RESEARCH PAPER

Unveiling the Anti-Arthritic Promise of *Zanthoxylum armatum*: Integrated In-Vitro and In-Vivo Evaluation in FCA-Induced Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that is accompanied by progressive joint destruction, pain and functional disability. The main objective of the present study was to examine the anti-arthritic activity of aqueous (ZAAQ) and ethanolic (ZAE) extract of *Zanthoxylum armatum* in both in-vitro and in-vivo experimental models. Preliminary phytochemical screening of the extracts showed the presence of alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and glycosides, which are known to possess anti-inflammatory properties. The protein denaturation and human red blood cell (HRBC) membrane stabilization assays were used to assess in-vitro anti-arthritic activity at concentration of (50-2000µg/mL). Strong membrane stabilization and protein denaturation inhibition effects were obtained by both extracts in a concentration-dependent manner, similar to diclofenac sodium. The in-vivo evaluation was performed on Wistar rats with the induction of rheumatoid arthritis (RA) using Freund's Complete Adjuvant (FCA). The extracts were orally given after 28 days at doses of 200, 400 and 600 mg/kg. The decrease in paw edema and joint diameter produced by treatment with ZAAQ and ZAE compared to the disease control group was significant and the increase in body weight in arthritic rats was also significantly improved. The therapeutic activity of the higher doses of both extracts was higher, with aqueous extract having comparatively higher activity. The result indicated that *Zanthoxylum armatum* has significant anti-inflammatory and anti-arthritic property which may be attributed to the combination effect of various phytoconstituents present in the plant. Hence, the plant can be a promising natural therapeutic agent for the control of RA.

Keywords: Rheumatoid arthritis, *Zanthoxylum armatum*, Anti-arthritic activity, FCA-induced arthritis, HRBC membrane stabilization, Protein denaturation assay, Phytochemicals, Diclofenac sodium

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1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by persistent inflammation that predominantly targets synovial joints. It is considered one of the most common inflammatory arthropathies worldwide and is esteemed to affect approximately 0.5-1% of the adult population across different regions, leading to significant joint damage and functional disability if left untreated [1]. The disease is marked by chronic synovial inflammation, gradual degradation of articular cartilage, focal bone erosions, and a range of extra-articular features that may involve the cardiovascular, pulmonary, and haematological systems, leading to multisystem morbidity beyond the joints [2]. The underlying pathophysiology is driven by a dysregulated immune response in which autoreactive T and B lymphocytes, activated macrophages, and synovial fibroblasts coordinate the release of multiple pro-inflammatory cytokines-especially tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1ß). This cytokine cascade promotes synovial lining hyperplasia, pannus formation, and progressive destruction of articular structures within the joint [3].

Contemporary pharmacological management of rheumatoid arthritis comprises non-steroidal anti-inflammatory drugs (NSAIDs), conventional disease-modifying antirheumatic drugs (DMARDs), corticosteroids, and biologic agents. Although these treatments offer symptomatic improvement and help retard disease progression, they are linked with substantial adverse effects such as gastrointestinal toxicity, hepatotoxicity, and immunosuppression, along with high treatment costs that pose particular challenges in low- and middle-income countries [4,5]. This unmet therapeutic need has fostered growing interest in natural product-derived medicines as potentially safer and more affordable alternative treatment options.

Ethnopharmacological approaches have led to the identification of several clinically validated anti-arthritic agents, such as salicylate-type compounds derived from *Salix alba*, colchicine

from *Colchicum autumnale*, and preparations of *Tripterygium wilfordii*, all of which have demonstrated therapeutic potential in inflammatory joint disorders [6]. The Himalayan and sub-Himalayan ethnobotanical traditions of India, Nepal, and Bhutan constitute an underutilized reservoir of medicinal plants, many of which have documented uses in managing musculoskeletal and arthritic conditions.

Zanthoxylum armatum DC. (Rutaceae), commonly referred to as Timur in Nepali and Hindi, Tejphal in Sanskrit, or Toothache Tree, is a spiny deciduous shrub or small tree distributed across the Himalayan and sub-Himalayan foothills, including Garhwal, Kumaon, Himachal Pradesh, Sikkim, and parts of Southeast Asia [7]. In traditional Ayurvedic and folk medicine, multiple plant parts—such as the fruits, bark, seeds, and leaves—have been used to manage fever, toothache, dyspepsia, intestinal worms, and various rheumatic and inflammatory disorders [8]. In Uttarakhand and Himachal Pradesh, traditional practitioners apply the fruit paste topically to alleviate joint pain and administer decoctions prepared from the bark for the management of arthritis.

Phytochemically, Z. armatum is characterized by a diverse array of secondary metabolites, including lignans (such as sesamin and asarinin), alkaloids (for example berberine, chelerythrine, and magnoflorine), terpenoids (including linalool, limonene, and sabinene), as well as polyphenolic compounds [9,10]. Preclinical evidence indicates that sesamin and chelerythrine exhibit cyclooxygenase (COX)-inhibiting effects and are capable of suppressing NF-κB signaling, both of which contribute to their anti-inflammatory and potentially anti-arthritic activity [11,12]. However, a comprehensive in vivo evaluation of the anti-arthritic potential of Z. armatum extract in a standardized experimental model, along with mechanistic analysis at the cytokine level, has not yet been adequately reported in the available literature.

In light of these gaps, the present study was designed to: (i) prepare and phytochemically characterize aqueous (ZAAQ) and ethanolic (ZAE) extracts derived from the fruits and bark of Z. armatum; (ii) assess their in vitro anti-inflammatory and antioxidant activities; and (iii) systematically evaluate anti-arthritic efficacy in Freund's complete adjuvant (FCA)-induced arthritic Wistar rats, with detailed assessment of inflammatory, hematological, biochemical, radiological, and histopathological parameters.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The leaves powder of Zanthoxylum *armatum* (Timur) was procured from Herbal Creations Village- Nayagaon, Distt. Nainital, Uttarakhand, India (Certificate of Analysis (COA) ID-HC/HE/F/06/2025/6130). A quantity of 1000 g of coarsely powdered leaves was used for extraction by the cold maceration method. The powder was divided into two equal portions and extracted separately with different solvents. For the aqueous extract (LZAAQ), the drug material was macerated with distilled water, whereas for the ethanolic extract (LZAE), it was macerated with ethanol. After two weeks, each mixture was filtered sequentially through a cotton bed and Whatman filter paper No. 1, and the filtrates were concentrated to dryness using a rotary evaporator maintained at 40°C under reduced pressure. The resulting extracts were subsequently evaluated for both in vitro and in vivo pharmacological activities.

