

Research Article

Anti-arthritic activity of solvent extracts of the bulbs of *Crinum pedunculatum* R.Br.

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ABSTRACT

Crinum pedunculatum (R.Br) has been used by traditional medicine practitioners in Ghana for the topical management of inflammation and associated disorders. This study investigated the anti-arthritic activity of the methanol, ethanol and ethyl acetate extract of the bulbs of *Crinum pedunculatum* using Sprague Dawley rats. The anti-arthritic activity of *Crinum pedunculatum* at various doses (100, 200 and 400 mg/kg) was evaluated using Complete Freund's Adjuvant (CFA) in rat model. After successful induction of the arthritis, the paw oedema, hematological parameters, pro-inflammatory cytokines and arthritis marker were assessed. Also, the radiological and histopathological evaluations were also performed. There was a significant inhibition ($P < 0.01$) of paw oedema after treatment with 100, 200 and 400 mg/kg of the methanol, ethanol and ethyl acetate *Crinum pedunculatum* extracts relative to the control. There was also a significant increase in haemoglobin and RBC levels as well as a decrease in WBC count. All the extracts of *Crinum pedunculatum* decreased IL-6 and Rheumatoid factor serum concentrations and improved joint and cartilage destruction in both contralateral and ipsilateral paw of CFA-induced rats. Significant anti-arthritic activity was observed by the 200 and 400 mg/kg ethanol and methanol *Crinum pedunculatum* extract. This study confirms that the bulb of *Crinum pedunculatum* has an anti-arthritic potential and the findings justify the folkloric use the plant as an anti-inflammatory agent.

Keywords:

Arthritis, *Crinum pedunculatum* R.Br., Complete Freund's Adjuvant, Cytokines, Rheumatoid factor

1. INTRODUCTION

Inflammation describes a response by vascularized tissues that transports leukocytes and molecules of host defense from the circulation to the site of infection or cell damage to terminate the offending agent¹. Inflammation forms part of the innate immune system and it requires inflammatory mediators such as histamine, bradykinin, and interleukins among others. It can be caused by pathogens (viruses, bacteria, fungi) as well as tissue necrosis from cells releasing their contents²⁻³. The inflammatory process can be acute or chronic. While acute inflammation occurs rapidly, chronic inflammation involves a more

prolonged duration that occurs simultaneously with tissue repair⁴. Chronic inflammation often leads to several conditions such as atherosclerosis, arthritis, and other autoimmune disorders⁵⁻⁶.

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease that is characterized by synovial hyperplasia and polyarthritis⁷⁻⁸. It is a debilitating or incapacitating disease that usually interferes with a patient's quality of life and it comes with a heavy familial and societal implication⁹. There is still no specific cure for rheumatoid arthritis, management of this condition depends on the use of non-steroidal anti-inflammatory drugs (NSAIDs) which have various side effects¹⁰.

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Disease modifying anti-rheumatic drugs (DMARDs) employed by some patients to manage this condition also cause severe adverse effects such as increased possibility of severe infections, retinopathy, hepatotoxicity among others¹¹. Due to the side effects associated with the long term use of NSAIDs, it is imperative that newer agents are developed and natural plants are a likely source¹².

Crinum pedunculatum R.Br. belongs to the Amaryllidaceae family and is also known as 'swamp lily'. Plants from the Amaryllidaceae family have been reported to contain several phytoconstituents most notably alkaloids with several pharmacological activities including anti-cancer and anti-cholinesterase inhibiting activities¹³⁻¹⁴. Phytochemical screening on the bulbs of *Crinum pedunculatum* confirmed the presence of alkaloids, saponins and tannins and a previous study described the analgesic, anti-pyretic and anti-inflammatory activities of the plant¹⁵. To the best of our knowledge, no research has been carried out on the anti-arthritic activity of the bulbs of *Crinum pedunculatum*. Therefore, this research is carried out to investigate the anti-arthritic activity of the different solvent extracts of the bulbs of *Crinum pedunculatum*.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The bulbs of *Crinum pedunculatum* (R.Br.) were harvested from Kwahu Asakrakra in the Eastern region of Ghana (Latitude: 6.62942 N 6°37'45.9048". Longitude: -0.68647 W 0°41'11.30253") and authenticated by Mr. Clifford Asare of the Department of Herbal Medicine Department, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. A voucher specimen with number KNUST/HMI/2021/001 of the plant has been deposited at the department's herbarium. The bulbs were dried (8.5 kg), ground to coarse powder and put through cold maceration process of extraction with ethanol (3.5 L), methanol (3 L), and 3.5 L of ethyl acetate solvents respectively for 7 days. After maceration, the solution was filtered and filtrate passed through the rotary evaporator to be concentrated. The final concentrated extracts were stored in the refrigerator until use.

