



Clerodendrum serratum (L.) Moon leaf extract and its chloroform fraction attenuates acute and chronic arthritis in albino rats

Shradhanjali Singh^{*}, Shanti Bhushan Mishra, Alok Mukerjee

United Institute of Pharmacy, Naini, Allahabad, 211010, Uttar Pradesh, India

ARTICLE INFO

Keywords:

Antiarthritic
Clerodendrum serratum
Complete freund's adjuvant
Histopathology HPTLC

ABSTRACT

The plant *Clerodendrum serratum* (L.) Moon has been traditionally claimed for the treatment of various inflammatory conditions including rheumatoid arthritis, hence in the present study the effect of the crude ethanolic extract (CSEE) from leaves of the plant and its chloroform fraction (CSCF) were evaluated in acute and chronic arthritic model. Preliminary phytochemical screening was performed for CSEE and chloroform fraction (CSCF). Formaldehyde (2%v/v), turpentine oil emulsion (10%v/v) and complete freund's adjuvant (CFA) were injected intraplantar in left hind paw of wistar albino rats. Paw swelling, body weight, serum enzyme markers, hematological parameters and histopathological analysis were observed in arthritic control group, extract and fraction treated groups. Phytochemical screening signified the presence of carbohydrates, steroids, saponins, alkaloids, tannins, phenolic compounds and flavonoids in CSEE and in CSCF. The plant extract and chloroform fraction possesses dose dependent antiarthritic effect in acute and CFA induced arthritic model by normalizing the level of serum enzyme biomarkers, hematological, body weight abnormalities and also amended the histopathological changes when compare to arthritic control group. Moreover, quantification of β -sitosterol was done by HPTLC technique in CSCF and the percentage area was found to be 18.57% (w/w). HPTLC fingerprinting of CSEE and CSCF revealed presence of various phytochemical constituents. The observations confirmed that the ethanolic extract and its chloroform fraction of leaves of *Clerodendrum serratum* (L.) Moon exhibits anti-arthritic potential.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease with predominant joint involvement which is characterized by inflammation of joints, devastation of synovium, pannus formation, bone and cartilage destruction. The inflammatory process in rheumatoid arthritis is due to the presence of cytokines such as IL-1, TNF α , TNF γ in the circulatory system Khader et al. (2018). Therapies for RA include pharmacological treatment including general therapies (NSAIDs) and targeted synthetic disease modifying antirheumatic drugs Pirmardvand Chegini et al. (2018). The allopathic medications exhibit side effects, hence to overcome with these issues today traditional and alternative medicine has been successfully opted by the population for the treatment of RA.

Medicinal plants are considered as important source of active ingredients in preventing various ailments. Numerous experimental approaches proved that *Cleodendrum serratum* (L.) Moon exhibits various pharmacological activities including hepatoprotective, antioxidant, anti-inflammatory, antinociceptive, anti-cancer Wang et al. (2018), antiasthmatic, antiallergic, antipyretic, analgesic, antibacterial, wound

healing, immunomodulatory, antiviral, vasorelaxant, alpha glucosidase inhibition Patel et al. (2014), antihistaminic, antispasmodic, antitussive (Khare, 2007), antidiabetic Kar et al. (2014) and neuroprotective Vazhayil et al. (2017). Chemically, the plant contains oleanolic acid, lupeol, ursolic acid, serratin, β -sitosterol, γ -sitosterol, spinasterol, α -spinasterol, stigmasterol, 7- β -coumaroyl-oxyugandoside, serratoside A, serratoside B, (+)-catechin, caffeic acid, ferulic acid, Apigenin-7-glucoside, luteolin 7-O- β -D-glucuronide, luteolin, glucose and sucrose Patel et al. (2014). The decoction of leaves traditionally claimed in ancient literature for the treatment of arthritis (Kirtikar and Basu, 2005).

Arthritic manifestations must be scientifically explored by using standard experimental animal models and should also resemble immune response as in human RA. Freund's complete adjuvant induced arthritis in rat model is the best and most widely used experimental model for arthritis. This model is sensitive to anti inflammatory and immune inhibiting medicines and considers being relevant for the study of pathophysiological and pharmacological control of inflammation process as well as for the evaluation of anti-nociceptive potential of drugs (Pandey, 2010). The present work establishes phytochemical

^{*} Corresponding author. United Institute of Pharmacy, A 31/1 UPSIDC, Industrial Area, Naini, Allahabad, 211010, Uttar Pradesh, India.

E-mail address: singhshradhanjali@gmail.com (S. Singh).

investigation of different compounds by fractionation of ethanolic extract of the leaves of *C. serratum* thereby quantitative estimation of β -sitosterol by HPTLC technique and evaluating antiarthritic activity.