2.2 Preparation of Aqueous Extract and Ethanolic Extract

The coarsely powdered plant material (500 g) was defatted with petroleum ether (60–80°C) for 48 h using a Soxhlet apparatus to remove non-polar, lipophilic components. The defatted marc was then air-dried and successively extracted with 70% ethanol (hydroalcoholic solvent, 1:10 w/v) by cold maceration for 72 h, with periodic shaking. The resulting extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary vacuum evaporator (Buchi R-300) at 45°C, and finally lyophilized to obtain a dry powder. The percentage yield was calculated, and the dried extract was stored at 4°C in amber vials pending further use [13].

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening of ZAAQ and ZAE was carried out using standard methods outlined by Harborne (1998) and Trease & Evans (2002) to detect alkaloids (with Dragendorff's and Hager's test), flavonoids (alkaline reagent test), tannins (ferric chloride test, alkaline reagent test), saponins (foam test), sterols (Salkowski and Liebermann–Burchard tests), as well as terpenoids, phenols, glycosides, and carbohydrates [14,15].

2.4 Organisms and Experimental Animals

Wistar rats (200–250 g) of both sexes were procured from the animal house facility of the Department of Pharmacology, KIET School of Pharmacy (KSOP), Ghaziabad. The animals were housed under standard laboratory conditions at 24–25 ± 2°C with a 12-hour light/dark cycle in polypropylene cages and provided ad libitum access to standard food pellets (Pranave Agro Industries Ltd, New Delhi) and drinking water. The study was initiated after obtaining approval (Reg. No. ……..) from the Institutional Animal Ethics Committee and was conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.5 Chemicals

The following chemicals and reagents were used in the study: Zanthoxylum *armatum* leaves powder, Freund's complete adjuvant (FCA), Fehling's solution, Mayer's reagent, sodium chloride, concentrated hydrochloric acid (HCl), sodium dihydrogen phosphate, disodium hydrogen phosphate, probe cleaner, pro-inflammatory cytokine ELISA kit, anti-collagen II IgG ELISA kit, and C-reactive protein assay kit.

2.6 In-Vitro Studies

2.6.1 Egg Albumin Denaturation Method

For the protein denaturation assay, 20 mL of either the aqueous or hydroalcoholic plant extract (prepared separately in distilled water) was mixed with 20 mL of phosphate-buffered saline (PBS, pH 6.4) and 20 mL of egg albumin to yield a reaction mixture of 50 mL. The egg albumin and PBS were kept constant across all samples; in the negative control, the extract was replaced with 20 mL of distilled water, while in the positive control it was replaced with 20 mL of diclofenac. The mixtures were incubated at 37±2°C for 15 minutes and then heated at 70°C for 5 minutes, cooled, and used to prepare three reaction systems—test, standard, and control—each at concentrations of 50, 100, 200, 400, 800, 1000, and 2000 µg/mL. Absorbance of the reaction mixtures was recorded at 680 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). The percentage inhibition of protein denaturation (%) was calculated according to the formula:

% inhibition = 100 × [(Vt / Vc) − 1], where

Vt denotes the absorbance of the test sample and Vc that of the control [16].

2.6.2 Membrane Stabilization Assay (HRBC Method)

The anti-arthritic potential of Zanthoxylum *armatum* was evaluated using a human red blood cell (HRBC) membrane stabilization assay. Blood was collected from a healthy human volunteer who had refrained from using any NSAIDs for at least 14 days prior to the experiment. A 10% w/v erythrocyte suspension was prepared by centrifuging the freshly drawn blood at 3,000 rpm for 15 minutes without anticoagulants; the supernatant layer was carefully discarded and the tightly packed RBC pellet was washed and resuspended in isotonic saline to obtain a 10% w/v suspension.

Various concentrations of aqueous and ethanolic extracts of Zanthoxylum *armatum* (50, 100, 200, 400, 800, 1000, and 2000 µg/mL) were prepared alongside diclofenac sodium as the reference standard. The test reaction mixture was prepared by combining 1 mL of phosphate buffer, 0.5 mL of 10% w/v HRBC suspension, 2 mL of isotonic saline, and 0.5 mL of the respective drug extract. The mixture was incubated at 37 ± 2°C in a BOD incubator for 25–30 minutes and then centrifuged at 3,000 rpm for 20 minutes. The standard reaction mixture was prepared similarly, replacing the plant extract with diclofenac sodium at equivalent concentrations, whereas the control mixture contained 1 mL of phosphate buffer, 2 mL of distilled water, and 0.5 mL of 10% w/v HRBC in isotonic saline. After centrifugation, the supernatant was collected and the released haemoglobin was quantified at 560 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800).

% inhibition of haemolysis = 100 X (OD1OD2/OD1)

where,

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution [17].

2.7 In-Vivo Studies

2.7.1 Induction of Rheumatoid Arthritis (FCA-Induced-Arthritis)

Freund's complete adjuvant (FCA)-induced arthritis is a well-established chronic immunological model of arthritis in experimental animals. Before induction, baseline parameters such as joint diameter, paw volume, and body weight were recorded on day 0. Arthritis was induced by a 0.1 mL sub-plantar injection of FCA into the left hind-paw. Following induction, the treatment regimen was continued for 28 days, with daily oral dosing administered via oral gavage using an 18G gavage needle in rats.

2.7.2 Experimental Design

A total of 54 healthy Wistar albino rats, weighing 200–250 g, were randomly divided into nine groups comprising six animals each. The test extracts were administered orally at three different dose levels (200, 400, and 600 mg/kg), while the control group received saline solution administered via oral gavage.

Groups	Rats/Group	Dose	Route
I	6	0.9 % Normal Saline	Oral
II	6	CFA 0.1ml + 0.9% Normal Saline	
III	6	CFA+[Diclofenac]10mg/kg	
IV	6	CFA+ LZAAQ 200 mg/kg	
V	6	CFA+ LZAAQ 400 mg/kg	
VI	6	CFA+ LZAAQ 600 mg/kg	
VII	6	CFA+ LZAE 200 mg/kg	
VIII	6	CFA+ LZAE 200 mg/kg	
IX	6	CFA+ LZAE 200 mg/kg	

CFA: Complete Freund's adjuvant, LZAAQ: Aqueous root extract of *Z. armatum*, LZAE: Ethanolic extract of *Z. armatum*.