2.2. Experimental animals

Animals were obtained from the animal house at Kwame Nkrumah University of Science and Technology. Wistar albino rats weighing 160-190 g were employed for the study. The animals were housed in the *vivarium* of the Pharmacology Department, KNUST in steel cages (57 x 34 x 40 cm³) and fed with commercial rat feed and water *ad libitum*. The following conditions were maintained at the laboratory: temperature: 25°C, relative humidity: 60-70%, 12-h light-dark cycle. These conditions were maintained throughout the days of the study. The

rats were treated humanely according to NIH Publication on Guide for Care and Use of Laboratory Animals⁸. All experimental procedures were authorized on the 6th of April 2020 by the Institutional Review Board on Animal Experimentation, Kwame Nkrumah University of Science and Technology with the ethics reference number FPPS/PCOL/012/2020.

2.3. Phytochemical Screening

The methanolic, ethanolic, and ethyl acetate extracts of *Crinum pedunculatum* were subjected to preliminary phytochemical examination using conventional procedures outlined by Trease and Evans¹⁶. Tannins, phlobatannins, saponins, and alkaloids were all screened qualitatively.

2.4. Complete Freund's adjuvant arthritis

Arthritis was induced by the injection of 0.1 ml of Complete Freund's Adjuvant (0.5 mg/ml mycobacterium emulsion) into the metatarsal area of the hind paw of each rat¹⁷. After an initial measurement of paw diameter, the rats were divided randomly into 5 groups. Group 1 was the negative control (normal saline), group 2 (diclofenac 10 mg/kg *p.o.*), groups 3-5 received *Crinum pedunculatum* extracts at 100, 200 and 400 mg/kg *p.o.* respectively. Treatment was initiated from the fourteenth day of CFA induction to the twenty-eighth day of the experiment. Paw diameter of both CFA injected paw and non-injected paw were measured on the 7th, 14th, 21st, and 28th day. On the 28th day, blood was taken from each animal through cardiac puncture for the evaluation of hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hb) and platelet concentrations (PLT). All hematological tests were carried out according to standard protocols using automated laboratory hematological analyzer. The levels of rheumatoid factor (RF) and interleukin-6 (IL-6) were evaluated from the serum according to the manufacturer's specifications with ELISA kits obtained from CUSABIO Technology Houston, Texas.

2.5. Radiological and histopathological analysis

On the 28th day, images of the hind paw were obtained using X-ray (Ecotron Any-Vet R-40KW, South Korea). Animals were placed under light anesthesia to facilitate this process and these images were assessed for radiographic changes. On the last day of the experiment, animals were sacrificed and the hind paws excised and fixed in 10% buffered formaldehyde and decalcified with 5% formic acid. Tissue sections were prepared and stained with hematoxylin and eosin (H&E). Infiltration of inflammatory cells, bone and cartilage erosion and other characteristics were evaluated¹⁸.

2.6. Statistical analysis

Results were analyzed using GraphPad Prism Version 8.0.2 (263), GraphPad Software, California USA. All results are stated as mean \pm SEM and differences among treatment groups were assessed using One-way Analysis of Variance (ANOVA). Multiple comparisons were carried out using Dunnett's test and $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Phytochemical screening

Saponins, and alkaloids were found in all solvent extracts of *Crinum pedunculatum* while phlobatannins were found in the methanol and ethyl acetate extracts (Table 1).

Table 1. Phytochemical constituents present in solvent extracts of *Crinum pedunculatum*.

