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Original Article
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Antiarthritic Activity and Inflammatory Mediators Modulation Effect of Traditional Ajmodadi Churna on Arthritis Experimental Model

Vikash Sharma[1], Shiv Shankar Shukla[2], Bina Gidwani[2], Ravindra Kumar Pandey[3]*

[1]Department of Pharmacology, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India
[2]Department of Analytical Chemistry, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India
[3]Department of Pharmacognosy, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India

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***Corresponding Author**
Ravindra Kumar Pandey
Department of Pharmacognosy, Columbia Institute of Pharmacy, Near Vidhan Sabha Road, Village Tekar, Raipur, Chhattisgarh 493111, India
Tel: +98-27246368
E-mail: ravindraiop@gmail.com

Objectives: The study was designed to evaluate anti-arthritic activity of Ajmodadi Churna (AC) and its effect on Complete freund's adjuvant (CFA)-induced arthritis in Wistar rats.
Methods: Arthritis was induced by injecting 0.2 mL CFA into sub plantar surface of left hind paw. Test sample AC-1 and AC-2, 200 and 400 mg/kg, respectively was given to the animals for 21 consecutive days. The increase in swelling was observed after induction of arthritis. The paw edema was measured on 0, 3, 7, 14 and 21 day using Vernier calliper after the induction of arthritis. The collected blood samples further used for the estimation of red blood cells (RBC), white blood cells (WBC), erythrocytes sedimentation rate (ESR), and hemoglobin (Hb), using hematology analyzer. Serum concentration of IL-6 and TNF-α were also measured using rat ELISA kits.
Results: Results showed that a significant reduction in paw edema was observed in AC-2 treated rats. The paw edema was restored on day 21 was 4.48 mm for AC-2, which is near to the control group. The arthritis score in treated rats was found to be considerably lower than in the control group i.e. 0.83 for AC-2 and 1.50 for AC-1. A decrease in levels of RBC and hemoglobin were observed in arthritic rats. Inflammation was significantly reduced and serum levels of IL-6 and TNF-α were lowered after treatment with the test drug.
Conclusion: It can be concluded from the study that AC possess significant anti-arthritic activity. Furthermore, this condition was linked to a reduction in abnormal humoral immune responses.

Keywords: Ayurvedic, arthritis, ELISA, interleukin, collagen, TNF-α

INTRODUCTION

The progressive deterioration of joints, deformity, disability, and early death caused by rheumatoid arthritis (RA) is the result of this chronic inflammatory illness [1]. The development of RA is influenced by many distinct mechanisms. Pro-inflammatory cytokines are very important in RA, and these include tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and IL-6. TNF-α is the major regulator of inflammatory cytokines [2].

Disease-modifying antirheumatic medications such as TNF-α or IL-1 are preferred over non-steroidal anti-inflammatory drugs because of their potential to stop or even reverse the disease [3]. However, the effectiveness of these treatments has several disadvantages related to adverse effects. Interestingly, there has been a significant rise in the screening of new therapeutic agents derived from natural materials that can be useful for arthritic patients due to their fewer side effects.

Ajmodadi churna (AC) is an ancient traditional Ayurvedic polyherbal formulation used in India for the treatment of inflammation and joint pain. It is also used as a carminative, antispasmodic, wormifuge, or for sciatica [4, 5]. The major ingredients—*Cedrus deodara*, *Trachyspermum ammi*, *Terminalia chebula*, *Zingiber officinale*, *Piper longum*, and *Argyreia*