2.8 Assessment Parameters

2.8.1 Physical Parameters

Paw Volume

Following FCA administration up to day 28, hind-paw volume was measured once weekly using a digital plethysmometer. The change in paw volume was calculated as the difference between the final and initial values for each paw. Measurements were recorded on days 0, 7, 14, 21, and 28 to assess the progression of arthritis-related edema.

Joint Diameter

The joint diameter was evaluated by placing a Vernier caliper around the joint and tightening it until the animal showed signs of leg withdrawl.

Body Weight

The animals were weighed carefully on days 0, 7, 14, 21, and 28 using a digital weighing balance to monitor changes in body weight throughout the experimental period.

2.8.2 Radiographic Analysis

Radiographic examination of the rats was performed under sedation or general anaesthesia to ensure immobility and image quality. The anesthetized animals were carefully positioned on the X-ray table in the appropriate orientation for the desired views. Appropriate lead shielding was used to limit radiation exposure to non-target body regions. After optimizing the X-ray parameters, images were acquired and subsequently reviewed for diagnostic evaluation. Throughout the procedure, the welfare, safety, and physiological stability of the animals were prioritized in accordance with established imaging and animal-care guidelines.

2.8.3 Haematological Parameters

Haematological analysis of rat blood samples was carried out using an automated haematological analyser. Prior to analysis, the instrument probe was cleaned with a suitable probe cleaner to remove any residual material. The blood sample was then introduced at the nozzle of the analyser and the start button was pressed; the machine automatically processed the sample and generated a complete haematological profile along with a corresponding histogram, which was printed for documentation and evaluation.

2.8.4 Behavioral Assessments

Actophotometer

Locomotor activity of arthritic control and treated rats was assessed using an Almicro digital actophotometer equipped with infrared light-sensitive photocells. Each animal was placed individually in the activity chamber, and motor activity was recorded over a 5-minute period. The instrument counted locomotor events whenever the rat interrupted an infrared beam falling on a photocell; if the animal remained immobile for 10–20 seconds, a mild electric shock (10–20 V) was applied to encourage movement and re-interception of the beam. The total counts per 5 minutes were taken as an index of locomotor performance.

3-Compartment Rota rods

In addition, locomotor activity and motor coordination were evaluated using a rotarod apparatus equipped with a rotating rod and a digital stopwatch. The rotational speed of the rod was maintained at 30 rpm, and each rat was placed on the rotating rod to assess balance and fall latency. The time each animal remained on the rod before falling was recorded as an index of motor performance and coordination.

2.9 Inflammatory Markers

After 28 days, blood samples were collected from rats under diethyl ether anesthesia into EDTA-coated tubes. The blood was centrifuged at 3000 rpm for 15 minutes and the separated serum was used for the biochemical estimation of pro-inflammatory markers, including IL-6, IL-1β, and TNF-α, as well as C-reactive protein (CRP) and anti-collagen type-II IgG antibodies.

2.10 Histopathological Evaluation

At the end of the study, animals were euthanized with an overdose of anesthesia, and paw tissues were harvested and fixed in 10% neutral buffered formalin for histological evaluation. The tissues were subsequently processed, sectioned at 5-µm thickness, and stained for microscopic examination. Histopathological assessment of the arthritic control group revealed marked signs of severe arthritis, including cartilage degradation and pronounced infiltration of inflammatory cells within the synovium and periarticular tissues.

2.11 Statistical Analysis

Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. All data are expressed as mean ± standard error of the mean (SEM), and graphs were generated using GraphPad Prism software, version 11.

3. RESULTS

3.1 *IN-VITRO* RESULTS

3.1.1 Protein denaturation method using egg albumin

In a protein denaturation inhibition assay, the standard drug diclofenac along with the aqueous and ethanolic leaf extracts of *Zanthoxylum armatum* demonstrated anti-inflammatory and anti-arthritic potential. Both extracts exhibited concentration-dependent anti-inflammatory effects, comparable to diclofenac, the reference drug. The observed inhibition of protein denaturation indicates that *Zanthoxylum armatum* extracts

possess anti-inflammatory activity. The in-vitro evaluation of these extracts in relation to rheumatoid arthritis-associated protein denaturation provides novel evidence supporting their potential as anti-inflammatory agents.

Concentration (µg/ml)	%Inhibition (Diclofenac)	%Inhibition (ZAAQ)	%Inhibition (ZAE)
50	48.5	40.8	35.2
100	55.4	48.9	42.6
200	63.2	52.4	50.3
400	72.5	62.66	59.8
800	81.4	75.9	68.2
1000	85.3	81.3	73.5
2000	92.8	88.1	79.6

Table 1 Effects of Diclofenac & Aqueous & Ethanolic leaf extracts of *Zanthoxylum armatum* on protein denaturation.

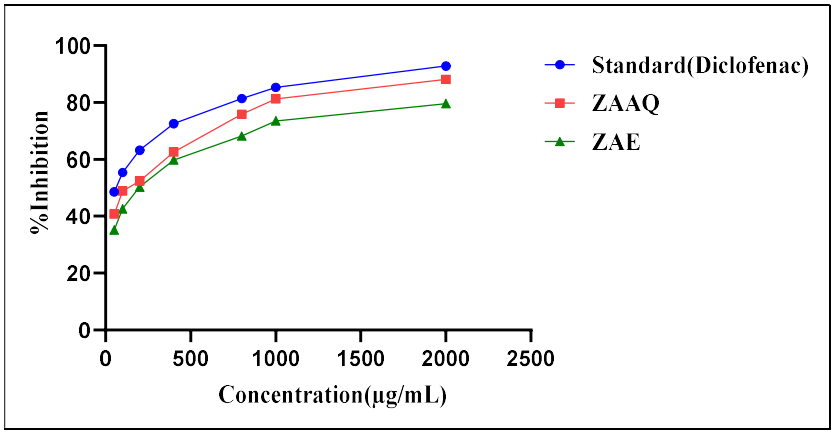

Fig. 1 % inhibition vs concentration plotted to evaluate the in-vitro anti-arthritic activity of Zanthoxylum armatum leaf extract and Diclofenac.

3.1.2 *In-Vitro* HRBC membrane stabilization results

Diclofenac and extracts of *Zanthoxylum armatum* showed anti-inflammatory properties as reflected by their ability to stabilize the human red blood cell (HRBC) membrane. In comparison with diclofenac, the extract exhibited a concentration-dependent anti-inflammatory effect. The in-vitro assessment of *Zanthoxylum armatum* extract in relation to rheumatoid arthritis, using the HRBC membrane stabilization test, provides novel insights into its potential anti-inflammatory and anti-arthritic activity.