Constituent	Ethanol <i>Crinum pedunculatum</i> extracts	Methanol <i>Crinum pedunculatum</i> extract	Ethyl acetate <i>Crinum pedunculatum</i> extract
Alkaloids	Present	Present	Present
Saponins	Present	Present	Present
Glycosides	Present	Absent	Absent
Flavonoids	Present	Absent	Absent
Tannins	Present	Absent	Absent
Phlobatannins	Absent	Present	Present

3.2. *Crinum pedunculatum* bulbs possess significant anti-arthritic activity against CFA-induced arthritis in rats

As shown in Figure 1, compared to the rats in the negative control group (normal saline), a decrease in paw swelling was observed by the rats treated with different solvent extracts of *Crinum pedunculatum*. Obvious symptoms of arthritis such as paw swelling were reduced significantly by all doses (100, 200 and 400 mg/kg) of the methanol and ethanol extract of *Crinum pedunculatum* ($P < 0.01$) while a significant decrease in paw diameter was observed by the 400 mg/kg of the ethyl acetate extract ($P < 0.05$). The methanol and ethanol extracts of *Crinum pedunculatum* were more effective in decreasing paw diameter compared with the ethyl acetate extract.

3.3. Effects of *Crinum pedunculatum* bulb extracts on hematological characteristics in CFA-induced rats

Rats administered solvent extracts of *Crinum pedunculatum* showed significant increase in haemoglobin

and RBC levels compared to the negative control. A significant decrease in WBC levels was observed by the 100 mg/kg methanol extract relative to the control. All doses of the ethanol and ethyl acetate *Crinum pedunculatum* extract also showed a decrease in WBC levels, this was however, not significant. Specific details are recorded in Table 2.

3.4. Effects of *Crinum pedunculatum* bulb extracts on inflammatory and arthritis markers

Serum concentrations of rheumatoid factor were significantly decreased in rats administered with ethanol and ethyl acetate *Crinum pedunculatum* extracts compared to the control. The ethanol extract showed a dose-dependent decrease in rheumatoid factor concentrations, a decrease was also observed with the methanol *Crinum pedunculatum* extract, this was however not significant. Serum levels of interleukin-6 were significantly decreased in rats administered with 400 mg/kg ethyl acetate *Crinum pedunculatum* extract ($P < 0.05$) relative to the negative control (Table 3).

Table 2. Hematological parameters in CFA induced arthritis in rats.

Treatment groups	Dose (mg/kg)	RBC (10 ⁶ /μL)	Hb (g/dL)	WBC (10 ³ /μL)	PLT (10 ³ /μL)
Normal saline	10	6.61 \pm 0.44	11.16 \pm 0.66	14.82 \pm 0.54	977.4 \pm 37.72
Diclofenac	10	7.09 \pm 0.00	12.20 \pm 0.00**	7.50 \pm 0.00**	837.0 \pm 0.00
MCP	100	7.94 \pm 0.07*	13.86 \pm 0.20**	9.68 \pm 1.83*	1166.0 \pm 24.71
	200	8.69 \pm 0.28***	14.16 \pm 0.56***	11.20 \pm 0.85	657.4 \pm 110.9*
	400	8.46 \pm 0.43**	13.72 \pm 0.42**	13.02 \pm 1.43	951.2 \pm 96.81
ECP	100	7.63 \pm 0.34	13.74 \pm 0.53**	11.52 \pm 1.21	876.2 \pm 42.26
	200	7.64 \pm 0.24	13.32 \pm 0.36*	10.92 \pm 0.92	977.8 \pm 89.50
	400	7.60 \pm 0.05	13.46 \pm 0.22**	12.48 \pm 2.06	888.4 \pm 69.25
EACP	100	7.73 \pm 0.44	12.94 \pm 0.31	10.80 \pm 1.25	988.8 \pm 65.89
	200	7.27 \pm 0.51	12.62 \pm 0.79	12.56 \pm 2.00	975.2 \pm 61.19
	400	7.23 \pm 0.50	12.36 \pm 0.69	10.84 \pm 2.11	1040.0 \pm 52.46

Results are expressed as mean \pm SEM (n=5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MCP-methanol *Crinum pedunculatum* extract, ECP-ethanol *Crinum pedunculatum* extract, EACP-ethyl acetate extract of *Crinum pedunculatum*.