nervosa have been examined separately for their anti-inflammatory effects. Antibacterial, analgesic, gastroprotective, fungal, parasitic, and viral properties have all been attributed to *Trachyspermum ammi* [6]. Antioxidant, antibacterial, antifungal, adaptogenic, anticonvulsant, antifertility, antihyperlipidemic, and anticancer properties are found in *Embelia ribes* [7]. It has been shown that *Cedrus deodara* has potent anticancer and antioxidant activities [8]. Antibacterial, anticancer, antileishmanial, anti-inflammatory, hypolipidemic, hyperglycemic, and antifungal properties have all been attributed to *Plumbago zeylanica* [9]. Hepatoprotective, antidepressant, antiulcer, antiviral, immunomodulatory, antiallergic, and anticancer properties have all been observed in *Piper longum* [10]. There have been reports that the herb *Anethum graveolens* can treat diabetes, cancer, and other diseases, as well as having antispasmodic, diuretic, antisecretory, and insecticidal effects [11]. Antiplatelet, antioxidant, anticancer, hepatoprotective, antihypertensive, antithyroid, and antiasthmatic properties are among the useful effects of *Piper nigrum* [12]. *Terminalia chebula* has been shown to have beneficial effects as an antibiotic, antiviral, antioxidant, radioprotective, immunomodulatory, and wound healing agent [13]. Uses for *Argyreia nervosa* include those of a diuretic and a contraceptive, as well as for the treatment of rheumatic illnesses, wound healing, syphilis, leukorrhea, cancer, and ulcers. Active components in *Zingiber officinale* include those with antitumorigenic, immunomodulatory, antiapoptotic, anti-inflammatory, antihyperglycemic, antiemetic, and antilipidemic properties [14]. Additionally, the Ayurvedic formulation of *Piper longum*, *Zingiber officinale*, and *Piper nigrum*, collectively referred to as Trikatu, is a powerful bioenhancer [15]. Thus, the protocol of this study was designed to evaluate the antiarthritic effect of the AC formulation on CFA-induced arthritis in rats and inflammatory immune cells.

MATERIALS AND METHODS

1. Preparation and extraction of AC

Trachyspermum ammi (12 gm), *Embelia ribes* (12 gm), rock salt (12 gm), *Cedrus deodar* (12 gm), *Plumbago zeylanica* (12 gm), *Piper longum* (12 gm), *Anethum graveolens* (12 gm), *Piper longum* (12 gm), *Piper nigrum* (12 gm), and *Terminalia chebula* (60 gm) were used to prepare the formulation of AC (Table 1). The medicines were verified and authenticated at the Department of Pharmacognosy, Columbia Institute of Pharmacy, Raipur, India. All of the voucher specimens have been deposited at the herbarium of the institute. AC was prepared according to the Indian Pharmacopeia and concentrated to the prescribed dosage ratio [16]. Two liters of distilled water were used to prepare 87 g of a finely powdered formulation over two hours. A stock solution of 300 mg/mL was made by dissolving 9.2 g of powder from the hydroalcoholic extract (60:40) into 30.7 mL of distilled water. Each plant utilized in the preparation of AC was analyzed by high-performance liquid chromatography fingerprint analysis.

Table 1. Ingredients used for the preparation of AC

Ingredients	Traditional name	Parts	Quantity (gm)
Trachyspermum ammi	Ajmoda	Fruit	12
Embelia ribes	Vidanga	Fruit	12
Rock salt	Saindhava lavana	Salt	12
Cedrus deodara	Devdaru	Wood	12
Plumbago zeylanica	Chitraka	Aerial parts	12
Piper longum	Pipalimula	Stem	12
Anethum graveolens	Satapuspa	Fruit	12
Piper longum	Pipali	Fruit	12
Piper nigrum	Marica	Fruit	12
Terminalia chebula	Pathya	Fruit	60
Argyreia nervosa	Vrddhadaruka	Root	120
Zingiber officinale	Nagara	Rhizome	120

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2. Experimental animals

In this study, male Sprague-Dawley rats weighing between 150 and 170 g were used. The animals used in this study were procured from the Columbia Institute of Pharmacy, Raipur, India. Institutional Animal Ethical Committee (IAEC) approval (Approval no. CIP/IAEC/2018/121) was obtained to perform experiments on animals. The animals had free access to food (Keval Sales Pvt. Ltd.) and water.

3. Induction of CFA-induced arthritis and AC treatment

Left hind paw arthritis was induced in animals by the sub plantar injection of 0.2 mL CFA. The animals were divided into five groups (n = 6/group). Group I was the normal control (5 mL/kg saline), group II (diseased control) received 0.2 mL CFA injected once into the sub plantar surface of the left hind paw along with saline, group III was administered indomethacin, and group IV and V animals were administered the test samples AC-1 and AC-2 (200 and 400 mg/kg, respectively) for 21 consecutive days (orally). Normal control and diseased control animals were treated with 5 mL/kg vehicle only [17].

