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Protective effect of ethanol extract of *Coriolopsis aspera* fruiting bodies against adjuvant-induced arthritis mice

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Abstract

Coriolopsis aspera is a medicinal mushroom that exerts several pharmacological and biological activities. However, the protective effect of the ethanol extract of C. aspera against arthritis has not been studied yet. In this study, we estimated some bioactive compounds in the ethanol extract of *C. aspera* fruiting bodies and used the extract with a dose of 400 mg.kg^{-1} body weight or the reference drug Mobic (0.2 mg.kg^{-1} body weight) to treat an adjuvant-induced arthritis model. Arthritis severity was determined via the changes in the diameters and volumes of hind paws, arthritic scores, total leukocyte count, ESR, biochemical parameters such as CRP and RF, as well as histological features of ankle joints. We found the presence of polyphenols and flavonoids with a high content (37.79 ± 0.88 mg GAE/g DW and 8.56 ± 0.37 mg QE/g DW, respectively) in the extract. Furthermore, the results showed that Freund's complete adjuvant-treated mice exhibited a severe arthritic score and histological features, an increase in the diameters and volumes of hind paws, along with an elevation of leukocyte count, erythrocyte sediment rate, and biochemical parameters such as rheumatoid factor and C-reactive protein compared to normal mice. On the contrary, treatment with ethanol extract at a dose of 400 mg.kg^{-1} body weight for 28 days exhibited a strong anti-arthritis effect and could improve all the testing parameters at the same efficacy as those of the reference drug (Mobic 0.2 mg.kg^{-1} body weight). These findings suggest a potential application of the ethanol extract of *C. aspera* for arthritis treatment.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that usually begins just in the small joints of the hands or feet and subsequently spreads to the larger joints (Safiri *et al*. 2019). The disease is characterized by chronic inflammation of the synovium, joint cartilage destruction, bone erosion, and joint deformation. According to Jean *et al*. (2017), the cumulative prevalence of RA in the Canadian population was 0.8 % in 2015, and the prevalence had a positive correlation with age. Women were more susceptible to RA than men, with a high incidence (about 65.6 cases per 100,000 people). RA patients suffer not only joint inflammation and damage but also extra-articular

manifestations leading to an increase in co-morbidities and premature mortality rates, cricoarytenoid arthritis, and pulmonary nodulosis, as well as a higher risk of cardiovascular disease (Meyer *et al*. 2018; Farquhar *et al*. 2019). Some synthetic medicines, such as meloxicam and celecoxib, have been proposed for the treatment of RA with high efficacy (Fidahic *et al.* 2017; Foeldvari *et al*. 2002).

The use of oriental herbal medicines has been proven to be a safe and promising therapeutic strategy for RA treatment. For example, Ramasamy *et al.* (2012) indicated that treatment with the ethanol extract of *Elaeocarpus sphaericus* at a dose of 250 mg.kg^{-1} body weight could inhibit paw edema and improve the alteration of hematological parameters such as hemoglobin, red blood cells, white blood cells, erythrocyte sediment rate (ESR), C-reactive protein (CRP) level in Freund's complete adjuvant-induced rheumatoid arthritis model. In a previous study, the anti-arthritic effect of the ethanol extract of *Justicia gendarussa* leaf, one of the remedies widely used in Chinese and Indian traditional medicines, was proved (Paval *et al*. 2009). In addition, (-)-epigallocatechin-3 gallate (EGCG), the anti-inflammatory compound extracted from green tea, has potential beneficial effects such as cardiovascular-protective and anti-rheumatic activities in RA (Riegsecker *et al*. 2013). Recently, Paul *et al.* (2016) have found some anti-inflammatory compounds such as 9-octadecenoic acid (Z), phenylmethyl ester, astaxanthin, α-N-Normethadol, fenretinide, and caryophyllene oxide in the flower of *Acmella uliginosa* (Sw.) Cass., the edible medicinal plant found in North Bengal, and the anti-arthritis activity of the combination of *A. uliginosa* flower and *Aloe vera* gel crude extracts has been proven *in vivo*.