2. Materials and methods

2.1. Collection of plant

The leaves of *Clerodendrum serratum* (L.) Moon (Verbenaceae) were collected from Arogyadham, Deendayal Research Institute, Chitrakoot, Satna, Madhya Pradesh in the month of August and identified by Dr. G.P Sinha, Scientist, Botanical Survey of India, CRC, Allahabad (U.P.) with accession No. (BSA-95442). The voucher specimen was deposited in the herbarium of BSI for future reference.

2.2. Chemicals and instruments

Complete Freund's adjuvant (CFA) each mL contains 1 mg *Mycobacterium tuberculosis* (H 37RA, ATCC 25177), heat killed and dried, 0.85 mL paraffin monooleate was purchased from Sigma-Aldrich, USA. Aspirin was obtained from CDH, New Delhi, as a gift sample. Formaldehyde, turpentine oil and other chemicals and solvents used are of AR grade (Merck, Mumbai). The instruments used were Plethysmometer (Kent Scientific), Vernier caliper (Asahi), CAMAG HPTLC system (Mutz, Switzerland) comprising of Hamilton 100 μ l syringe, Linomat IV applicator, twin trough developing chamber (20 \times 20 cm), Camag TLC Scanner 3 with Wincats 3 Software, UV Cabinet with UV lamp, Silica Gel G60 F₂₅₄, 5 cm \times 10 cm TLC plate as stationary phase.

2.3. Extraction and fractionation

The powdered leaves (500 g) were extracted using 95%v/v ethanol by hot percolation method at 55 °C. The percentage yield was found to be 28% w/w. Fractionation was performed by mixing 100g of ethanolic remnant with water and chloroform (1:1) in a separating funnel and by liquid partitioning method, chloroform fraction was obtained which further concentrated on rotavapour (Buchi, USA) at a temperature of <40 °C and then dried in lyophilizer (Labconco, USA) under reduced pressure. The percentage yield of fraction was found to be 24%w/w.

2.4. Preliminary phytochemical screening

CSEE and CSCF were subjected to preliminary phytochemical screening for the identification of various active constituents by using standard procedures (Harborne, 1998).

2.5. Characterization of ethanolic extract and its chloroform fraction

The ethanolic extract and its chloroform fraction were analyzed by HPTLC fingerprinting method. 10 μ l of 1 mg/ml pure β -sitosterol (marker compound) and 10 μ l of chloroform fraction as a test solution were spotted on the HPTLC plates. The plates were developed using Toluene: Ethyl acetate: Formic acid (7:2:1v/v/v) as mobile phase.

2.6. Experimental animals

Healthy adult male wistar albino rats, weighing 180–210 g were kept in polypropylene cages in animal facility of United Institute of Pharmacy, Allahabad under standard environmental conditions as per CPCSEA (committee for the purpose of control and supervision of experiments on animal's regulations), 12 h light and 12 h dark cycle at 25 \pm 2 °C. Before and through the experiments, the rats were fed with standard laboratory pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) with approval number (REG. No: UIP/IAEC/April-2015/06).

2.7. Toxicity study of the plant extract

The lethal median dose (LD₅₀) estimation was executed in rats by OECD guidelines for testing of chemicals 423 by method of (OECD, 2001). A single dose of the extract (5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg) in 1% gum acacia was given orally to all groups of animals (three each). The animals were allowed free access to food and water. The animals were initially monitored continuously for any adverse effects for 4 h and then monitored at 1-h intervals. They were later monitored twice daily for any abnormal changes throughout the study period (which lasted 14 days).

2.8. Induction of arthritis

Arthritis was induced after 30 min of drug/vehicle administration to all the rats. Suspensions of Standard drug aspirin, CSEE and CSCF were prepared in 1%w/v gum acacia and administered orally to rats. Formaldehyde solution (2%v/v) and Turpentine emulsion (10%v/v) were made in 0.9% saline. The rats paw volume and diameter was measured by Vernier caliper as the baseline Chaudhary et al. (2014).

2.9. Acute arthritic model

2.9.1. Formaldehyde and turpentine oil induced arthritic model

Seven groups of rats (6 each) were used for the study. Group I was Positive control rats, which received vehicle, Group II was arthritic control rats received 0.1 ml of 2% Formaldehyde solution, Group III was Arthritic rats treated with the standard drug Aspirin at 100 mg/kg body weight, Group IV was arthritic rats treated with extract (CSEE) 100 mg/kg body weight, Group V was arthritic rats treated with extract at (CSEE) 200 mg/kg body weight, Group VI was arthritic rats treated with fraction (CSCF) 100 mg/kg body weight, Group VII was arthritic rats treated with fraction (CSCF) 200 mg/kg body weight. 0.1 ml of Formaldehyde was injected intra-plantar in left hind paw of rats on 0th day. The injection was repeated on the 3rd day. Anti-arthritic activity of CSEE and CSCF were evaluated on injected paw on the following parameters paw volume, joint diameter on day 0, 2, 4, 6, 8 and 10. (Newbould, 1963).