4. Evaluation of paw edema and arthritic score

The paw diameter was measured on days 0, 3, 7, 14, and 21 using electronic digital calipers. Redness, swelling, and erythema are all morphological features of arthritis that were tracked using predetermined visual criteria: normal paw = 0, mild swelling and erythema of digits = 1, swelling and erythema of the digits = 2, severe swelling and erythema of the digits = 3, and gross deformity and inability to use the limb = 4.

5. Serum preparation

Blood was taken from the retro-orbital sinus of the experimental animals into Eppendorf Micro-centrifuge tubes and immediately placed in a cooling Micro-centrifuge apparatus at 7,000 rpm at 4℃ for 15 minutes to obtain clear serum. The resultant serum was transferred to fresh sterilized Eppendorf Micro-centrifuge tubes and kept in a deep fridge away from artificial light and sunlight. It was stored for biochemical parameter studies.

6. Hematological parameters

Hematology analyzers were then used to determine red blood cells (RBC), white blood cells (WBC), erythrocyte sedimentation rate (ESR), and hemoglobin (Hb) in the collected blood samples. Blood samples were analyzed in a hematological analyzer (Procan Electronics PE6800).

7. Arthritis evaluation and ELISA measurement

Paw edema and arthritic scores were measured on days 0, 3, 7, and 21 to determine the anti-arthritic efficacy of AC. Retro-orbital puncture was used to obtain blood at the end of the 21st day. Standard ELISA kits were used to quantify cytokines in the samples. Rat ELISA kits were used to determine the levels of IL-6 and TNF-α in serum. We used an ELISA microplate reader (Erba) to quantify the absorbance and compared the results to a standard curve to determine the levels of each cytokine [18].

8. Histopathology

Tissues from rat paws were fixed in 10% formalin solution for histological analysis. The tissues were impregnated in molten paraffin wax for predetermined times after being cleaned in toluene and dehydrated in various concentrations of alcohol. Fresh, molten paraffin wax was used to implant the processed tissues, which were then left to harden. Hematoxylin and 1% aqueous eosin staining of 3 μm sections dried on a hot plate for 15 minutes showed the general structure of the tissue. The slides were dehydrated in a series of increasing alcohol concentrations, cleaned in xylene, and mounted in *Canada balsam*. Microscopic examination of cut sections was performed after the preparation of tissues [19, 20].

9. Statistical analysis

Data were evaluated as the mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA using Bonferroni's post hoc test in GraphPad Prism software. Significant differences are shown by $^{*}p < 0.05$, $^{**}p > 0.01$, and $^{***}p < 0.001$ in comparison to the control group.

RESULTS

1. *In vivo* anti-arthritic activity in the CFA-induced paw edema model

Left hind paw edema caused by CFA was observed in animals with arthritis. The demineralization of the extremities became more widespread as the disease progressed. CFA-induced arthritic rats consistently had larger paw volumes than control group animals. Results showed that the inhibition of edema formation was a direct effect of the continuous injection of AC-1 and AC-2 to the animals. Additionally, arthritic scores were significantly ($p < 0.01$) lower in animals as shown in Fig. 1. Edema was increased in the CFA-induced arthritic group (6.43 mm) and reduced significantly after treatment with AC-2 at 400 mg/kg dose. Paw edema was restored on day 21 (4.48 mm) in the AC-2 group compared with the control group. However, the standard group receiving indomethacin had the highest anti-arthritic activity by restoring the paw edema (4.25 mm). Fig. 2 shows the morphological condition of paw edema after the administration of the test drug.

2. Arthritic score

Rats in all groups were examined for RA scores after treatment with the test drug. The arthritic index score was significantly higher in the CFA-induced arthritis rats than in the control group. The test drug effects on the arthritis index score are depicted in Fig. 3. The arthritis index in treated rats was found considerably lower than in the control group when AC-1 and

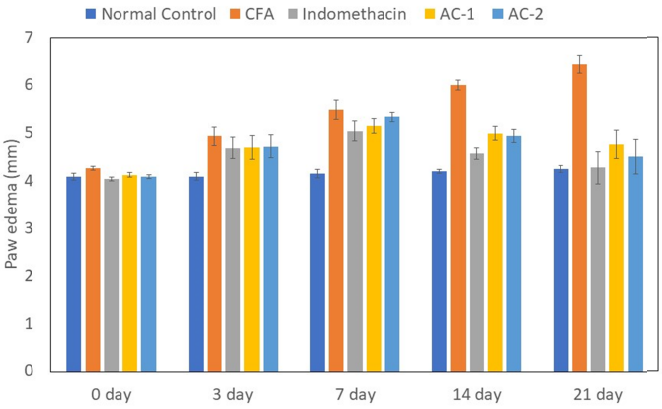

Figure 1. Effect of AC on CFA-induced arthritis in rats. Values are expressed as mean ± SD at n = 6, One-way ANOVA followed by Bonferroni test, significantly different at *p < 0.05, **p > 0.01 and ***p < 0.001, compared to the CFA induced.