Table 3. The effect of *Crinum pedunculatum* on inflammatory and arthritis marker in CFA-induced rats.

Treatment groups	Dose (mg/kg)	Rheumatoid factor (ng/ml)	Interleukin-6 (ng/ml)
Normal saline	10	1.668 ± 0.13	0.09 ± 0.02
Diclofenac	10	1.020 ± 0.05*	0.00 ± 0.00**
MCP	100	0.980 ± 0.04	0.00 ± 0.00
	200	1.480 ± 0.48	0.20 ± 0.20
	400	1.230 ± 0.23	0.07 ± 0.03
ECP	100	0.960 ± 0.04*	0.05 ± 0.05
	200	0.770 ± 0.04**	0.07 ± 0.00
	400	0.670 ± 0.16**	0.18 ± 0.10
EACP	100	1.140 ± 0.06*	0.05 ± 0.001
	200	0.740 ± 0.01**	0.05 ± 0.004
	400	0.910 ± 0.11**	0.04 ± 0.01*

Results are expressed as mean±SEM. * $P < 0.05$, ** $P < 0.01$ relative to the negative control. MCP-methanol *Crinum pedunculatum* extract, ECP-ethanol *Crinum pedunculatum* extract, EACP-ethyl acetate extract of *Crinum pedunculatum*.