In case of Turpentine oil induced arthritis, 0.1% turpentine oil emulsion was injected into intra-plantar in left hind paw of rats on 0hr. Anti-arthritic activity of CSEE and CSCF was evaluated by measuring paw volume and joint diameter at every hour up to 6 h. Di Rosa et al. (1971).

2.10. Chronic arthritic model

2.10.1. Complete freund's adjuvant arthritic model

Seven groups of rats were used for the study as discussed in acute arthritic model. 0.1 ml of CFA was injected intra-plantar in left hind paw of rats on 0th day Tatiya et al. (2017). Anti-arthritic activity of CSEE and CSCF were evaluated on injected paw on the following parameters paw volume, joint diameter and body weight on day 0, 1, 4, 8, 12, 16, 20, 24, and day 28. On 28th day the blood was withdrawn by retro-orbital puncture for the estimation of various parameters Foyet et al. (2014).

2.11. Hematological and serum parameters

On day 28, blood of the rats was withdrawn by retro-orbital puncture. The collected blood samples were centrifuged at 2500 rpm for 15 min. The serum was collected in fresh serum tubes and stored in refrigerator (2–4 °C) after tightly capped and subjected to biochemical and hematological examination for AST, ALT, ALP (serum enzyme biomarkers), total protein levels, Albumin/Globulin ratio, red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and platelets (PLT). Serum C-reactive protein (CRP) and Rheumatoid factor (RF) level were also measured also measured using diagnostic kits (Erba Lachema).

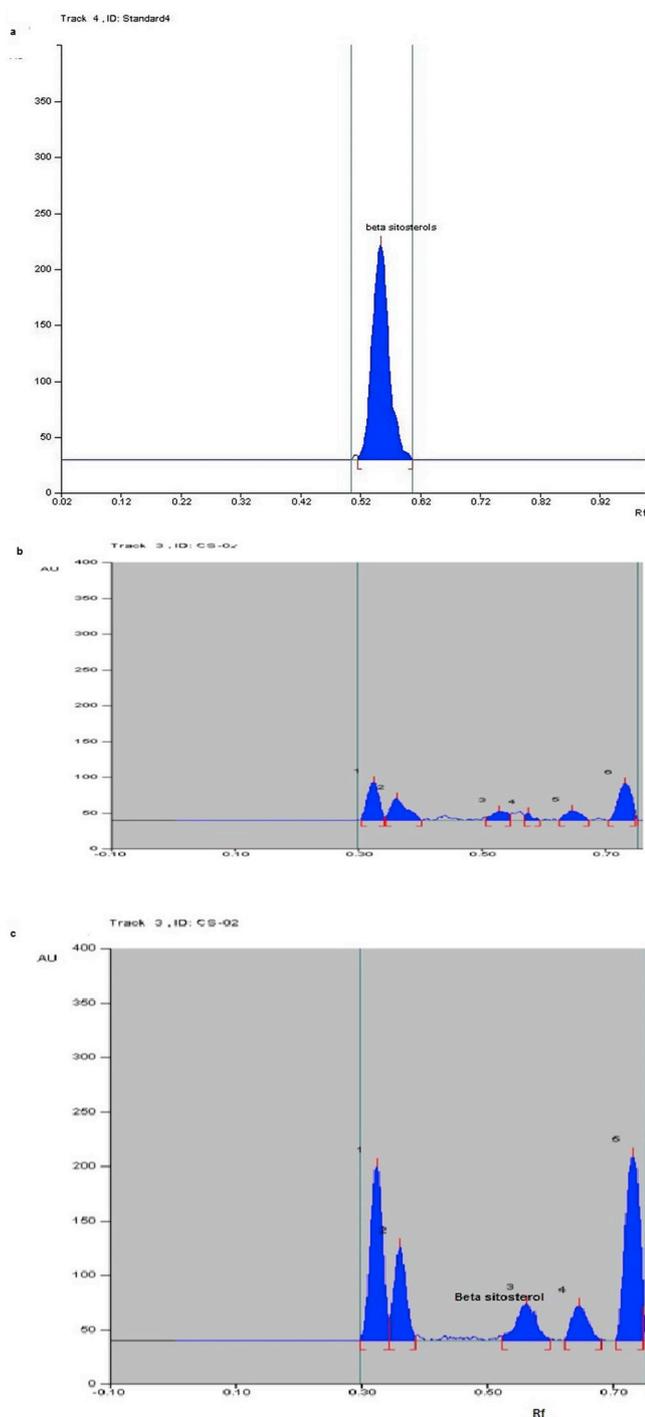


Fig. 1. (a) HPTLC fingerprinting of marker compound (β -sitosterol) (b) HPTLC fingerprinting of CSEE (c) HPTLC fingerprinting of CSCF.