Coriolopsis is a genus of the Polyporaceae family, belonging to the division of Basidiomycota, and consists of several wood-decaying fungi that play an important role in forest ecosystem, such as *Coriolopsis gallica*, *Coriolopsis strumosa*, *Coriolopsis trogii*, *Coriolopsis occidentalis* (Homolka *et al*. 1994; Jang *et al*. 2014; Cartabia *et al*. 2021). Some of them could be used as medicinal mushrooms due to their pharmacological and biological activities. For instance, *C. gallica* has been employed in traditional medicine for the treatment of diabetes (Bautista-González *et al*. 2022). In addition, *Coriolopsis polyzona* was reported for its anti-fungal, anti-bacterial, and antioxidant effects (Al-Fatimi *et al*. 2013). During the investigation project on the new metabolites of medicinal mushrooms in Vietnam, *Coriolopsis aspera* was found and collected in Nghe An Province, Vietnam. In the previous study, some bioactivities of *C. aspera* such as antioxidant, anticancer, and anti-inflammatory propertie,s have been reported (Nguyen *et al*. 2020). Furthermore, several bioactive compounds, including trametenolic acid B, ergosterol, cerevisterol, trans-p-hydroxycoumaric acid, methyl ferulate, and umbelliferone, have been identified in *C. aspera*, which suggests *C. aspera* as a medicinal mushroom and a natural source to extract bioactive compounds (Nguyen *et al.* 2020). However, the anti-arthritis effect of the ethanol extract of the fruiting bodies of *C. aspera* (EtCA) has not been studied yet. In this study, we established Freund's complete adjuvant-induced arthritis model and evaluated the arthritis effect of EtCA on the adjuvant-induced arthritis model.

Experimental

Preparation of ethanol extract of Coriolopsis aspera

Coriolopsis aspera was collected from Ky Son District of Nghe An Province, Vietnam, in May 2022 (Fig. 1A). The mushroom specimen was authenticated by a taxonomist from the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam, and the voucher specimen was deposited in the institutional herbarium (sample code CA110522VST). After collection, the fresh fruiting bodies of *C. aspera* (Fig. 1B) were cut into small pieces, washed, and dried in a drying cabinet at 55 ^{o}C to obtain the dried samples with a moisture content of less than 4 %, then the samples were ground into a fine powder by blender. The powder of mushrooms was stored in a desiccator until used in the next steps (Fig. 1C).

Briefly, 480 g of powdered mushrooms were completely macerated in 1.2 L of ethanol. The

mixture was subsequently kept in the refrigerator with shaking for 30 h to extract soluble components. The solution was filtrated by Whatman No.1 filter paper to collect the supernatant and remove solid materials, and the procedure was repeated twice. The filtrate was condensed in a rotary evaporator under reduced pressure (130 mmBar) to obtain the ethanol extract (12.2 g). The procedure was repeated several times until reaching the desired amount of ethanol extract from *C. aspera* (named EtCA). The resulting extract from several times of extraction was combined and stored in a sealed glass beaker at 4 °C until further use (Fig. 1D).

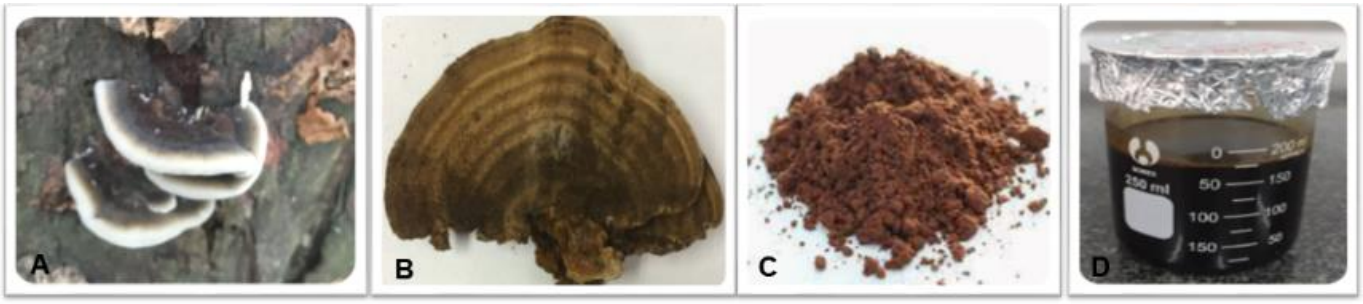

Fig.1. The ethanol extract of the fruiting bodies of *Coriolopsis aspera*. **A** – *C. aspera* in its habitat; **B** – fruiting bodies of *C. aspera*; **C** – mushroom powder; **D** – the ethanol extract of fruiting bodies of *C. aspera* (EtCA).