Concentration (µg/ml)	% Haemolysis ZAAQ	% Protection ZAAQ	% Haemolysis (Diclofenac)	% Protection (Diclofenac)
50	38.5	61.5	35.2	64.82
100	34.7	65.3	31.4	68.6
200	30.2	69.8	27.3	72.7
400	25.8	74.2	22.8	77.2
800	21.6	78.4	18.5	81.5
1000	17.8	82.2	15.2	84.8
2000	12.6	87.4	10.4	89.6

Table 2 Effects of Aqueous leaf extract of *Zanthoxylum armatum* on HRBC membrane stabilization with standard Diclofenac.

Concentration (µg/ml)	% Haemolysis ZAE	% Protection ZAE	% Haemolysis (Diclofenac)	% Protection (Diclofenac)
50	42.8	57.2	35.2	64.82
100	39.5	60.5	31.4	68.6
200	35.8	64.2	27.3	72.7
400	31.6	68.4	22.8	77.2
800	27.2	72.8	18.5	81.5
1000	23.4	76.6	15.2	84.8

2000	18.1	81.9	10.4	89.6

Table 3 Effects of Ethanolic leaf extract of *Zanthoxylum armatum* on HRBC membrane stabilization with standard Diclofenac.

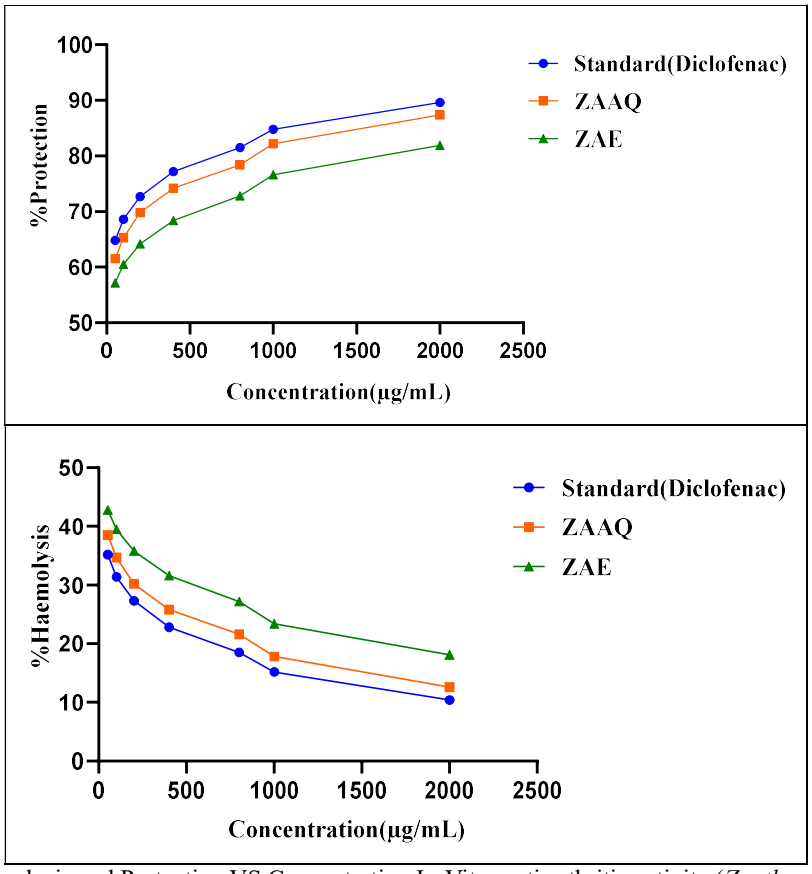

Fig. 2 % Haemolysis and Protection VS Concentration In-Vitro anti-arthritic activity (*Zanthoxylum armatum* leaf extract and Diclofenac) on HRBC membrane stabilization method.

3.2 *IN-VIVO* RESULTS

a) Effect of ZAAQ and ZAE extracts on body weight

The body weights of the rats were recorded on days 0, 7, 14, 21, and 28. From day 14 to day 28, the body weight of the arthritic rats increased in a dose-dependent and statistically significant manner ($P < 0.01$ and $P < 0.001$) compared with the disease control group.

Groups	Days				
	0	7	14	21	28
Normal	225.60± 0.82^{b}	224.90± 0.78^{b}	226.30± 0.86^{b}	225.90± 0.74^{b}	226.70± 0.80^{b}
Diseased	224.30± 1.88	205.80± 1.80	198.20± 1.85	191.00± 1.90	185.20± 1.72
Standard	226.00± 1.42^{a}	220.50± 1.50^{a}	229.80± 1.55^{a}	236.90± 1.60^{a}	244.80± 1.58^{a}
ZAAQL	225.20± 1.60^{a}	214.80± 1.72^{a}	218.60± 1.65^{a}	222.40± 1.58^{a}	227.10± 1.62^{a}
ZAAQM	225.80± 1.55^{a}	217.90± 1.60^{a}	222.80± 1.68^{a}	227.60± 1.64^{a}	233.20± 1.66^{a}
ZAAQH	226.20± 1.50^{a}	219.80± 1.58^{a}	226.40± 1.62^{a}	232.90± 1.70^{a}	239.60± 1.72^{a}

Table 4 A table comparing the effects of standard and ZAAQ treatments on changes in body weight in adjuvant injected rats compared to the arthritic disease control group (with statistical significance denoted as $^{b}p<0.01$ and $^{a}p<0.001$). The result expressed in mean ± SEM, with n=6.