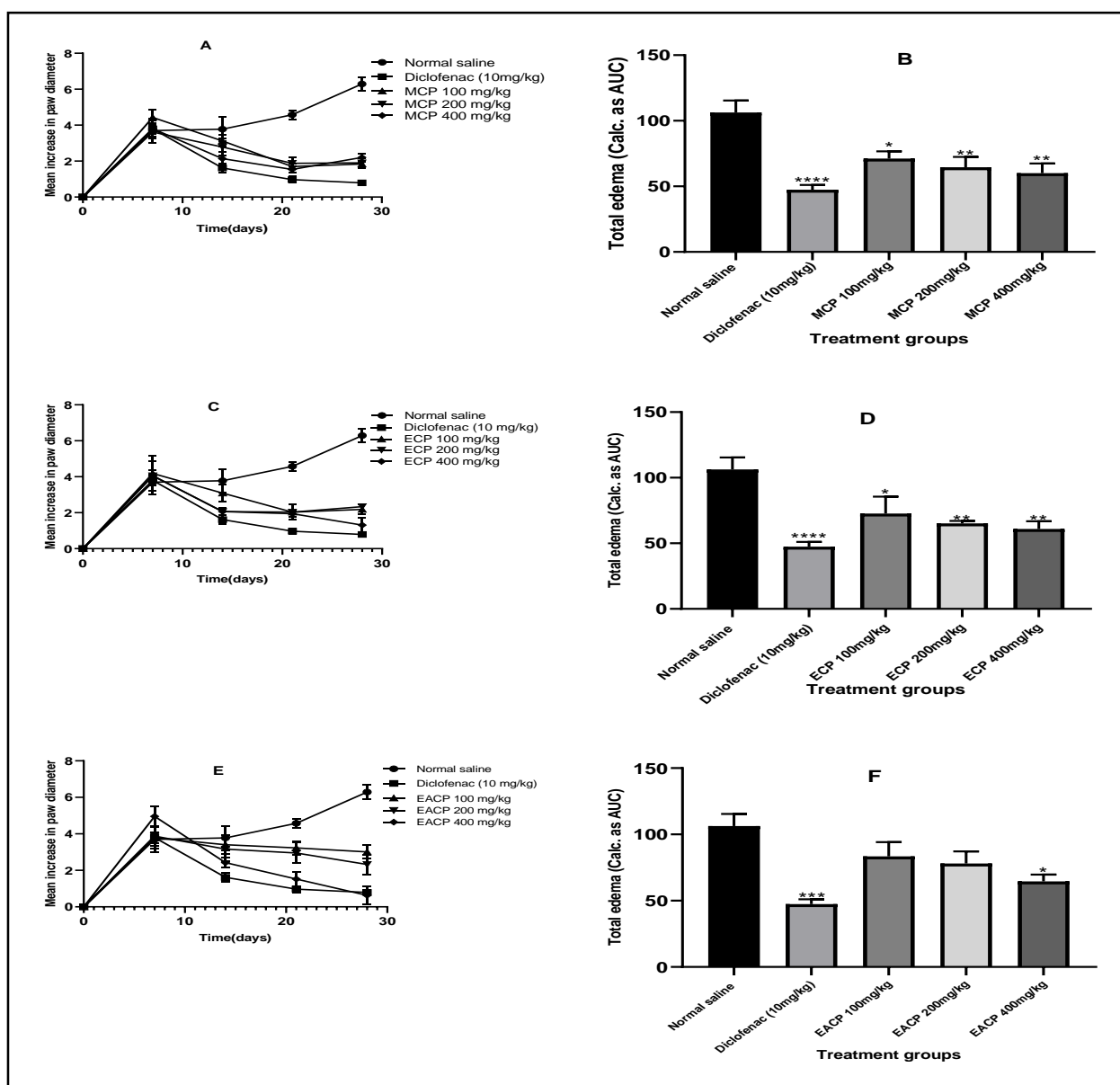


Figure 1. *Crinum pedunculatum* inhibits the hind paw swelling in CFA-induced arthritic animals. Results are expressed as mean±SEM. A, C, and E- Mean increase in paw diameter by methanol, ethanol, and ethyl acetate *Crinum pedunculatum* extract respectively. B, D, and F- Total oedema (calculated as AUC) observed by methanol, ethanol, and ethyl acetate *Crinum pedunculatum* extract respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ relative to the negative control (normal saline).



Figure 2. The effect of *Crinum pedunculatum* on the radiology of the right hind paw of animals. Key: 1B- Normal saline; 1G-Diclofenac (10 mg/kg); 4B- Methanol 100 mg/kg; 3G- Methanol 200 mg/kg; 4G- Methanol 400 mg/kg; 2R- Ethanol 100 mg/kg, 3R- Ethanol 200 mg/kg; 2B- Ethanol 400 mg/kg; 2B1G- Ethyl acetate 100 mg/kg; 3R1G- Ethyl acetate 200 mg/kg; 2B2G- Ethyl acetate 400 mg/kg.

3.5. Radiological modifications by *Crinum pedunculatum* on the paw of CFA-induced rats

Radiographical evaluations of the right hind paw of the animals in the control group showed significant soft tissue oedema with narrowing of joint spaces which are indicative of bone destruction associated with rheumatoid arthritis. Methanol, ethanol and ethyl acetate *Crinum pedunculatum* extracts decreased joint and paw (soft tissue) swelling as well as reduce joint narrowing relative to the negative control group (Figure 2).

3.6. Histopathological changes by *Crinum pedunculatum* extracts on the right hind paw of CFA-induced rats

For investigation of the histopathological modifications observed of the right hind paw of rats treated with *Crinum pedunculatum* extracts showed reduced infiltration of inflammatory cells, presence of osteoblasts, preserved cartilage and bone architecture. On the other hand, rats in the control group showed complete bone and cartilage destruction. There is evidence of acute inflammation and extensive abscess in the bone parenchyma (normal saline). This suggests that the administration of *Crinum pedunculatum* could suppress the damage to the cartilage and the bone and also reduce the progression of disease caused by the administration of CFA.

3.7. Histopathological changes by *Crinum pedunculatum* extracts on the contralateral paw of CFA-induced rats

Histopathological evaluations of the left hind paw (contralateral paw) of rats treated with different solvent extracts of *Crinum pedunculatum* showed presence of normal cortical bone architecture (ECP, MCP, EACP 100 mg/kg); preserved cartilage (MCP 200 mg/kg, EACP 200 mg/kg, MCP 400 mg/kg); absence of infiltration of inflammatory cells (MCP 400 mg/kg); preserved cartilage

(EACP 400 mg/kg).