Chemicals and reagents

Freund's complete adjuvant (FCA, F5881), containing heat-inactivated *Mycobacterium tuberculosis* emulsified in mineral oil, was provided by Sigma-Aldrich Chemical Company, USA. Mobic or meloxicam [4-hydroxy-2-metyl-N-(5-metyl-2-tiazolyl)-2H-1, 2-benzothiazin-3-cacboxamit-1,1-dioxit] is a nonsteroidal anti-inflammatory drug that could reduce the pain and inflammation caused by rheumatoid arthritis and osteoarthritis. Mobic (Boehringer Ingelheim, Germany) was used as the reference drug in this study. All reagents used in this study were at analytic grade unless otherwise stated.

Phytochemical screening

Preliminary phytochemical screening of EtCA was performed using standard methods as described by Gupta *et al*. (2013) and Yadav *et al*. (2014). Then total polyphenol content was determined via a colorimetric assay using the Folin-Ciocalteu reagent (Nguyen and Tran 2018). The absorbance of the reaction mixture at 750 nm was measured, and the results were presented as milligrams of gallic acid equivalents per gram of dry weight of extract (mg GAE/g DW). Moreover, the total flavonoid content of the extract was estimated using the spectrophotometric method based on aluminium chloride complex formation in compliance with Pham *et al.* (2022) procedure. The absorbance of the reaction mixture at 415 nm was recorded, and the results were expressed as milligrams of quercetin equivalents per gram of dry weight of extract (mg QE/g DW).

Experimental animal

Swiss *albino* mice (male, 7-week-old, 29 ± 2 g) were obtained from the Pasteur Institute of Ho Chi Minh City. The mice were housed in glass cages with wooden shaving bedding. They were maintained under standard husbandry conditions in the institutional animal facility (Vietnam), water was supplied via the water bottle, and the animals were fed with a normal diet (Anifood, Institute of Vaccine and Medical Biologicals, Nha Trang City, Vietnam). The mice were acclimated to laboratory conditions for 7 days before the experiment. All animals received humane care and use during the experiment according to Vietnamese legislation (Law of Animal Husbandry, Law No.32/2018/QH14), and the experimental procedure was in compliance with the Vietnam National Guideline for Preclinical and Clinical Trials of Traditional Medicines and Pharmaceuticals (Decision 141/QD-K2DT of Administration of Science Technology and

Training, The Ministry of Health of Vietnam, 2015).

Experiment design

A total of 24 male Swiss albino mice were randomly divided into 4 groups, with 6 mice per group. Group I (Control): the mice were orally received a volume of saline (100 µL) and injected with 0.1 mL of saline into their hind paws after 1 h (day 0). They were subsequently treated with the same volume of saline for 28 days p.o., and this group was used as the normal control. Group II (RA): The pathological model was established via the Ruckmani *et al.* (2018) method. Briefly, the animals were administered a volume of saline (100 µL) via oral gavage, and 0.1 mL of FCA was successively injected into the hind paws of the mice after 1 hour. Then they were orally received the same volume of saline once daily for 28 days. Group III (EtCA + RA) the animals were treated similarly to the FCA-induced rheumatoid arthritis model and treated with given doses of EtCA (400 $mg.kg^{-1}$ body weight) p.o. once daily for 28 days. Group IV (Mobic+RA) was the reference drug group, in which the mice developed rheumatoid arthritis as Group II, and were treated with Mobic (0.2 $mg.kg^{-1}$ body weight) for 28 days. The dose selection of EtCA (400 $mg.kg^{-1}$ body weight) was determined based on the previous study, in which the safety of ethanol extract of *Coriolopsis aspera* was proven (up to 6,000 $mg.kg^{-1}$ body weight) (Nguyen *et al.* 2021).

Measurement of joint diameters, paw volumes, and arthritis scores

Determination of joint diameters and paw volumes: the severe degree of arthritis was evaluated via the change of joint diameters and paw volumes. The joint diameters of groups were determined using the electronic vernier caliper and the paw volumes were measured using a plethysmometer (UGO Basile) at the end of experiment.