2.12. Histopathological analysis

At the end of the treatment period the control as well as treated rats were sacrificed by using cervical dislocation and the ankle joint was removed, immediately fixed in bouin's fluid for 24 h and washed in running tap water to remove colour of bouin's fluid and dehydrated in alcohol embedded in paraffin and cut at 5 μ m in a rotary microtome. The joint sections were then deparaffinized in xylene and stained with eosin-haematoxylin stain and viewed under 40 \times magnifications [Petkova et al. \(2006\)](#). The histopathological slides were examined and photographs were captured with a digital stereomicroscope (Olympus, B061).

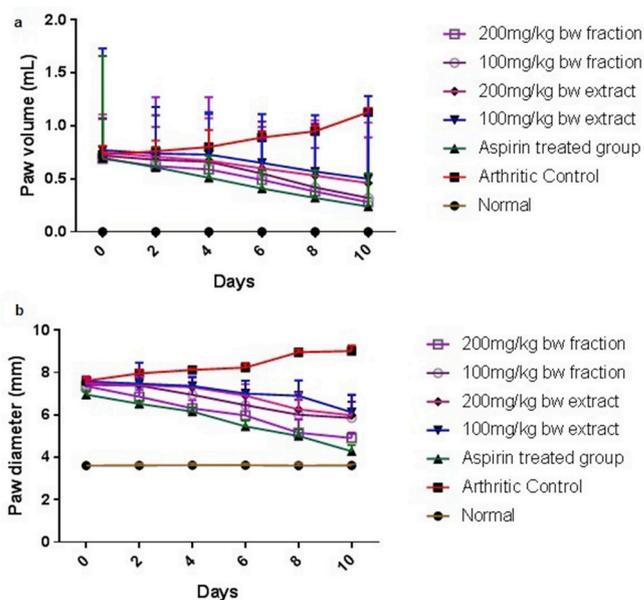


Fig. 2. (a) Effect of ethanolic extract and its fraction of *C. serratum* on paw volume of formaldehyde induced arthritic model (b) Effect of ethanolic extract and its fraction of *C. serratum* on paw diameter of formaldehyde induced arthritic model.

2.13. Statistical analysis

Data were expressed as mean \pm SD and statistical analysis was carried out by using GraphPad Prism 7.05 software by applying two-way ANOVA with Newman-Keuls method. $P < 0.001$ was considered to be significant.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of carbohydrates, steroids, saponins, alkaloids, tannins, phenolic compounds and flavonoids in CSEE and presence of alkaloid, deoxysugars, and phytosterols in CSCF fraction.

3.2. Toxicity study of the plant extract

The LD₅₀ of the extract was found to be 1000 mg/kg. Hence for further study one-tenth of the maximum dose of the extract as well as fraction was selected for the study, i.e. 100 mg/kg and its double strength 200 mg/kg body weight.

3.3. High performance thin layer chromatography fingerprinting analysis

HPTLC fingerprinting analysis identifies flavonoids (R_f 0.55–0.73), phenolics (R_f 0.73) and phytosterols (R_f 0.40) related compounds in CSEE. Moreover, quantification of β -sitosterol was done by HPTLC technique and the percentage area in CSCF was found to be 18.57% (w/w). The plates were scanned densitometrically at 631 nm as shown in ([Fig. 1](#)). HPTLC fingerprinting of marker compound (β -sitosterol) was shown in ([Fig. 1a](#)), HPTLC fingerprinting of CSEE was shown in ([Fig. 1b](#)) and HPTLC fingerprinting of CSCF was shown in ([Fig. 1c](#)).