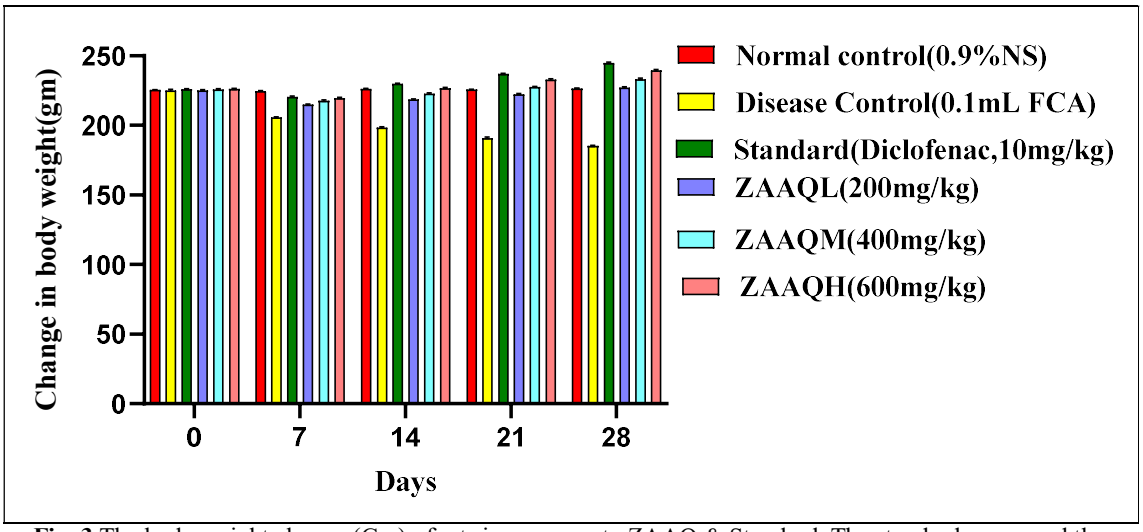

Fig. 3 The body weight change (Gm) of rats in response to ZAAQ & Standard. The standard group and the group receiving treatment were contrasted with the arthritic disease control group. For n = 6, the data was shown as mean ± SEM. Dunnett's comparison test was performed after the OneWay ANOVA analysis, producing a result between $^{b}p<0.01$ & $^{a}p<0.001$.

Groups	Days				
	0	7	14	21	28
Normal	225.60± 0.82^{b}	224.90± 0.78^{b}	226.30± 0.86^{b}	225.90± 0.74^{b}	226.70± 0.80^{b}
Diseased	224.30± 1.88	205.80± 1.80	198.20± 1.85	191.00± 1.90	185.20± 1.72
Standard	226.00± 1.42^{a}	220.50± 1.50^{a}	229.80± 1.55^{a}	236.90± 1.60^{a}	244.80± 1.68^{a}
ZAEL	225.10± 1.65^{a}	213.80± 1.75^{a}	216.90± 1.70^{a}	220.60± 1.62^{a}	224.80± 1.68^{a}
ZAEM	225.70± 1.60^{a}	216.20± 1.68^{a}	220.80± 1.72^{a}	225.30± 1.65^{a}	230.40± 1.70^{a}
ZAEH	226.10± 1.55^{a}	218.00± 1.65^{a}	223.60± 1.70^{a}	229.10± 1.68^{a}	234.60± 1.72^{a}

Table 5 A table comparing the effects of standard and ZAE treatments on changes in body weight in adjuvant injected rats compared to the arthritic disease control group (with statistical significance denoted as $^{b}p<0.01$ and $^{a}p<0.001$). The result expressed in mean ± SEM, with n=6.

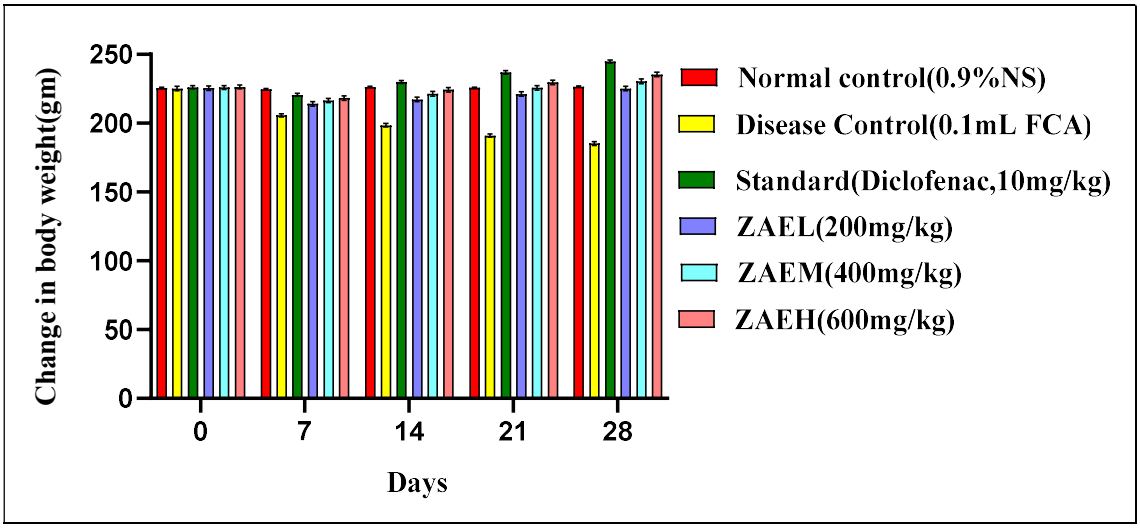

Fig. 4 The body weight change (Gm) of rats in response to PLE & Standard. The standard group and the group receiving treatment were contrasted with the arthritic disease control group. For n = 6, the data was shown as mean ± SEM. Dunnett's comparison test was performed after the OneWay ANOVA analysis, producing a result between $^{b}p<0.01$ & $^{a}p<0.001$.

b) Effects of ZAAQ and ZAE extracts on paw volume

Paw volume or degree of edema reduction is commonly used as an index for evaluating the anti-arthritic effect of a drug. Both ZAAQ and ZAE demonstrated a significant, dose-dependent reduction in paw swelling from day 14 to day 28 when compared with the standard treatment group (diclofenac) and the disease control (arthritic) group. The results are illustrated in (Fig. 6.28 and 6.29).

Groups	Days				
	0	7	14	21	28
Normal	0.78 ± 0.02[a]	0.79 ± 0.03[a]	0.80 ± 0.02[a]	0.79 ± 0.01[a]	0.80 ± 0.02[a]
Diseased	0.77 ± 0.03	2.35 ± 0.32	2.45 ± 0.28	2.30 ± 0.15	2.10 ± 0.12
Standard	0.79 ± 0.10[b]	1.30 ± 0.12[b]	1.20 ± 0.11[b]	1.00 ± 0.08[b]	0.85 ± 0.05[b]
ZAAQL	0.76 ± 0.03[c]	1.85 ± 0.20[c]	1.65 ± 0.18[c]	1.40 ± 0.12[c]	1.15 ± 0.10[c]
ZAAQM	0.77 ± 0.03[c]	1.65 ± 0.18[c]	1.40 ± 0.16[c]	1.15 ± 0.10[c]	0.95 ± 0.08[c]
ZAAQH	0.78 ± 0.02[c]	1.45 ± 0.15[c]	1.20 ± 0.12[c]	0.95 ± 0.08[c]	0.82 ± 0.06[c]

Table 6 Tabular comparison of standard and ZAAQ treatments on paw volume changes in adjuvant injected rats to the arthritic disease control group (between [a]p<0.001, [b]p<0.01, [c]p<0.5). The result expressed in mean ± SEM, with n=6.