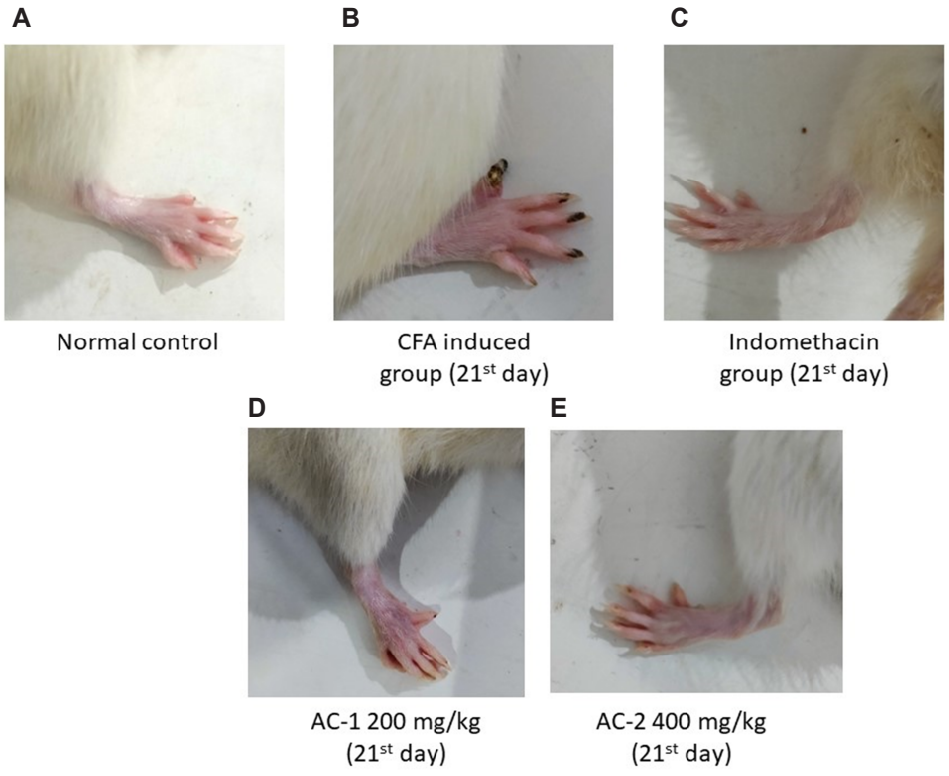

Figure 2. Morphology of paw after administration of test drug. (A) Normal control group, (B) CFA induced group, (C) Indomethacin group, (D) AC-1 test group, and (E) AC-2 test group.

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AC-2 were used. The arthritic scores were 0.83 for AC-2 and 1.50 for AC-1. However, the lowest arthritic score was observed in the indomethacin-treated group (0.67).

3. Effects of AC-1 on the levels of RBCs, WBCs, Hb, and ESR in arthritic rats

Blood profiles of CFA-induced arthritis rats were examined to determine their RBC, WBC, Hb counts, and ESR. The blood profile was drastically changed after the induction of arthritis. However, it was restored after the administration of test drugs AC-1 and AC-2. The Hb level was 14.42 and 13.62 for AC-1 and AC-2, respectively, in comparison to the control group. Similarly, the RBC, WBC, and ESR values were 12.17, 5.75, and 5.33, respectively for AC-2 as compared to the control group (Table 2). However, the standard group receiving indomethacin had the most significant effect on all blood parameters.