4. DISCUSSION

Rheumatoid arthritis is a chronic condition that affects a patient's quality of life and there is still no treatment for this debilitating condition. Traditional healers in the Eastern and Southern region of Ghana have employed *Crinum pedunculatum* plant for the management of chronic inflammatory conditions. Therefore, this study investigated the anti-arthritis activity of different solvent extracts of the bulb of *Crinum pedunculatum* R.Br. in rats to substantiate its traditional use. CFA-induced arthritis is an appropriate model for evaluating anti-arthritis activity because it has been hypothesized to happen through cell-mediated immunity and also as a result of its structural mimicry observed with cartilage peptidoglycans and mycobacterium in rats¹⁸⁻²⁰. This effect was observed when animals administered with only CFA showed highest paw oedema (Figure 1), highest RF values (Table 3), as well as complete bone and cartilage destruction (Figure 3). The results from this study shows that *Crinum pedunculatum* was able to offer protection in rats against arthritis induced by the administration of CFA. Paw diameter was significantly decreased dose dependently in rats administered with methanol, ethanol and ethyl acetate extract of *Crinum pedunculatum*. A consistent increase in paw diameter was observed by the animals in the control group throughout the duration of the experiment which is consistent with previous studies²¹⁻²². This indicates the potential of *Crinum pedunculatum* to reduce oedema associated with acute and chronic inflammation which is consistent with previous studies on other *Crinum* species²³. CFA is reported to cause chronic inflammation through up-regulated migration of macrophages and lymphocytes as well as the overproduction of inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α)²⁴⁻²⁷ which are responsible for the damage