3.4. Effect of CSEE and CSCF in acute arthritic model

The injection of inducers in left hind elicited paw swelling including edema of tissue, proliferation of synovial cells, inflammatory cell

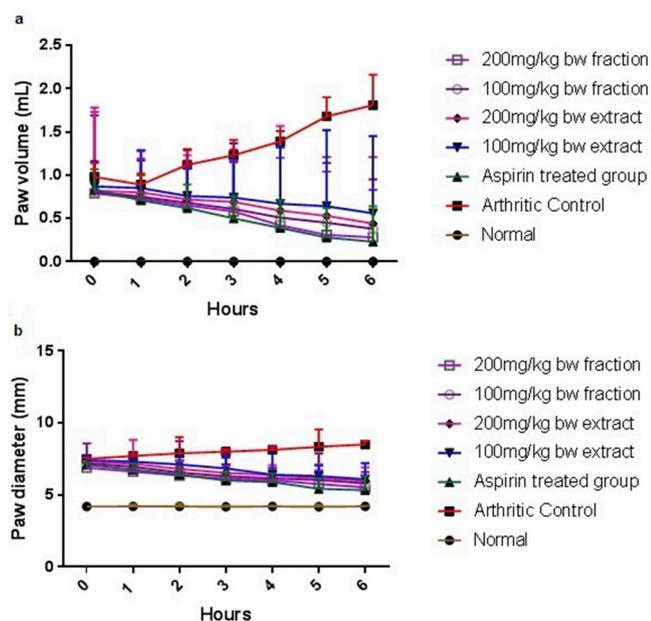


Fig. 3. (a) Effect of ethanolic extract and its fraction of *C. serratum* on paw volume of turpentine induced arthritic model (b) Effect of ethanolic extract and its fraction of *C. serratum* on paw diameter of turpentine induced arthritic model.

infiltration, fibrosis suggesting the successful establishment of acute unilateral mechanical arthritis in both models. All the treated groups were compared with the arthritic control group for acute and chronic arthritic models. Effect of formaldehyde induced joint edema in rats was shown in (Fig. 2). The paw volume in Group II was increased by $(0.73 \pm 0.08-1.13 \pm 0.04)$. Group III showed decrease in paw volume to be $(0.70 \pm 0.11-0.24 \pm 0.31, p < 0.001)$. On 10th day, decrease in paw swelling were observed in Group VII 200 mg/kg b. w $(0.69 \pm 0.38-0.28 \pm 0.61, p < 0.001)$ when compared with Group VI 100 mg/kg b. w $(0.72 \pm 0.35-0.32 \pm 0.81, p < 0.01)$, Group V 200 mg/kg $(0.75 \pm 0.36-0.46 \pm 0.57)$ and Group IV 100 mg/kg b. w $(0.77 \pm 0.96-0.50 \pm 0.78, p < 0.01)$. Effect of ethanolic extract and its fraction of *C. serratum* on paw volume of formaldehyde induced arthritic model were shown in (Fig. 2a). The extent of edema was also measured by measuring paw diameter of Group II $(7.61 \pm 0.16-9.02 \pm 0.26)$. Group III showed decrease in swelling $(6.96 \pm 0.13-4.29 \pm 0.29, p < 0.001)$. On 10th day, decrease in paw edema in Group VII was $(7.36 \pm 0.37-4.91 \pm 0.25, p < 0.001)$, in Group VI was $(7.43 \pm 0.35-5.86 \pm 0.77, p < 0.01)$, in Group V was $(7.50 \pm 0.33-5.99 \pm 0.98, p < 0.01)$ and in Group IV was $(7.59 \pm 0.17-6.13 \pm 0.81, p < 0.05)$. Effect of ethanolic extract and its fraction of *C. serratum* on paw diameter of formaldehyde induced arthritic model were shown in (Fig. 2b). Effect of turpentine induced paw swelling in rats is shown in (Fig. 3). On measuring paw volume Group II showed increased swelling $(0.98 \pm 1.16-1.81 \pm 0.35)$. After 6hrs in fraction treated group at 200 mg/kg b. w., decrease in edema $(0.79 \pm 0.94-0.28 \pm 0.55, p < 0.001)$ was observed in large extent as compared with Group VI 100 mg/kg b. w $(0.81 \pm 0.88-0.38 \pm 0.57, p < 0.001)$, Group V 200 mg/kg b. w $(0.82 \pm 0.96-0.44 \pm 0.77, p < 0.01)$, Group IV 100 mg/kg b. w $(0.87 \pm 0.29-0.56 \pm 0.89, p < 0.01)$. Effect of ethanolic extract and its fraction of *C. serratum* on paw volume of turpentine induced arthritic model were shown in (Fig. 3a). Paw diameter in Group II showed increased edema $(7.50 \pm 0.10-8.50 \pm 0.50)$. The edema progressively falls for Group III over 6 h $(7.10 \pm 0.17-5.33 \pm 0.96, p < 0.001)$. Paw edema in Group VII $(6.89 \pm 0.45-5.53 \pm 0.81, p < 0.001)$ was immensely reduced as compared with Group VI $(7.21 \pm 1.36-5.81 \pm 0.78, p < 0.01)$, Group V $(7.33 \pm 0.31-5.98 \pm 0.94, p < 0.01)$, Group IV $(7.43 \pm$

