatory cells, destruction of articular cartilage, fragments of necrotic bone surrounded by osteoblasts with sclerosis of marrow spaces, proliferation of connective tissue in synovial cavity that destructed the articular cartilage and extended to subchondral bone (punness reaction), severe inflammatory cells infiltration the subcutaneous tissue and muscular layer and apoptosis of cartilage cells (Figures 4-11). In mice of this group, there was a moderate distance between articular cartilage and bone marrow, subchondral sclerosis, dead fragments of bone and sclerosis in subchondral bone, thickness of bone trabeculae with infiltration of inflammatory cells in bone marrow, limited abscess in subcutaneous tissue, proliferation of

chondrocytes with regular tidemark, infiltration of inflammatory cells, sclerosis of bone marrow and marked increase in number of osteoclast that made bit space in bone trabeculae, and moderate infiltration of inflammatory cells in dermis and subcutaneous tissues (Figures 12-15).

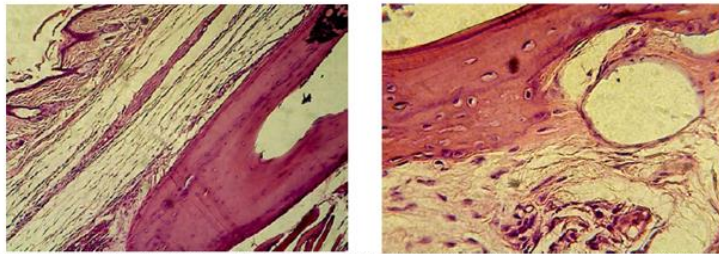


Figure (3): Histopathological section of NC mice shows the absence of lesions in skin, H & E stain at 100X (Left) and 400X (Right)

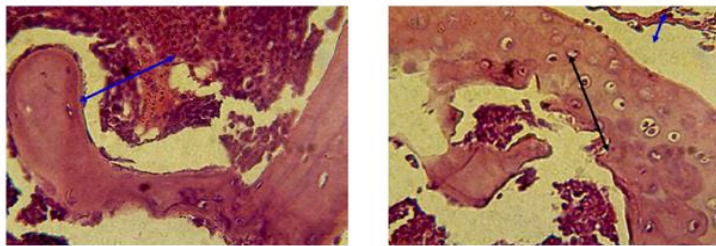


Figure (4): Histopathological section of PC mice shows inflammatory cells filled bone marrow spaces with osteoblasts (Blue arrow), (Left); degenerative changes in articular cartilage (Blue arrow) and necrosis (Black arrow) of subchondral bone trabeculae (Rghit), H & E stain, 400X

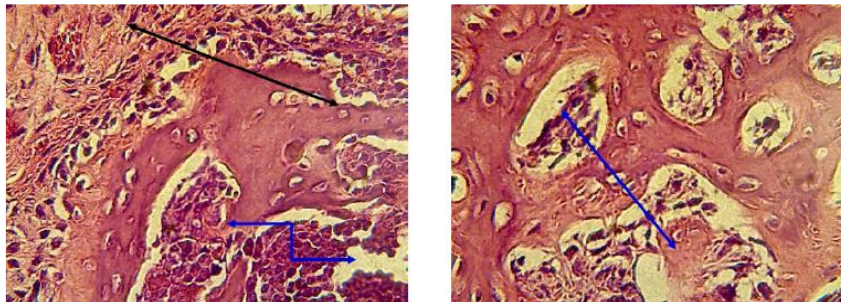


Figure (5): Histopathological section of PC mice shows infiltration of inflammatory cells and fibrosis of synovial membrane (Black arrow), degeneration of cartilage and destruction of bone trabeculae (Blue arrow), (Left); sclerosis of marrow spaces (Right), H & E stain 400X

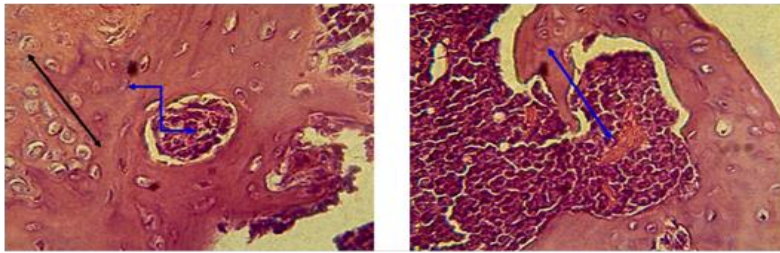


Figure (6): Histopathological section of PC mice shows degenerative changes in articular cartilage, irregular of Tidemark line (Black arrow), increase distance between bone marrow filled with inflammatory cells (Blue arrow), (Left); thickness trabecular bone with severe inflammatory cells filled bone marrow (Blue arrow), (Right), H & E stain 400X