Determination of arthritis score: Arthritis scoring was conducted using the Endale *et al.* (2013) method. Clinical assessment was performed by a pathological expert with the scoring system as follows: 0 – no symptom of erythema or swelling, 1 – mild swelling, erythema in the tarsals or the ankle, 2 – mild swelling, erythema spread out from the tarsals to the ankle, 3 – moderate swelling, erythema spread out from the ankle to metatarsal joints, 4 – severe swelling, erythema throughout the foot, or gross deformity and inability to use the limb. The arthritic score in each mouse was evaluated according to the variation of erythema, edema of the injected joint, and the involvement of other non-injected joints. The maximum of the combined arthritis scores of both hind limbs of each mouse was 8.

Determination of hematological and biochemical parameters

At the end of the experiment, the mice were euthanized using an overdose of carbon dioxide inhalation. The blood was collected via cardiac puncture and divided into two parts, one part was stored in a tube containing EDTA for hematological analysis, and the other was kept in a serum separator tube used for biochemical analysis. The leukocyte count was carried out by a hematology analyser, and erythrocyte sedimentation rate (ESR) was determined by the method described by Westergren (1957). CRP was measured via the Voila *et al.* (1981) procedure, and rheumatoid factor (RF) was determined using the Johnson and Fraulk (1976) method. Cervical dislocation was used as a secondary physical method to verify the death before carcass disposal.

Histological study

The joints were taken and fixed in 10 % formalin for further histological studies with Hematoxylin and Eosin Staining routine procedures with minor modifications (Liu *et al.* 2017; Tran *et al.* 2018). Briefly, the specimens were prepared via a series of steps including decalcification, dehydration, and embedding in paraffin wax. After that, the embedded samples were sliced into 4 – 6 um sections and placed on adhesive pre-coated slides. The sections were subsequently deparaffinized, rehydrated, and stained with Hematoxylin and eosin. The histological features were analysed under a light microscope (Nikon, Japan, x200).

Statistical analysis

Results were presented as mean ± standard deviation (Mean ± SD). The differences among groups were determined by the One-way analysis of variance ANOVA followed Fisher's least significant difference using the Stagraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, Virginia, USA), and the criterion of statistical significance was set as $P < 0.05$.

Results and Discussion

The presence of bioactive compounds in EtCA

In the present study, it was found that there were alkaloids, flavonoids, saponins, terpenoids, tannins, phlobatannins, glycosides, and carbohydrates in the extract, whereas neither fats, oils, nor proteins were detected in the extract (Table 1). Furthermore, the extract possessed a rich content of bioactive compounds such as polyphenols and flavonoids (37.79 ± 0.88 mg GAE/g DW and 8.56 ± 0.37 mg QE/g DW, respectively). These results are in line with the previous study (Nguyen *et al.* 2020), in which the authors report the presence of bioactive compounds including triterpenoids (trametenolic acid B), sterols and steroid derivatives (cerevisterol, ergosterol, ergosterol peroxide), phenols (methyl-(2-hydroxyphenyl)acetate), trans-p-hydroxycoumaric acid, umbelliferone, 8-hydroxy-3,4-dimethylisocoumarin, methyl ferulate. Furthermore, the polyphenol content of the extract is significantly higher than those of peeled black garlic (4.7 times), a well-known functional food, and two other mushrooms, including *Sarcodon imbricatus* (2.9 times) and *Russula chloroides* (12.1 times) (Nguyen and Tran 2018; Shomali *et al.* 2019). According to Van *et al.* (2020), flavonoid contents of *Amorphophallus opertus* and *Amorphophallus lanceolatus* tubers are 0.313 and 0.032 mg QE/g DW, respectively, while the flavonoid content of *C. aspera* is 8.56 mg QE/g DW. Notably, the flavonoid contents of some edible mushrooms, such as *Lactarius indigo, Cantharellus cibarius, Amanita caesarea,* and *Boletus edulis,* have been reported in the range of 0.25-0.92 mg QE/g (López-Vázquez *et al.* 2017). As demonstrated above, *C. aspera* possesses high contents of polyphenol and flavonoid, two well-known bioactive compound groups.

Table 1. Phytochemical screening of ethanol extract of *C. aspera.*

No.	Compounds	EtCA
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Terpenoids	+
5	Tannins	+
6	Phlobatannins	+
7	Glycosides	+
8	Carbonhydrates	+
9	Proteins	-
10	Fats and oils	-

Notes: (+) Detect in EtCA; (-) Not detect in EtCA.