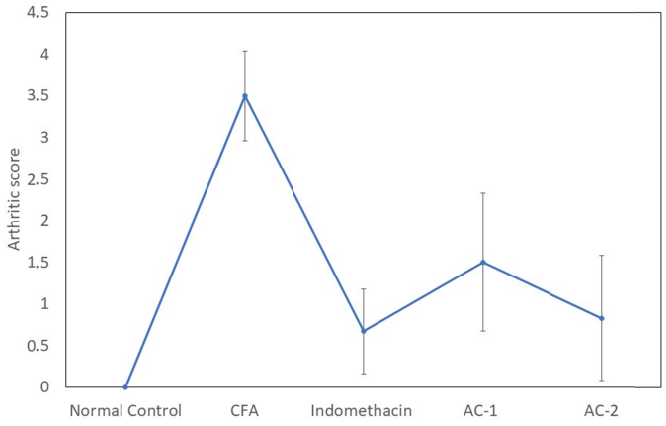

Figure 3. Effect of test drug on arthritis index score in CFA induced arthritis rats.

4. *In vivo* estimation of TNF-α and IL-6

ELISA was used to calculate the concentrations of TNF-α and IL-6. There was an increase in TNF-α and IL-6 in rats from group II compared to controls. Serum levels of TNF-α and IL-6 were significantly reduced in rats in groups III, IV, and V after AC administration compared to those in the CFA groups. The serum TNF-α level was increased to 109.53 pg/mL after the induction of arthritis. Administration of the test drugs AC-1 and AC-2 significantly restored the raised serum TNF-α level to 82.33 and 66.60 pg/mL, respectively (Table 3). However, it was most significantly restored in the standard group (58.33 pg/mL). The serum IL-6 level was increased to 222.95 pg/mL after the induction of arthritis. The administration of AC-1 and AC-2 significantly restored the raised serum IL-6 level to 99.44

Table 2. Effect of test drug on hematological parameters on CFA-induced arthritic animals

Group no.	Group	Parameter			
		Hb	RBC	WBCs	ESR
I	Normal control	15.72 ± 1.619	11.93 ± 1.940	6.18 ± 0.578	5.43 ± 0.635
II	CFA (0.2 mL)	9.40 ± 1.022	5.27 ± 0.619	11.47 ± 0.607	10.64 ± 0.857
III	Indomethacin (10 mg/kg)	16.08 ± 1.086***	11.48 ± 1.111*	5.06 ± 0.529**	5.00 ± 1.310***
IV	AC-1 200 mg/kg	13.62 ± 1.295*	10.13 ± 0.712*	6.38 ± 0.935**	5.35 ± 0.787**
V	AC-2 400 mg/kg	14.42 ± 0.801**	12.17 ± 2.137***	5.75 ± 0.720***	5.33 ± 0.516**

Mean standard deviation; n = 6; one-way analysis of variance with Bonferroni's post hoc test; *p < 0.05, **p > 0.01, and ***p < 0.001 indicate statistically significant differences from CFA-induced controls.

Table 3. Effect of AC on serum TNF-α and IL-6 in different groups

S. no.	Group	TNF-α (pg/mL)	IL-6 (pg/mL)
I	Normal control	94.66 ± 3.893	87.742 ± 2.594
II	CFA (0.2 mL)	109.53 ± 0.814	222.954 ± 1.753
III	Indomethacin (10 mg/kg)	58.33 ± 0.838***	84.52 ± 0.630***
IV	AC-1 200 mg/kg	82.33 ± 7.223*	99.44 ± 2.295**
V	AC-2 400 mg/kg	66.60 ± 0.630***	72.40 ± 5.255***

Mean standard deviation; n = 6; one-way analysis of variance with Bonferroni's post hoc test; *p < 0.05, **p > 0.01, and ***p < 0.001 indicate statistically significant differences from CFA-induced controls.

and 72.40 pg/mL, respectively. However, it was most significantly restored in the standard group (84.52 pg/mL) (Table 3).

5. Effects of AC on histopathological changes

The analysis of histological samples taken at week 6 revealed the significant degeneration of cartilage and bone, in addition to the widespread proliferation of synovium and inflammatory infiltration. Synovium proliferation and inflammatory infiltration were reduced ($p < 0.05$) after 2 weeks of treatment with a high dose of AC; however, no change was seen in the cartilage and bone. A lower AC dose had no beneficial effects (Fig. 2A). At week three, CFA-induced arthritic rats demonstrated extensive synovium proliferation and inflammatory infiltration, severe cartilage and bone breakdown with loss of fragmented cartilage and huge areas of bone, and widespread bone neoformation in the affected joints. Interestingly, AC therapy for 3 weeks significantly reduced such pathogenic conditions. AC treatment at a lower dose significantly reduced the course of histological lesions in CFA-induced arthritis in rats compared to the control group (Fig. 4B and C).