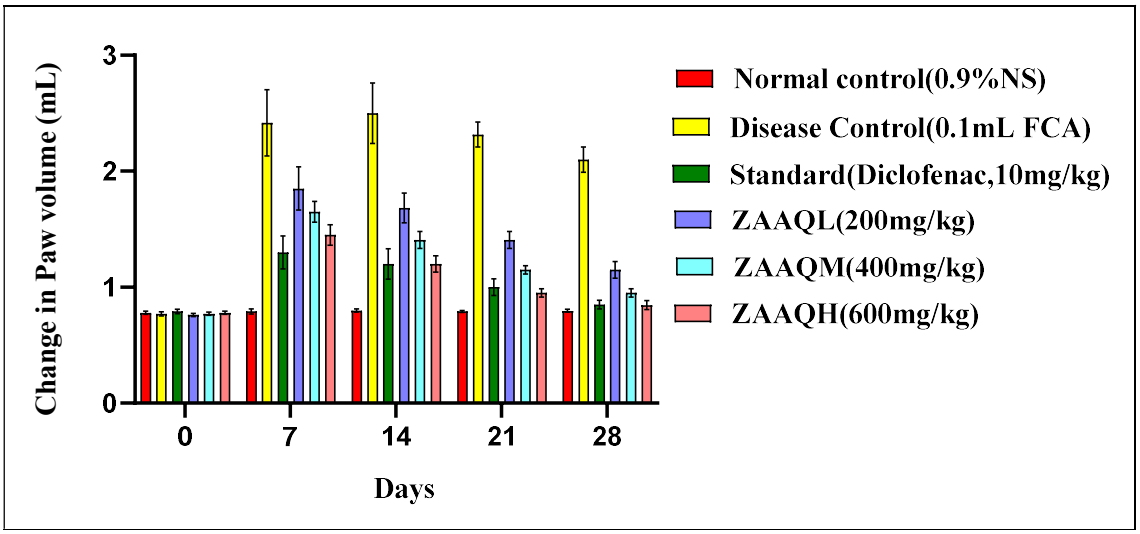

Fig. 5 Rats' Paw Volume varies (mL) according to ZAAQ & Standard. The standard group and the therapy group were contrasted with the arthritic disease control group. For n =6, the data was shown as mean ± SEM. Dunnett's comparison test was performed after the OneWay ANOVA analysis, and the results showed that [c]p<0.5, [b]p<0.01, and [a]p<0.001.

Groups	Days				
	0	7	14	21	28
Normal	0.78 ± 0.02[a]	0.79 ± 0.03[a]	0.80 ± 0.02[a]	0.79 ± 0.01[a]	0.80 ± 0.02[a]
Diseased	0.77 ± 0.03	2.35 ± 0.32	2.45 ± 0.28	2.30 ± 0.15	2.10 ± 0.12
Standard	0.79 ± 0.10[b]	1.30 ± 0.12[b]	1.20 ± 0.11[b]	1.00 ± 0.08[b]	0.85 ± 0.05[b]
ZAEL	0.76 ± 0.04[c]	1.95 ± 0.22[c]	1.75 ± 0.20[c]	1.55 ± 0.14[c]	1.30 ± 0.12[c]
ZAEM	0.77 ± 0.03[c]	1.75 ± 0.20[c]	1.55 ± 0.18[c]	1.30 ± 0.12[c]	1.10 ± 0.10[c]
ZAEH	0.78 ± 0.02[c]	1.60 ± 0.18[c]	1.40 ± 0.15[c]	1.15 ± 0.10[c]	0.98 ± 0.08[c]

Table 7 Tabular comparison of standard and ZAE treatments on paw volume changes in adjuvant injected rats to the arthritic disease control group (between [a]p<0.001 & [b]p<0.01). The result expressed in mean ± SEM, with n=6.

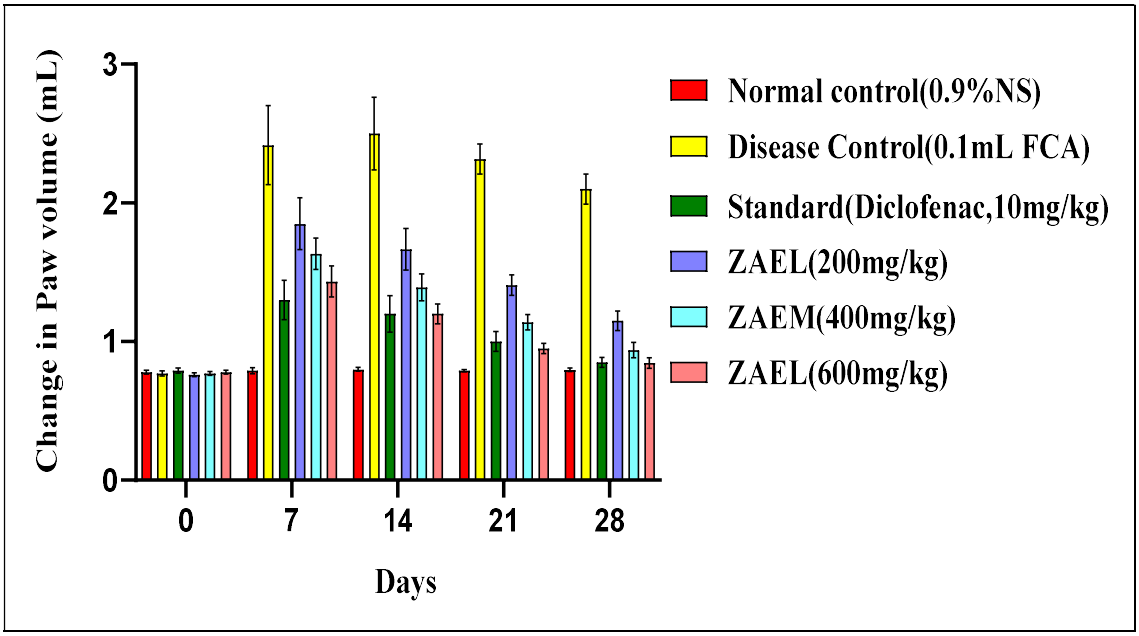

Fig. 6 The change in paw volume (mL) in response to Standard and ZAE treatment was compared between the arthritic disease control group, the standard group, and the treatment group. The data were presented as mean ± SEM for n=6. Following the One- Way ANOVA analysis, Dunnett's comparison test was conducted, revealing statistical significance denoted as $^{b}p<0.01$ and $^{a}p<0.001$.

c) Effects of ZAAQ and ZAE extracts on joint diameter

Administration of PLAQ and PLE at doses of 100, 200, and 400 mg/kg resulted in a significant reduction in joint diameter from day 14 to day 28 when compared with both the control and arthritic groups. The overall pattern of these findings is summarized in **(Fig. 6.30 and 6.31)**.