Dietary flavonoids have been shown to have a strong anti-arthritis effect in both preclinical and clinical studies, which may be attributed to their suppression of pro-inflammatory cytokines, chemokines, matrix metalloproteinases, and proliferation, as well as their augmentation of inflammatory cells (Hughes *et al.* 2017; Lim *et al.* 2019). According to Sun *et al.* (2022), triterpenoids isolated from *Gypsophila oldhamiana* are potential anti-inflammatory constituents. In a previous study, Sun *et al.* (2016) also proved that treatment with saponins from *Clematis henryi* Oliv. led to a decrease in paw volume and thickness, as well as a decrease in IgG, IL-1β, and TNF-α levels, and an improvement in the histological features of the joints in collagen-induced arthritis rats. Moreover, the anti-inflammatory effect of polyphenols is well documented (Oliviero *et al.* 2018). Polyphenols can block important signalling pathways, which in turn lowers the expression of mediators involved in RA pathogenesis like matrix metalloproteases, receptor activator of nuclear factor kappa B ligand, and pro-inflammatory cytokines (Christman and Gu 2020). According to Nguyen *et al.* (2020), *C. aspera* also possesses some anti-inflammatory compounds, such as cerevisterol, umbelliferone, 8-hydroxy-3,4-dimethylisocoumarin, methyl ferulate, trametenolic acid B, methyl-(2-hydroxyphenyl)acetate. Thus, the presence of these compounds in the extract gives a basis for the anti-arthritis activity of *C. aspera*.

Treatment of EtCA improves the alteration of paw diameters, volumes, and arthritis scores

The joint diameters of FCA-treated mice were significantly increased as compared to normal control mice (P <0.05). These results indicated that FCA induced swelling and inflammation around the joints of mice, and the arthritis model was successfully established (Table 2). On the contrary, the joint diameters of EtCA-treated mice were remarkably reduced as compared to those of the FCA-untreated group (P <0.05). The efficacy of the reference drug against the increase in joint diameter (3.35 ± 0.14 mm) was equivalent to mice treated with 400 mg.kg^{-1} b.w. (3.42 ± 0.11 mm) after 28 days (P >0.05).

Furthermore, the anti-arthritis effect of EtCA was also evaluated via the decrease in paw volumes of mice after treatment with FCA and EtCA (Table 2). In this study, the paw volumes of FCA-treated mice were approximately twice as high as those of the normal control group (P <0.05). After 28 days, treatment with EtCA improved the alteration of paw volumes induced by FCA. Notably, the paw volumes of EtCA-treated mice (400 mg.kg^{-1} b.w.) and Mobic-treated mice showed no significant difference (0.52 ± 0.03 versus 0.49 ± 0.04 mm, respectively, P >0.05). In addition, the arthritis score of FCA treated mice was remarkably higher than that of normal mice (P <0.05). On the other hand, EtCA-treated mice showed a decrease in their arthritis scores as compared to the FCA-treated only group (1.33 ± 0.82 and 5.50 ± 0.84, respectively, P <0.05). Moreover, treatment with EtCA (400 mg.kg^{-1} b.w.) could reduce the arthritis score toward the range of reference drug-treated mice (1.17 ± 0.75, P >0.05). These results indicated that treatment with EtCA could improve the arthritis scores and alleviate the paw oedema (paw diameters and volumes) in the arthritis group, which is in agreement with the data of macroscopic morphology of paws (Fig. 2A). That suggests preliminary evidence for the anti-arthritis effect of ethanol extract.

Table 2. The effect of EtCA on the joint diameters, the paw volumes, arthritis scores.

Groups	Joint diameters [mm]	Paw volumes [mL]	Arthritis scores
Control group	3.21^{a} ± 0.12	0.43^{a} ± 0.02	0.00^{a} ± 0.00
RA group	6.53^{c} ± 0.18	0.85^{c} ± 0.04	5.50^{c} ± 0.84
EtCA + RA group	3.42^{b} ± 0.11	0.52^{b} ± 0.03	1.33^{b} ± 0.82
Mobic + RA group	3.35^{ab} ± 0.14	0.49^{b} ± 0.04	1.17^{b} ± 0.75

The data were expressed as mean ± SD. The superscript letters ($^{a, b, c}$) in the same column denote the significant differences among different treatments (P <0.05).