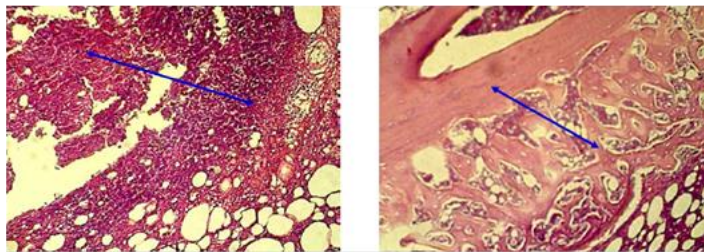


Figure (7): Histopathological section of PC mice shows abscess formation in subcutaneous tissue of the skin (Blue arrow), (Left); thickness subchondral bone with several trabecular bone formation (Blue arrow), H & E stain 400X (Left), X100 (Right)

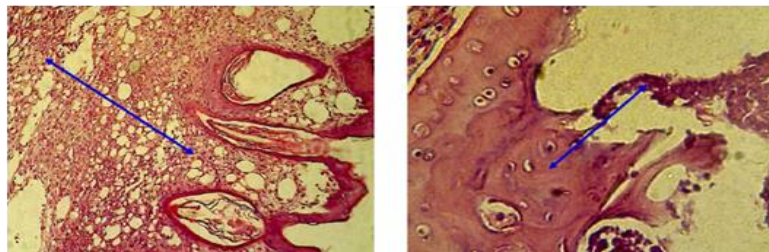


Figure (8): Histopathological section of PC mice shows severe infiltration of inflammatory cells in dermis and subcutaneous tissue (Blue arrow), (Left); destruction of subchondral trabeculae with intra trabecular cartilage (Blue arrow), (Right), H & E stain 100X (Left), 400X (Right)

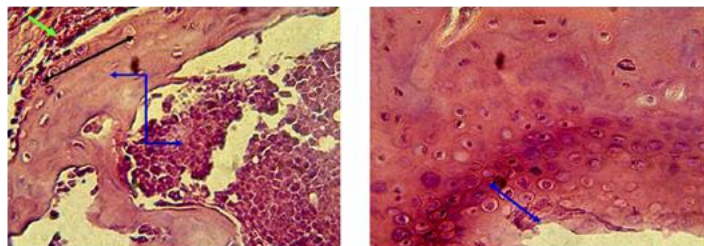


Figure (9): Histopathological section of PC mice shows proliferation of chondrocytes (Black arrow), with increasing distance between bone marrow (Green arrow) and cartilage filled with the inflammatory cells (Blue arrow), (Left); destruction of articular cartilage with proliferation of chondrocytes (Blue arrow), (Right), H & E stain 400X

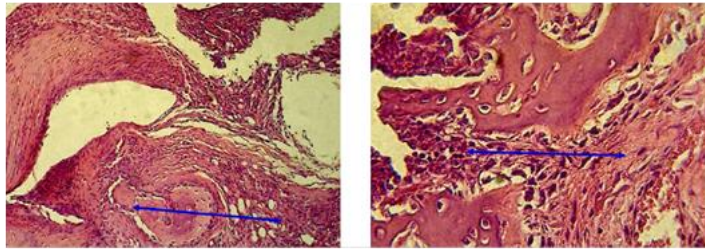


Figure (10): Histopathological section of PC mice shows fragments of necrotic bone surrounded by osteoblasts with sclerosis of marrow spaces (Blue arrow), (Left); inflammatory reaction and proliferation of connective tissue in synovial cavity (Blue arrow), (Right), H & E stain 100X (Left), 400X (Right)

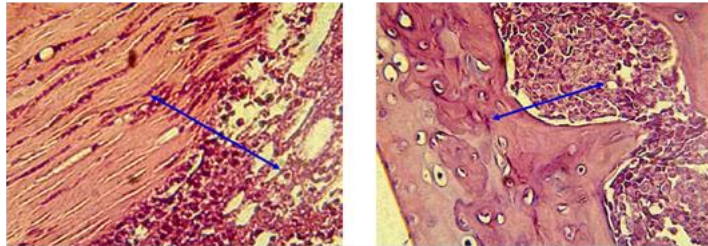


Figure (11): Histopathological section of PC mice shows severe infiltration of inflammatory cells in subcutaneous tissue and muscular layer (Blue arrow), (Left); apoptosis of cartilage cells (Blue arrow), (Right), H & E stain 400X

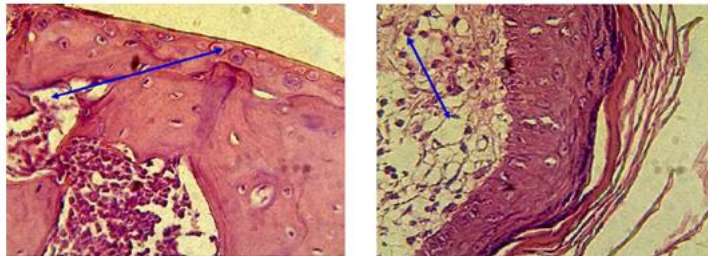


Figure (12): Histopathological section of TG4 mice shows proliferation of chondrocytes of articular cartilage that near to moderate cellular subchondral marrow space (Blue arrow), (Left); infiltration of few inflammatory cells in dermis (Blue arrow), (Right), H & E stain, 400X