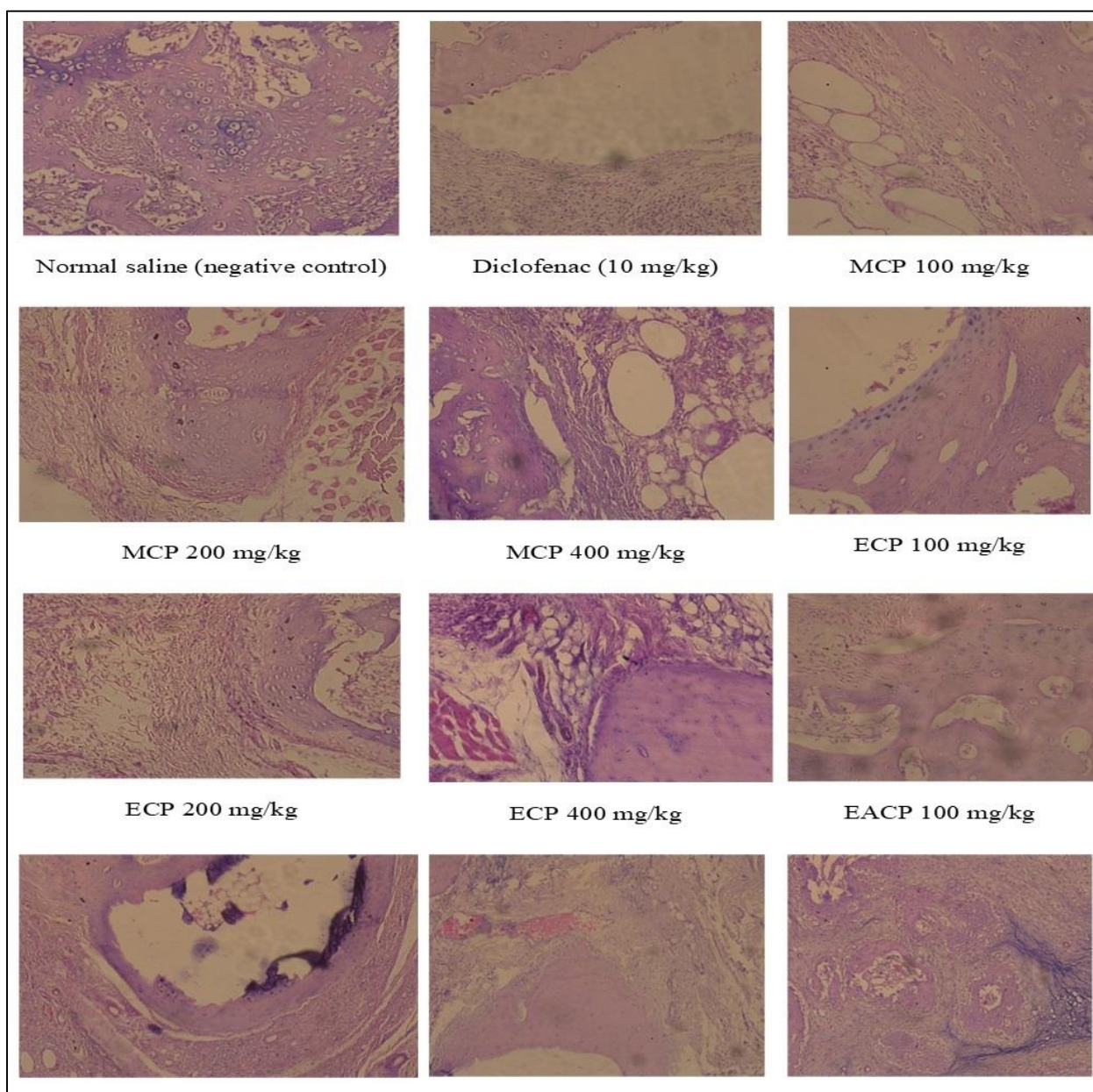


Figure 3. Modulating effect of *Crinum pedunculatum* on the right hind paw of CFA-induced rats. Tissue sections of the right hind paw were stained with H&E. MCP- methanol *Crinum pedunculatum* extract, ECP- ethanol *Crinum pedunculatum* extract, and EACP- ethyl acetate extract of *Crinum pedunculatum*. Original magnification 20x.

to tissue and cartilage that results in arthritis²⁸. *Crinum pedunculatum* extract decreased the infiltration of inflammatory cells (IL-6) and mediators which indicates its ability to reverse the arthritis induced by the administration of CFA. Studies have suggested that the presence of rheumatoid factor in patients with arthritis increases the potential for severe disability and also causes a delay in remission²⁹⁻³⁰. The ethanol and ethyl acetate *Crinum pedunculatum* extracts at all doses caused a significant decrease in rheumatoid factor which could indicate its potential for inhibiting severe disease progressions and bone degradation.

Arthritis is usually linked to anemia caused by the deformation of red blood cells and decreased levels of erythropoietin this results in decreased haemoglobin

levels³¹. It has also been reported that an increase in WBC concentration during induction of arthritis is associated with an increase in inflammatory cytokines^{3,32}. *Crinum pedunculatum* solvent extracts caused significant increase in haemoglobin and RBC levels and also decreased WBC levels relative to the control. Histopathological analysis also show that *Crinum pedunculatum* was able to ameliorate the damage of the cartilage and the bone on both ipsilateral and contralateral rat paw compared to the negative control (Figures 3 and 4). Preliminary phytochemical analysis on the ethanol, methanol and ethyl acetate extracts of *Crinum pedunculatum* revealed the presence of saponins, alkaloids, tannins among others (Table 1). Flavonoids, alkaloids and tannins are some of the phytoconstituents that may be responsible for the