Treatment of EtCA improves the changes in hematological and biochemical parameters

White blood cell count, ESR, RF, and CRP are key indicators for systemic inflammatory response; therefore, the changes in these parameters indicate the complication and improvement of systematic inflammation at the hematological and biochemical levels. According to Yukawa and Mimori (2013), leukocytosis, the term used to describe an elevation of white blood cell count in the blood, is a common phenomenon observed in rheumatoid arthritis patients. Moreover, Atzeni *et al*. (2017) suggest RF along with anti-citrullinated protein antibodies as the diagnosis and prognosis biomarkers for rheumatoid arthritis, which are used to determine a patient susceptibility to the disease and propose ESR and CRP as the disease monitoring biomarkers. As shown in Table 3, the white blood cell count of RA group was higher than that of the control group (10.07 ± 0.13 versus 5.95 ± 0.10 ×10^{3} cells/mm^{3}, respectively). Furthermore, other parameters of FCA-treated mice, such as CRP, RF, and ESR, were elevated as compared to normal mice (P <0.05). That implies the arthritis model has been successfully established. On the contrary, treatment with EtCA (6.56 ± 0.14 ×10^{3} cells/mm^{3}) inverted the alteration of leukocyte count induced by FCA (P <0.05). Furthermore, there is no significant difference between the WBC of EtCA-treated mice (400 mg.kg^{-1} b.w.) and that of reference drug treated group (6.42 ± 0.15 ×10^{3} cells/mm^{3}, P >0.05). In addition, the levels of CRP, RF, and ESR of EtCA-treated mice (400 mg.kg^{-1}

b.w.) were similar to those of reference drug-treated mice (P >0.05), and both were less than those of the FCA-treated group (P <0.05).

Several medicinal mushrooms, such as *Lentinus polychorus, Flammulina velutipes,* and *Ganoderma lucidum* have been reported for their anti-inflammatory effect via inhibition of a variety of inflammatory mediators, including TNFα, NO, IL6, and IL-1β (Rowaiye *et al.* 2022). Notably, Nguyen *et al.* (2020) also prove that ethyl acetate and chloroform extracts *of C. aspera* can suppress LPS-induced nitrite oxide production, which suggests anti-inflammatory and anti-arthritis activity of *C. aspera*. The results of plasma concentrations of hematological and biochemical parameters are in line with the previous study and provide evidence for the anti-inflammatory effect of the extract at the biochemical level (Nguyen *et al.* 2020). Furthermore, these findings suggest that the efficacy of ethanol extract (400 mg.kg^{-1} B.W.) is identical with the efficacy of the reference drug (Mobic 0.2 mg.kg^{-1} B.W. for 28 days). Further histological analysis needs to be conducted to investigate the protective effect of EtCA against adjuvant-induced arthritis at the cellular and tissue levels.

Table 3. Hematological and biochemical parameters of experimental mice.

Groups	WBC [10^3 cells.mm^{-3}]	CRP [mg.L^{-1}]	RF [mg.L^{-1}]	ESR [mm.h^{-1}]
Control group	5.95^a ± 0.10	0.53^a ± 0.08	0.34^a ± 0.06	5.04^a ± 0.21
RA group	10.07^c ± 0.13	7.95^c ± 0.22	8.62^c ± 0.24	7.74^c ± 0.28
EtCA + RA group	6.56^b ± 0.14	2.83^b ± 0.20	3.06^b ± 0.16	5.69^b ± 0.20
Mobic + RA group	6.42^b ± 0.15	2.64^b ± 0.17	2.85^b ± 0.22	5.46^b ± 0.16

The data were expressed as mean ± SD. The superscript letters (^{a,b,c}) in the same column denote the significant differences among different treatments (P <0.05).

Histological analysis

To explore the regional inflammatory response of joints, anatomical and histological analyses of joints were performed. In the arthritis group, FCA induced joint swelling and inflammation, stiffness, and redness in the joints (Fig. 2A; Table 2). Histological examination also showed that the joints of arthritis mice had an abnormal synovial cavity with an unclear and rough surface of articular cartilage (cartilage erosion), which indicated chondrocyte destruction, as well as severe inflammatory cell infiltration and synovial hyperplasia (Fig. 2B).