Group 1: Normal control showing normal histological structures of paw tissues. Group 2: the CFA group showed cartilage destruction, detachment of the epidermal layer, mononuclear inflammatory infiltration, synovial hyperplasia with increased vascularity, severe edema, and necrosis. Group 3: the indomethacin group showed intact cartilage, mild hyperplasia, meta tarsus, sweat glands, and almost retained the normal paw size. Group 4: AC-1 200 mg/kg treated rats showed mild edema, decreased inflammation, and decreased vascularity. Group 5: AC-2 400 mg/kg treated rats showed intact cells, tarsus, and decreased vascularity.

DISCUSSION

A variety of physiological mechanisms such as acute and chronic inflammation and other immune responses contribute to the pathophysiology of RA. A very rapid erosive disease is characteristic of the CFA-induced arthritis model, which is widely used to study the etiology of RA and to evaluate treatments. Adjuvant arthritis is triggered by the CFA bacterial peptidoglycans and muramyl dipeptides. Biological agents are regarded as the most promising therapeutic agents to overcome RA as protein therapy [21, 22]. Herbal medicines often employ multiple factors and targets to achieve their desired pharmacological effects. However, the use of "polyherbal therapy" for RA may be helpful in mitigating arthritic conditions as a contemporary treatment [23]. Thus, the approach of treating RA using polyherbal therapy may be a potent anti-rheumatic herbal remedy formula. The anti-arthritic efficacy of polyherbal formula-

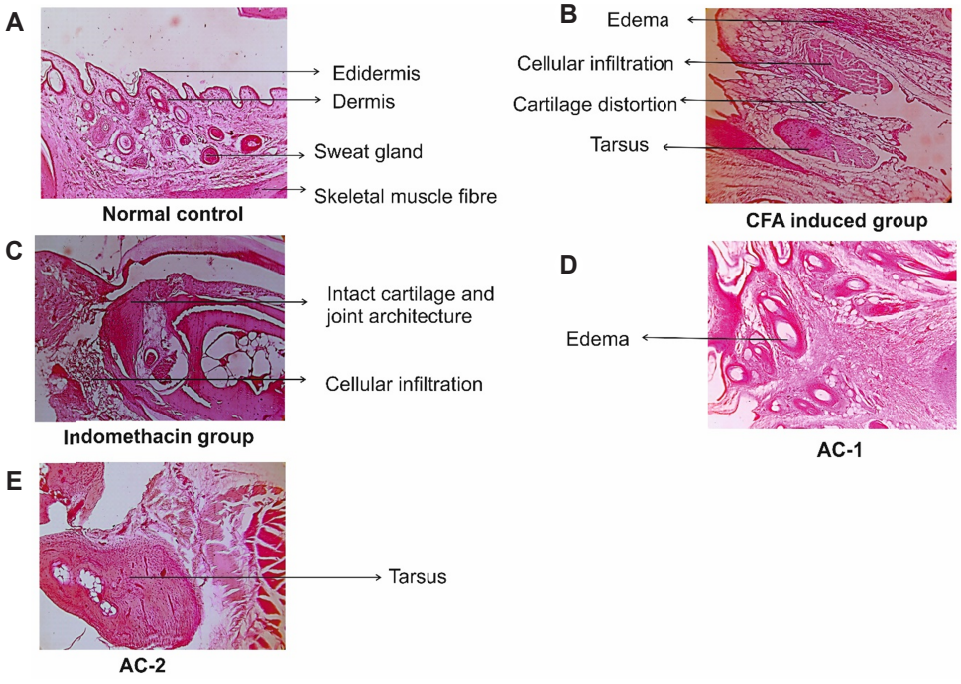

Figure 4. Histopathological slides of rat paw after administration of test drug. (A) Normal control group, (B) CFA induced group, (C) Indomethacin group, (D) AC-1 test group, and (E) AC-2 test group.

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tions has been reported to be higher than that of individual plant extracts [24].