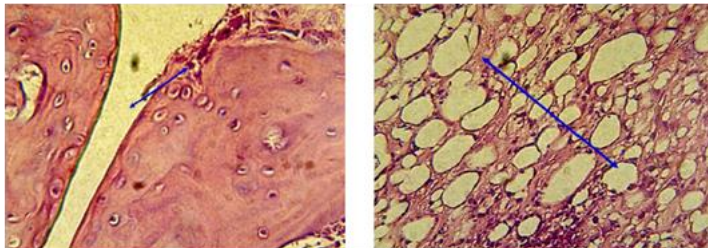


Figure (13): Histopathological section of TG4 mice shows proliferation of chondrocytes in articular cartilage with absence of inflammatory reaction in synovial cavity (Blue arrow), (Left); moderate infiltration of inflammatory cells in subcutaneous tissue (Blue arrow), (Right), H & E stain 400X

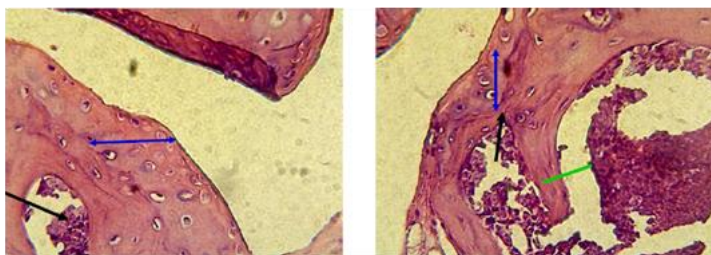


Figure (14): Histopathological section of TG4 mice shows nearly normal articular cartilage (Black arrow) with regular tidemark and absence of sclerosis in marrow spaces (Blue arrow), (Left); regenerative articular cartilage (Black & Blue arrow) with presence of very narrow distance between articular cartilage and marrow bone (Green arrow), (Right), H & E stain 400X

Stimulation and destruction of immune cells, pro-inflammatory cytokines and other inflammatory mediators are associated with the pathogenesis of arthritis (Sokolove et al., 2012). Therefore, changes in immune response and inflammation are considered one of the most important mechanisms explaining the true incidence of arthritis lesions and have been shown in various publications and experimental studies of co-arthritis models (Barsante et al., 2005; Kamel, 2018 a, b). Frasnél et al. (2005) assessed the severity of histological signs of arthritis, and showed that there low cellular infiltration and cartilage destruction at day 8; while at day 25, there was an increasing in cellular infiltration but cartilage destruction remained low with less cell infiltration. Based on the results of this study, El-Tanbouly and Abdelrahman (2022) found the normal synovium, articular cartilage surface, and underlying bone in tissue sections of the hind limbs of healthy rats; whereas in arthritis, CFA mice were showed the signs of synovial hyperplasia and destruction of cartilage and bone, and inflammation that characterized by synovial vascular hypertrophy. This phenomenon was reduced in the treatment group with less proliferative synovial hyperplasia and chondrocyte vacuolization compared to the CFA group. In the present study, tissue sections of EG1 were showed a significant improvement in their structure, demonstrating the effect of *C. spinosa* L. roots extract as it rich by glucosinolates (glucosinolates, glucosides, myrosins, glucosinolates), flavonoids, phenolic acids and important alkaloids and a variety of other secondary metabolites that having diverse biological activities (Tlili et al., 2017). Hence, these contents result in several biological properties including antioxidant, anti-inflammatory, anti-cancer, antibacterial, anti-mutagenic, and anti-diabetic properties (Nabavi et al., 2016; Yu et al., 2017). In addition, *C. spinosa* is one of the most abundant sources of calcite and is considered a major antioxidant, hepatoprotective, and nephroprotective biological factor for chemical cytotoxicity (Kalantari et al., 2018). Interestingly, *C. spinosa* is safe and has no scientific evidence of side effects or toxicity (Hassan and Mohammad, 2010; Vahid et al., 2017). Based on the composition of the active ingredient of *C. spinosa*, Al-Anazi et al. (2021) believed that this plant may have potential protective effects against oxidative stress, genotoxicity and cytotoxicity in animal models.

Conclusions

This study demonstrated anti-arthritic effects of *C. spinosa* L. roots extract *in vivo*, and justified the using of this extract as an anti-inflammatory and anti-

arthritic crude drug. Alkaloids seemed to be the major constituent of *C. spinosa* L. roots extract. Histological examination of tissue section revealed that *C. spinosa* L. roots extract can be treated effectively the injured tissues caused by arthritis, suggesting its activity in medication of other health problems and injuries. Therefore, furthermore studies to detect therapeutic effects of *C. spinosa* L. roots extract on other systemic or local diseases are needed. Also, therapeutic effects of other *C. spinosa* L. parts such as leaves and seeds should be aimed.

Authors' contributions

RJAJ: Collection, preparation of *C. spinosa* roots extract, management of study mice and clinical measurement of paw thickness during the experimental period, and collection of tissue samples at the end of the study. AHC: Phytochemical and histopathological examination of collected samples in addition to statistical analysis of study results. Both authors read and approved the final manuscript.

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