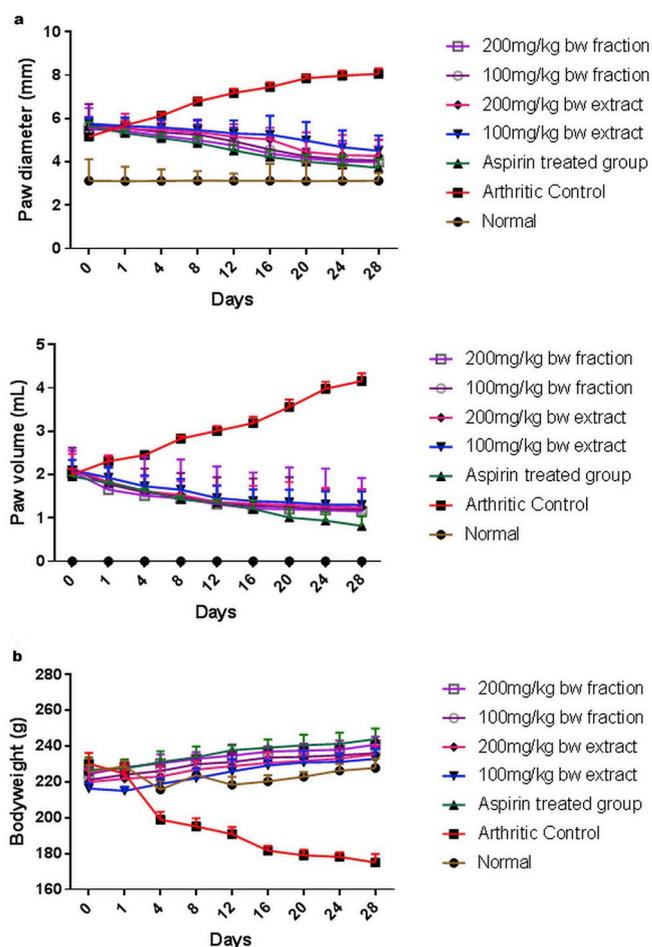


Fig. 4. (a) Effect of ethanolic extract and its fraction of *C. serratum* on paw edema in CFA induced arthritic model (b) Effect of ethanolic extract and its fraction of *C. serratum* on body weight in CFA induced arthritic model.

$0.28-6.09 \pm 1.12, p < 0.05)$. Effect of ethanolic extract and its fraction of *C. serratum* on paw diameter of turpentine induced arthritic model were shown in (Fig. 3b).

3.5. Effect of CSEE and CSCF in CFA induced arthritic model

3.5.1. Paw swelling & body weight

The effect of ethanolic extract and its fractions of *C. serratum* on body weight and in paw volume were shown in (Fig. 4). Group II showed extensive swelling immediately after injection. Group III showed significant reduction in paw swelling $(1.96 \pm 0.02-0.82 \pm 0.22, p < 0.001)$. On 28 day, significant decrease in paw volume were observed in Group VII 200 mg/kg b. w $(2.07 \pm 0.48-1.15 \pm 0.77, p < 0.001)$ when compared with Group VI 100 mg/kg b. w $(2.11 \pm 0.51-1.19 \pm 0.47, p < 0.001)$, Group V CSEE 200 mg/kg b. w $(2.13 \pm 0.35-1.24 \pm 0.42, p < 0.001)$ and Group IV CSEE 100 mg/kg b. w $(2.10 \pm 0.24-1.30 \pm 0.31, p < 0.01)$.

On measuring the paw diameter of induced and treated groups, aspirin treated group showed significant reduction in paw swelling $(5.65 \pm 0.18-3.73 \pm 0.39, p < 0.001)$, the fraction treated rats manifests decreased paw swelling in Group VII $(5.51 \pm 0.98-3.99 \pm 0.73, p < 0.001)$. At 100 mg/kg b. w the swelling in fraction treated rat decreased significantly $(5.70 \pm 0.96-4.07 \pm 0.95, p < 0.001)$. Group V $(5.53 \pm 0.69-4.28 \pm 0.74, p < 0.01)$ and Group IV $(5.76 \pm 0.29-4.51 \pm 0.70, p < 0.01)$ had moderate effect on plant extract and its fraction. Effect of ethanolic extract and its fraction of *C. serratum* on paw edema of CFA induced arthritic model were shown in (Fig. 4a).