In the present study, the arthritic score, paw measurement, and inflammatory cytokines were significantly increased in CFA-treated rats compared with the normal control group. AC was used to reduce arthritis induced by CFA in the animals. The test drug was effective at reducing inflammation and arthritic scores in rats. The significant reductions of lesions by these drugs indicate their possible anti-inflammatory effects. In the current study, TNF-α and IL-6 serum levels were considerably increased by CFA. However, AC was effective at reducing the levels of inflammatory mediators that cause arthritis. It was reported that RA can be reduced or inhibited when pro-inflammatory cytokines such as TNF-α and IL-6 are inhibited [25]. Mojtabavi et al. [26] evaluated the effect of curcumin on the expressions of TNF-α and IL-6. They found that the herbal drug significantly reduced the expressions of inflammatory mediators. This was in agreement with our results showing a reduction in inflammatory mediators after treatment with a polyherbal formulation [26].

The histopathological observations also correlated with these parameters, which further support the anti-inflammatory effects of the polyherbal formulation. Similar findings were reported by Barua et al. [27] who evaluated the anti-inflammatory activity of a polyherbal formulation. In the histopathology analysis of arthritic animals, AC-1-treated rats showed mild edema and decreased inflammation with reduced vascularity; however, AC-2-treated rats showed intact cells, tarsus, and decreased vascularity by histopathological analysis. These results indicated that AC ameliorates joint histopathological changes of RA by reducing pro-inflammatory mediators. Guo et al. [1] showed that herbal medications lower collagen-induced arthritis in rats via anti-inflammatory and apoptotic actions, which is in agreement with our findings.

CONCLUSION

The findings of this study showed that AC has significant anti-arthritic activity. Paw edema was significantly reduced in AC-2 400 mg/kg-treated rats. Arthritic rats had lower levels of RBCs and Hb; however, AC-2-treated animals had significantly higher amounts of Hb and RBCs compared to the control group. When AC-2 was administered, the arthritic group showed a dramatic reduction in their WBC and ESR levels. These results were associated with a reduction in acute arthritis-related TNF-α and IL-6 levels. Furthermore, this condition was linked to a reduction in abnormal humoral immune responses. However, the role of AC in treating arthritis and its long-term therapeutic benefits in RA have not been fully explored. Further work is required to determine the mechanism of action of AC in reducing arthritis.

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CONFLICTS OF INTEREST

We declared no conflict of interest.

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ORCID

Vikash Sharma, https://orcid.org/0000-0001-6396-7583
Shiv Shankar Shukla, https://orcid.org/0000-0001-5291-577X
Bina Gidwani, https://orcid.org/0000-0003-1257-723X
Ravindra Kumar Pandey, https://orcid.org/0000-0001-8284-6269

REFERENCES

1. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res. 2018;6:15.
2. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci. 2019;20(23):6008.
3. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. Biochem Pharmacol. 2020;180:114147.
4. Government of India Department of Indian System of Medicine and Homoeopathy. The Ayurvedic formulary of India, part 1. 2nd ed. Delhi: Controller of publications; 2003. 488 p.
5. Kulkarni PH. Bronchial asthma care in ayurveda and holistic systems. 2nd ed. Delhi: Sri Satguru Publications; 2001. 155 p.
6. Goyal S, Chaturvedi V, Dhingra G, Tanwar S, Sharma K, Singh S. Trachyspermum ammi: a review on traditional and modern