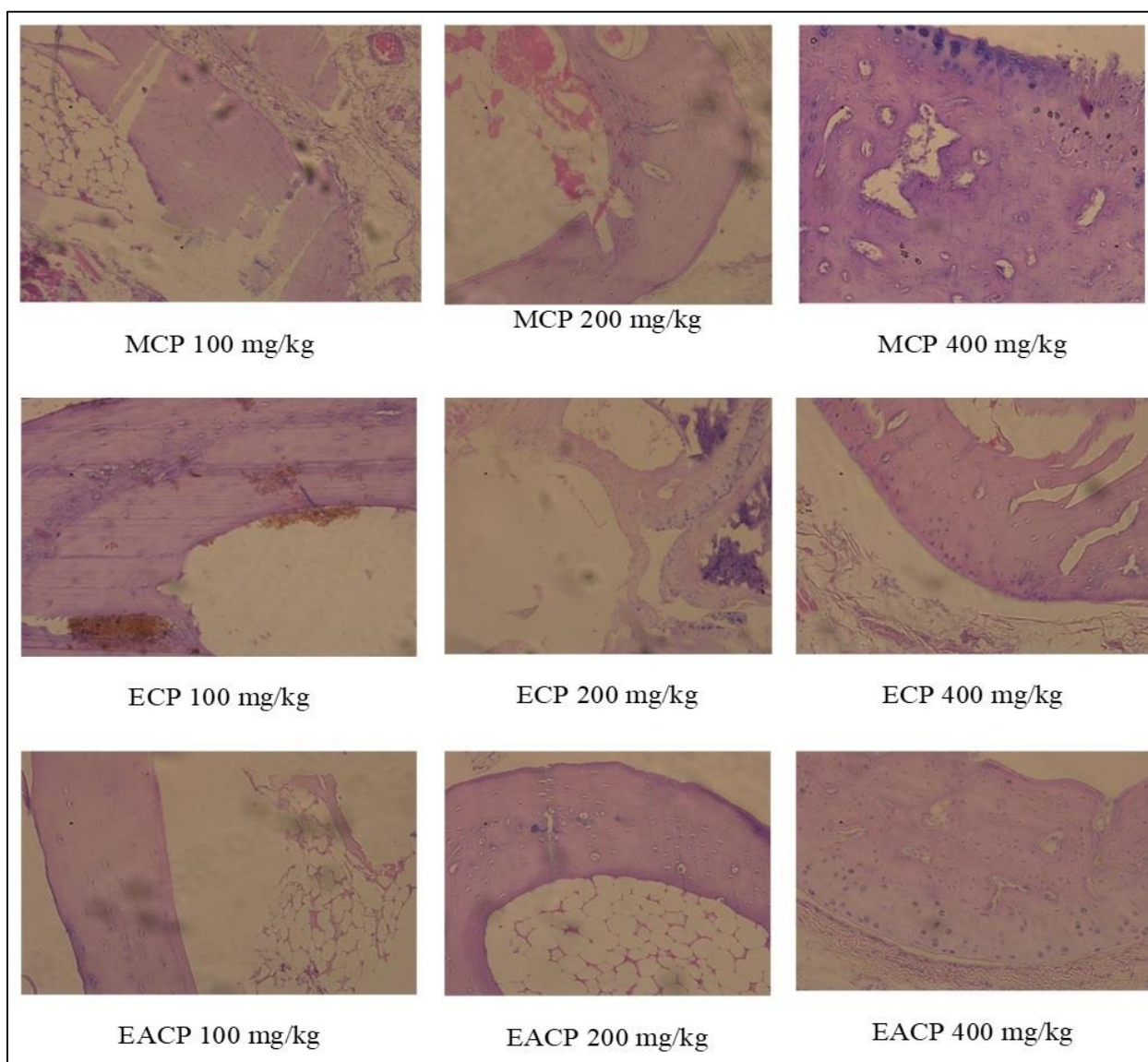


Figure 4. The effect of *Crinum pedunculatum* on the contralateral hind paw of CFA-induced rats. MCP- methanol *Crinum pedunculatum* extract, ECP- ethanol *Crinum pedunculatum* extract, EACP- ethyl acetate extract of *Crinum pedunculatum*.

observed anti-arthritic activity. Studies have reported the potential of flavonoids to inhibit some of the mediators of inflammation such as nuclear factor-kappa B, phospholipase A₂ and C³³.

The results obtained from this study indicate the anti-arthritic activity of the methanol, ethanol and ethyl acetate extract of *Crinum pedunculatum* in CFA-induced rats. The possible mechanisms for this activity could be due to the inhibition of inflammation and the suppression of infiltration by inflammatory cells. However, further studies are required to determine the exact mechanism of anti-arthritic activity.

5. CONCLUSION

This study provides pharmacological support for *Crinum pedunculatum*'s claimed traditional use in the management of chronic inflammation. Further research is needed to understand the specific mechanism of action

of *Crinum pedunculatum* for the treatment of arthritis and studies are ongoing to isolate compounds from this plant to confirm its bioactivity.

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Conflict of interest

None to declare.

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None to declare.

Ethics approval

None to declare.

Author contribution

PD and CD: study conceptualization and design. PD, CD, KO, MO, SA, YA: methodology. PO- methodology and interpretation of histopathological findings. PD, CD, KO, MO, SA, YA: article structuring and writing. PD, CAD, YA- data analysis and interpretation. CAD and KAO: revision and supervision. All authors have read and approved the manuscript for publication.

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