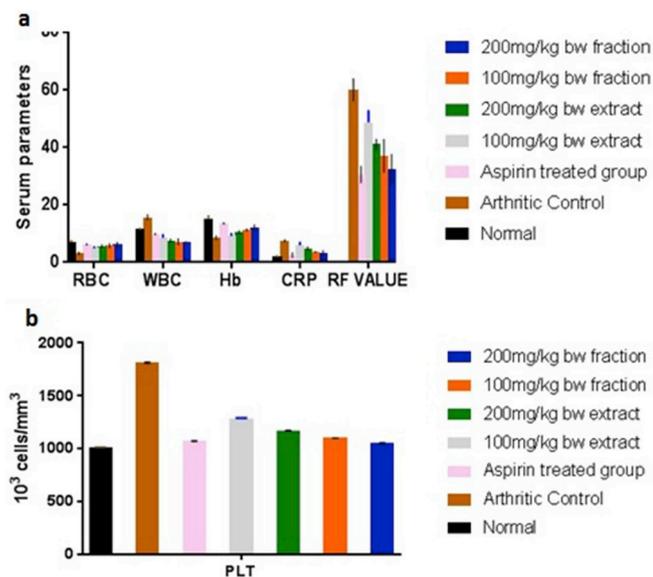


Fig. 5. Effect of ethanolic extract and fractions of *C. serratum* on (a) RBC's, WBC's, platelets, Hb, CRP, RF (b) Platelets parameters after 28 days.

The decrease in the body weight in Group II (230.23 ± 5.98 – 175.00 ± 4.76) confirmed that arthritis was induced on all groups whereas in extract and fraction treated groups changes in body weight were not statistically significant. The effect of ethanolic extract and its fraction of *C. serratum* on body weight of CFA induced arthritic model were shown in (Fig. 4b).

3.6. Hematological parameters

The parameters including RBC, Platelet, WBC, Hb, CRP and Rf were assessed in the serum samples in all groups on 28 day. The effect of ethanolic extract and its fraction of *C. serratum* on hematological and serum parameters were shown in (Fig. 5a) and (Fig. 5b).

CFA treated group showed increase in WBC (11.50 ± 0.23 – 15.63 ± 0.89), Platelet count (1010.00 ± 4.35 – 1813.00 ± 6.36), CRP levels (2.01 ± 0.16 – 7.51 ± 0.34) and Rf value (0.00 – 60 ± 3.76) but decrease in Hemoglobin (15.20 ± 0.76 – 8.55 ± 0.64) and RBC count (7.20 ± 0.26 – 3.12 ± 0.36). Upon comparison with arthritic control group, aspirin treated group showed decreased WBC count (15.63 ± 0.89 – 9.86 ± 0.16 , $p < 0.001$), platelet count (1813.00 ± 6.36 – 1072.00 ± 3.16 , $p < 0.001$), CRP levels (7.51 ± 0.34 – 2.53 ± 0.78 , $p < 0.001$) and Rf value (60.00 ± 3.76 – 30.51 ± 2.76 , $p < 0.001$) but increased Hb level (8.55 ± 0.64 – 13.50 ± 0.22 , $p < 0.001$) and RBC count (3.12 ± 0.36 – 6.23 ± 0.18 , $p < 0.001$). The fraction treated groups at 200 mg/kg b. w showed significant increase in RBC count

(3.12 ± 0.36 – 6.10 ± 0.78 , $p < 0.001$) and Hemoglobin (8.55 ± 0.64 – 12.05 ± 0.84 , $p < 0.001$) but decrease in WBC (15.63 ± 0.89 – 6.99 ± 0.14 , $p < 0.001$), platelet count (1813.00 ± 6.36 – 1053.12 ± 4.78 , $p < 0.001$), CRP levels (7.51 ± 0.34 – 3.09 ± 0.95 , $p < 0.001$) and Rf value (60.00 ± 3.76 – 32.53 ± 4.88 , $p < 0.001$) as compared with fraction at 100 mg/kg b. w dose in RBC count (3.12 ± 0.36 – 5.81 ± 0.63 , $p < 0.001$), Hemoglobin (8.55 ± 0.64 – 11.26 ± 0.28 , $p < 0.001$), WBC (15.63 ± 0.89 – 7.10 ± 0.99 , $p < 0.001$), platelet count (1813.00 ± 6.36 – 1101.01 ± 1.61 , $p < 0.01$), CRP levels (7.51 ± 0.34 – 3.55 ± 0.15 , $p < 0.05$) and Rf value (60.00 ± 3.76 – 37.00 ± 5.65 , $p < 0.001$). The extract treated groups at 200 mg/kg b. w. showed less changes in RBC count (3.12 ± 0.36 – 5.51 ± 0.53 , $p < 0.01$) Hemoglobin (8.55 ± 0.64 – 10.51 ± 0.45), WBC (15.63 ± 0.89 – 7.50 ± 0.55 , $p < 0.001$), platelet count (1813.00 ± 6.36 – 1169.72 ± 4.76), CRP levels (7.51 ± 0.34 – 4.85 ± 0.54) and Rf value (60.00 ± 3.76 – 41.00 ± 1.68 , $p < 0.01$) as compared with 100 mg/kg b. w treated dose in RBC count (3.12 ± 0.36 – 4.99 ± 0.34 , $p < 0.05$) Hemoglobin (8.55 ± 0.64 – 9.23 ± 0.65 , $p < 0.01$), WBC (15.63 ± 0.89 – 8.50 ± 0.98 , $p < 0.01$), platelet count (1813.00 ± 6.36 – 1287.00 ± 2.15), CRP levels (7.51 ± 0.34 – 5.98 ± 0.88 , $p < 0.01$) and Rf value (60.00 ± 3.76 – 48.61 ± 3.88) showed a moderate effect on the parameters.