pharmacological aspects. Biol Sci. 2022;2(4):324-37.
7. Sharma V, Gautam DNS, Radu AF, Behl T, Bungau SG, Vesa CM. Reviewing the traditional/modern uses, phytochemistry, essential oils/extracts and pharmacology of *Embelia ribes* Burm. Antioxidants (Basel). 2022;11(7):1359.
8. Shi X, Du R, Zhang J, Lei Y, Guo H. Evaluation of the anti-cancer potential of Cedrus deodara total lignans by inducing apoptosis of A549 cells. BMC Complement Altern Med. 2019;19(1):281.
9. Aleem M. Anti-inflammatory and anti-microbial potential of *Plumbago zeylanica* L.: a review. J Drug Deliv Ther. 2020;10(5S):229-35.
10. Biswas P, Ghorai M, Mishra T, Gopalakrishnan AV, Roy D, Mane AB, et al. Piper longum L.: a comprehensive review on traditional uses, phytochemistry, pharmacology, and health-promoting activities. Phytother Res. 2022;36(12):4425-76.
11. Haidari F, Zakerkish M, Borazjani F, Ahmadi Angali K, Amoochi Foroushani G. The effects of Anethum graveolens (dill) powder supplementation on clinical and metabolic status in patients with type 2 diabetes. Trials. 2020;21(1):483.
12. Zhang C, Zhao J, Famous E, Pan S, Peng X, Tian J. Antioxidant, hepatoprotective and antifungal activities of black pepper (Piper nigrum L.) essential oil. Food Chem. 2021;346:128845.
13. Meher SK, Panda P, Das B, Bhuyan GC, Rath KK. Pharmacological profile of *Terminalia chebula* Retz. and Willd. (Haritaki) in Ayurveda with evidences. Res J Pharm Pharmacodyn. 2018;10(3):115-24.
14. Yücel Ç, Karatoprak GŞ, Açıkara ÖB, Akkol EK, Barak TH, Sobarzo-Sánchez E, et al. Immunomodulatory and anti-inflammatory therapeutic potential of gingerols and their nanoformulations. Front Pharmacol. 2022;13:902551.
15. Tiwari A, Mahadik KR, Gabhe SY. Piperine: a comprehensive review of methods of isolation, purification, and biological properties. Med Drug Discov. 2020;7:100027.
16. Marshall AC. Traditional Chinese medicine and clinical pharmacology. In: Hock FJ, Gralinski MR, editors. Drug discovery and evaluation: methods in clinical pharmacology. Cham: Springer; 2020. p. 455-82.
17. Li Y, Kakkar R, Wang J. In vivo and in vitro approach to anti-arthritic and anti-inflammatory effect of crocetin by alteration of nuclear factor-E2-related factor 2/hem oxygenase (HO)-1 and NF-κB expression. Front Pharmacol. 2018;9:1341.
18. Li SH, Kobayashi Y, Yamauchi Y, Gonda T, Tsunematsu T. Suppressive effects of a herbal formula, TBL-1, on type II collagen-induced arthritis in DBA/1J mice. Clin Exp Pharmacol Physiol. 1996;23(3):236-40.
19. Joosten LA, Lubberts E, Durez P, Helsen MM, Jacobs MJ, Goldman M, et al. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. Arthritis Rheum. 1997;40(2):249-60.
20. Keyszer G, Redlich A, Häupl T, Zacher J, Sparmann M, Engethüm U, et al. Differential expression of cathepsins B and L compared with matrix metalloproteinases and their respective inhibitors in rheumatoid arthritis and osteoarthritis: a parallel investigation by semiquantitative reverse transcriptase-polymerase chain reaction and immunohistochemistry. Arthritis Rheum. 1998;41(8):1378-87.
21. Evans CH, Robbins PD. Pathways to gene therapy in rheumatoid arthritis. Curr Opin Rheumatol. 1996;8(3):230-4.
22. Chernajovsky Y, Annenkov A, Herman C, Triantaphyllopoulos K, Gould D, Dreja H, et al. Gene therapy for rheumatoid arthritis. Theoretical considerations. Drugs Aging. 1998;12(1):29-41.
23. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov. 2003;2(6):473-88.
24. Petchi RR, Parasuraman S, Vijaya C, Gopala Krishna SV, Kumar MK. Antiarthritic activity of a polyherbal formulation against Freund's complete adjuvant induced arthritis in female Wistar rats. J Basic Clin Pharm. 2015;6(3):77-83.
25. Maini RN, Taylor PC. Anti-cytokine therapy for rheumatoid arthritis. Annu Rev Med. 2000;51:207-29.
26. Mojtabavi S, Saed A, Aboulfazli S, Kheirandish A, Najafi M, Jafari-Sabet M, et al. Evaluation of curcumin effect on Il6, Sirt1, TNFα and NFkB expression of liver tissues in diabetic mice with STZ. J Diabetes Metab Disord. 2022;22(1):205-15.
27. Barua CC, Bodduluru LN, Haloi P, Purukayastha A, Patowary P, Hussain MI, et al. Anti-arthritic and anti-inflammatory activity of a polyherbal formulation against Freund's complete adjuvant induced arthritis in Wistar rats. Indian J Tradit Knowl. 2017;16(3):482-9.