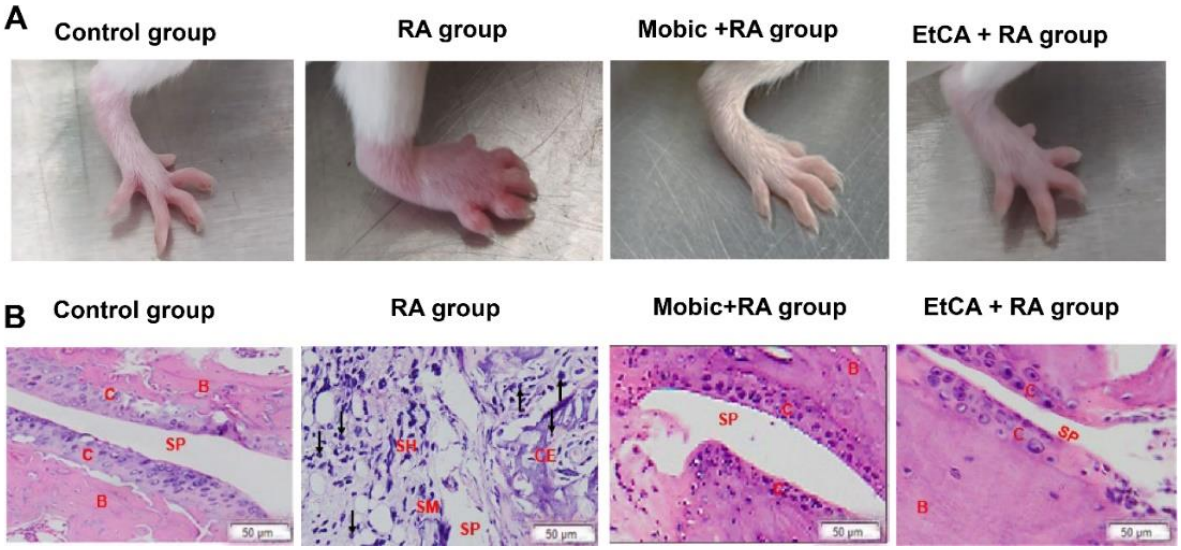

Fig. 2. Anatomical and histological features of FCA treated mice (HE, × 200) and EtCA treated mice for 28 days. Anatomical features of the ankle joints (**A**), histological features of the ankle joints (**B**). B – denotes for bone; C – indicates for cartilage; CE – cartilage erosion; SH – synovial hyperplasia; SP – joint space; → immune cell infiltration.

In contrast, treatment with EtCA reduced the swelling and inflammatory response, and the stiffness and redness in the joints of EtCA mice were also decreased (Fig. 2A; Table 2). Furthermore, the structure of the synovial cavity of EtCA- or reference drug-treated mice was improved, and the surface of the articular cartilage was smooth, and clear along with a normal joint space (Fig. 2B). These results prove the potential of the extract against adjuvant-induced arthritis at cellular and tissue levels. Our study not only provides more information about the bioactivity of the ethanol extract of *C. aspera* but also explores the further application of the medicinal mushroom in medicine and the pharmaceutical industry, especially for arthritis treatment.

Conclusion

In this study, we detected the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, phlobatannins, glycosides, carbohydrates, as well as polyphenols and flavonoid (37.79 ± 0.88 mg GAE/g DW and 8.56 ± 0.37 mg QE/g DW, respectively) in the ethanol extract of *C. aspera*. Moreover, the extract exerted a strong anti-arthritis effect on FCA-induced rheumatoid arthritis. After 28 days of arthritis induction, treatment with EtCA reduced the paw diameters, volumes, and arthritis scores, as well as inverted the alteration of WBC, RF, CRP, and ESR. The histological analysis revealed that EtCA could inhibit chondrocyte destruction and inflammatory cell infiltration. The mice treated with EtCA (400 $mg.kg^{-1}$ BW) showed similar efficacy as those treated with the reference drug (Mobic, 0.2 $mg.kg^{-1}$ BW). These results suggest *C. aspera* as a promising remedy for supporting the treatment of arthritis or as a potential source to extract some bioactive compounds, such as flavonoids and polyphenols, use in medicine and the pharmaceutical industry.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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