Groups	Days				
	0	7	14	21	28
Normal	0.62 ± 0.03[a]	0.61 ± 0.02[a]	0.62 ± 0.02[a]	0.61 ± 0.02[a]	0.63 ± 0.02[a]
Diseased	0.70 ± 0.04	0.95 ± 0.05	1.02 ± 0.06	0.98 ± 0.05	0.96 ± 0.04
Standard	0.63 ± 0.03[b]	0.80 ± 0.04[b]	0.75 ± 0.04[b]	0.70 ± 0.03[b]	0.65 ± 0.03[b]
ZAAQL	0.64 ± 0.03[c]	0.88 ± 0.05[c]	0.82 ± 0.05[c]	0.78 ± 0.04[c]	0.74 ± 0.04[c]
ZAAQM	0.63 ± 0.03[c]	0.85 ± 0.05[c]	0.78 ± 0.04[c]	0.73 ± 0.04[c]	0.70 ± 0.03[c]
ZAAQH	0.62 ± 0.02[c]	0.82 ± 0.04[c]	0.74 ± 0.04[c]	0.69 ± 0.03[c]	0.66 ± 0.03[c]

Table 8 Tabular comparison of standard and ZAAQ treatments on joint diameter changes in adjuvant injected rats to the arthritic disease control group ($^{c}p<0.5$). The result expressed in mean ± SEM, with n=6.

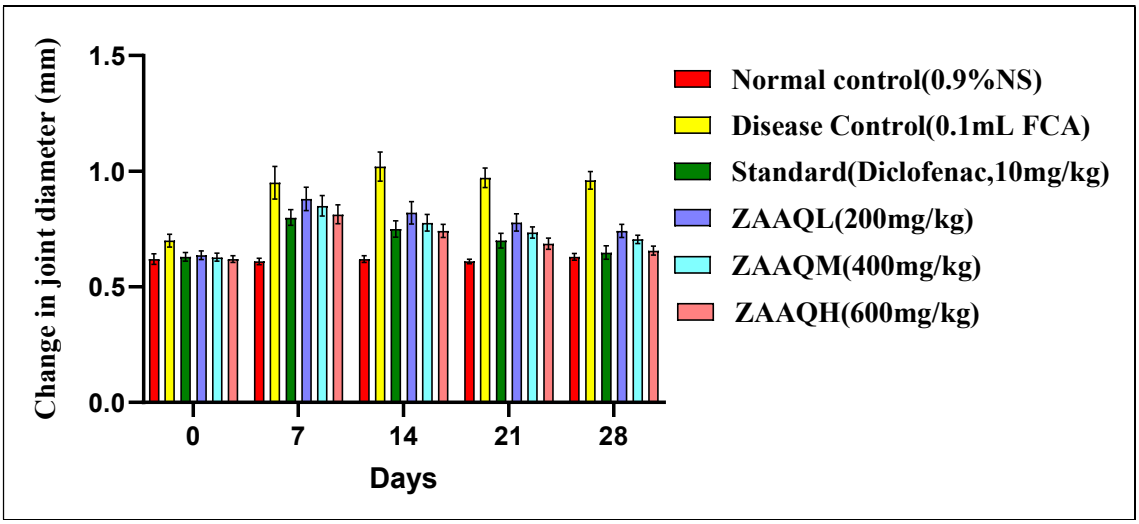

Fig. 7 Changes in rats' joint diameter (mm) in response to Standard and ZAAQ treatment were assessed by comparing the arthritic disease control group with the standard group and the treatment group. The data were presented as mean ± SEM for n = 6. After conducting the One-Way ANOVA analysis, Dunnett's comparison test was performed, revealing statistical significance indicated as $^{c}p < 0.05$.

Groups	Days				
	0	7	14	21	28
Normal	0.62 ± 0.03[a]	0.61 ± 0.02[a]	0.62 ± 0.02[a]	0.61 ± 0.02[a]	0.63 ± 0.02[a]
Diseased	0.70 ± 0.04	0.95 ± 0.05	1.02 ± 0.06	0.98 ± 0.05	0.96 ± 0.04
Standard	0.63 ± 0.03[b]	0.80 ± 0.04[b]	0.75 ± 0.04[b]	0.70 ± 0.03[b]	0.65 ± 0.03[b]
ZAEL	0.65 ± 0.03[c]	0.90 ± 0.05[c]	0.85 ± 0.05[c]	0.80 ± 0.04[c]	0.76 ± 0.04[c]
ZAEM	0.64 ± 0.03[c]	0.88 ± 0.05[c]	0.82 ± 0.05[c]	0.77 ± 0.04[c]	0.73 ± 0.04[c]
ZAEH	0.63 ± 0.02[c]	0.85 ± 0.04[c]	0.79 ± 0.04[c]	0.74 ± 0.03[c]	0.70 ± 0.03[c]

Table 9 Tabular comparison of standard and ZAE treatments on joint diameter changes in adjuvant injected rats to the arthritic disease control group ($^{b}p<0.01$ & $^{c}p<0.5$). The result expressed in mean ± SEM, with n=6.

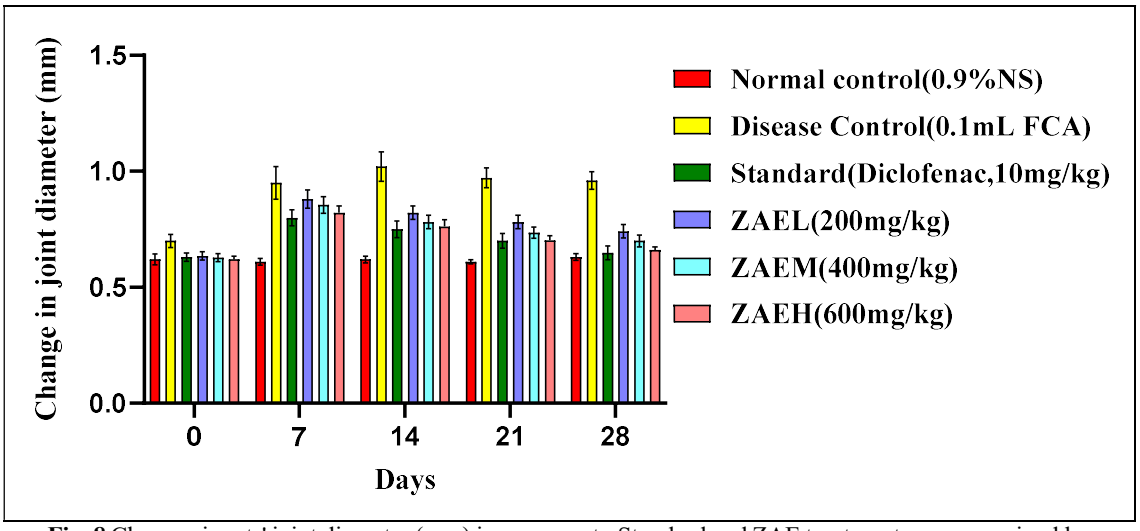

Fig. 8 Changes in rats' joint diameter (mm) in response to Standard and ZAE treatment were examined by comparing the arthritic disease control group with the standard group and the treatment group. The data were presented as mean ± SEM for n = 6. After conducting the One-Way ANOVA analysis, Dunnett's comparison test was performed, revealing statistical significance indicated as $^{c}p < 0.5$ and $^{b}p < 0.01$.

4. DISCUSSION

The current study showed that the aqueous extract (ZAAQ) and ethanolic extract (ZAE) of *Zanthoxylum armatum* exhibited considerable anti-arthritic activity in Freund's Complete Adjuvant (FCA)-elicited arthritis Wistar rats. RA is a continuous inflammatory disease that involves

synovial inflammation, cartilage destruction, oxidative stress and eventual joint deformity. The FCA model is a suitable immunological model of human rheumatoid arthritis with similar immunological and pathological changes and is used extensively to test anti-arthritic drugs. Phytochemical screening of both extracts indicated the presence of flavonoids, alkaloids, tannins, terpenoids, phenolic compounds and glycosides that are known to have anti-inflammatory and antioxidant properties. The possible therapeutic activity of the plant extracts could be attributed to these bioactive ingredients in combination. Sesamin, chelerythrine, and other polyphenolic compounds in Zanthoxylum armatum have been reported to inhibit inflammatory mediators such as NF-κB signaling pathways and cyclooxygenase (COX) enzymes, which helps to suppress inflammation. Both extracts showed concentration-dependent inhibition of protein denaturation in the egg albumin assay in the present investigation. Protein denaturation has been identified as one of the primary factors underlying production of autoantigens and inflammation in RA. Thus, the extracts' denaturation inhibition activities indicate protein stabilization and inflammation prevention. Likewise, the HRBC membrane stabilization assay showed that the erythrocyte membranes were well protected from hemolysis. The erythrocyte membrane is similar to lysosomal membrane and stabilization of HRBC membranes implies capability of the extracts to prevent the release of lysosomal enzymes and inflammatory mediators under arthritic conditions. In the in-vivo study, FCA administration resulted in significant arthritic changes such as reduction in body weight, swelling of paws and increase in joint diameter. The pathological changes were significantly reduced in a dose dependent manner by treatment with ZAAQ and ZAE. Loss of paw volume and swelling of its joints suggest reduction in inflammatory oedema and synovial hyperplasia. Enhanced body weight improvements also indicate better overall health and recovery from systemic inflammatory stress of the animals. The aqueous extract exhibited the most potent anti-arthritic activity when compared to the ethanolic extract, possibly due to the higher concentration of the polar bioactive compounds in the aqueous extract, among the extracts tested. The anti-arthritic activity observed may be related to the ability to inhibit pro-inflammatory cytokines (e.g. tumour necrosis factor α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6)), which are involved in the pathogenesis of rheumatoid arthritis. Furthermore, antioxidant phytoconstituents found in the plant can help lower oxidative stress and tissue damage that may occur due to chronic inflammation. The results of this study are also in line with the previous reports of medicinal plants with higher levels of flavonoids and phenolic compounds showing noteworthy anti-inflammatory and immunomodulatory properties. Overall, the results of the present study scientifically validate the traditional use of Zanthoxylum armatum in the management of inflammatory and rheumatic disorders. The study indicates that both aqueous and ethanolic extracts could exhibit excellent anti-arthritic activity, where aqueous extract exhibited comparatively high anti-arthritic activity. However, additional studies on the isolation of active constituents, molecular mechanistic studies, and clinical trials need to be conducted to demonstrate its safety, efficacy, and therapeutic potential in human RA.

5. CONCLUSION

The present study was successful in elucidating the potential of aqueous (ZAAQ) and ethanolic (ZAE) extracts of *Zanthoxylum armatum* to possess anti-arthritic effects with its in-vitro and in-vivo experimental models. The phytochemical analysis also revealed the presence of various bioactive compounds, such as flavonoids, alkaloids, tannins, terpenoids, phenolic compounds, and glycosides, that could contribute to the therapeutic effects observed. The in-vitro studies revealed that both extracts exhibited significant protein denaturation inhibitory and HRBC membrane stabilizing activity in a concentration-dependent manner, which is of considerable anti-inflammatory and membrane protective activity. The treatment with ZAAQ and ZAE successfully decreased paw edema, the volume of the joints and arthritis-associated body weight loss in the FCA-induced arthritic rat when compared to the arthritic disease control group. Both extracts showed dose-dependent therapeutic effects, and the high doses of both extracts showed good anti-arthritic responses. The aqueous extract showed comparatively higher activity as compared to other extracts tested and close to the activity of standard drug diclofenac. The results of the study are scientifically substantiated to confirm the traditional medicinal applications of *Zanthoxylum armatum* in treatment of inflammatory and rheumatic ailments. The anti-arthritic activity demonstrated could be due to synergic effect of phytoconstituents which can inhibit inflammatory mediators and oxidative stress. Thus, Zanthoxylum armatum could be a potential natural source for therapeutic intervention in the treatment of rheumatoid arthritis. Nevertheless, additional research to isolate active compounds, elucidate detailed molecular mechanisms, evaluate chronic toxicity and clinical trials is required to prove its safety and therapeutic potential in humans.

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