3.7. Biochemical parameters

AST, ALP, ALT, total protein and albumin/globulin ratio were estimated in all groups on 28 day. Effect of ethanolic extract and its fraction of *C. serratum* on biochemical parameters were shown in (Fig. 6).

CFA induced rat significantly showed an elevated AST (50.01 ± 6.73 – 120.00 ± 5.17), ALT (40.31 ± 3.14 – 150.73 ± 2.66), ALP (76.05 ± 4.12 – 483.56 ± 6.89) levels except total proteins (7.12 ± 0.45 – 4.93 ± 0.59) and A/G ratio (1.80 ± 0.13 – 0.68 ± 0.03). The Aspirin treated group shows decreased levels in biochemical parameter except total proteins and A/G ratio which showed increased level when compared with arthritic control as in AST (120.00 ± 5.17 – 75.56 ± 3.12 , $p < 0.001$), ALT (150.73 ± 2.66 – 112.98 ± 1.89 , $p < 0.001$), ALP (483.56 ± 6.89 – 218.69 ± 3.55 , $p < 0.001$), total proteins (4.93 ± 0.59 – 6.98 ± 0.23) and A/G ratio (0.68 ± 0.03 – 0.96 ± 0.17). Upon treatment with the extract at 100 mg/kg b. w. the levels of AST (120.00 ± 5.17 – 112.01 ± 2.17), ALT (150.73 ± 2.66 – 129.98 ± 4.61 , $p < 0.01$), ALP (483.56 ± 6.89 – 378.61 ± 2.11) decreases except total proteins (4.93 ± 0.59 – 6.51 ± 0.66) and A/G ratio (0.68 ± 0.03 – 1.01 ± 0.05). Treatment with extract at 200 mg/kg b. w. the levels of AST (120.00 ± 5.17 – 100.65 ± 4.13 , $p < 0.01$), ALT (150.73 ± 2.66 – 116.68 ± 2.56 , $p < 0.001$), ALP (483.56 ± 6.89 – 308.98 ± 4.55 , $p < 0.01$) also decreases except total proteins (4.93 ± 0.59 – 6.12 ± 0.33) and A/G ratio (0.68 ± 0.03 – 1.12 ± 0.12). Significant changes were observed in

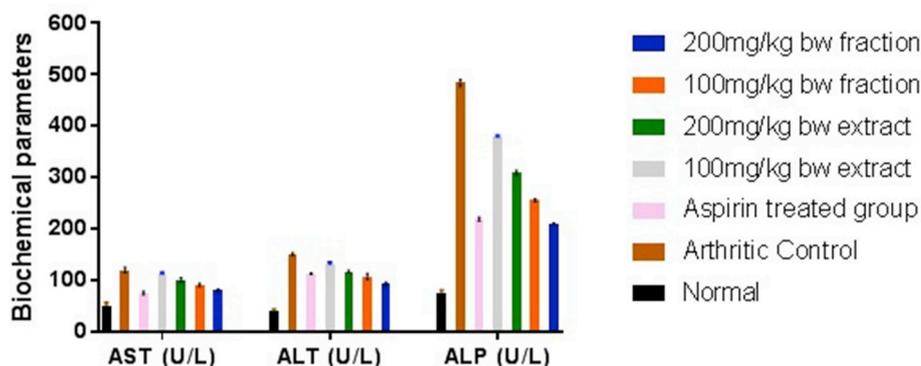


Fig. 6. Effect of ethanolic extract and fractions of *C. serratum* on biochemical parameters after 28 days.

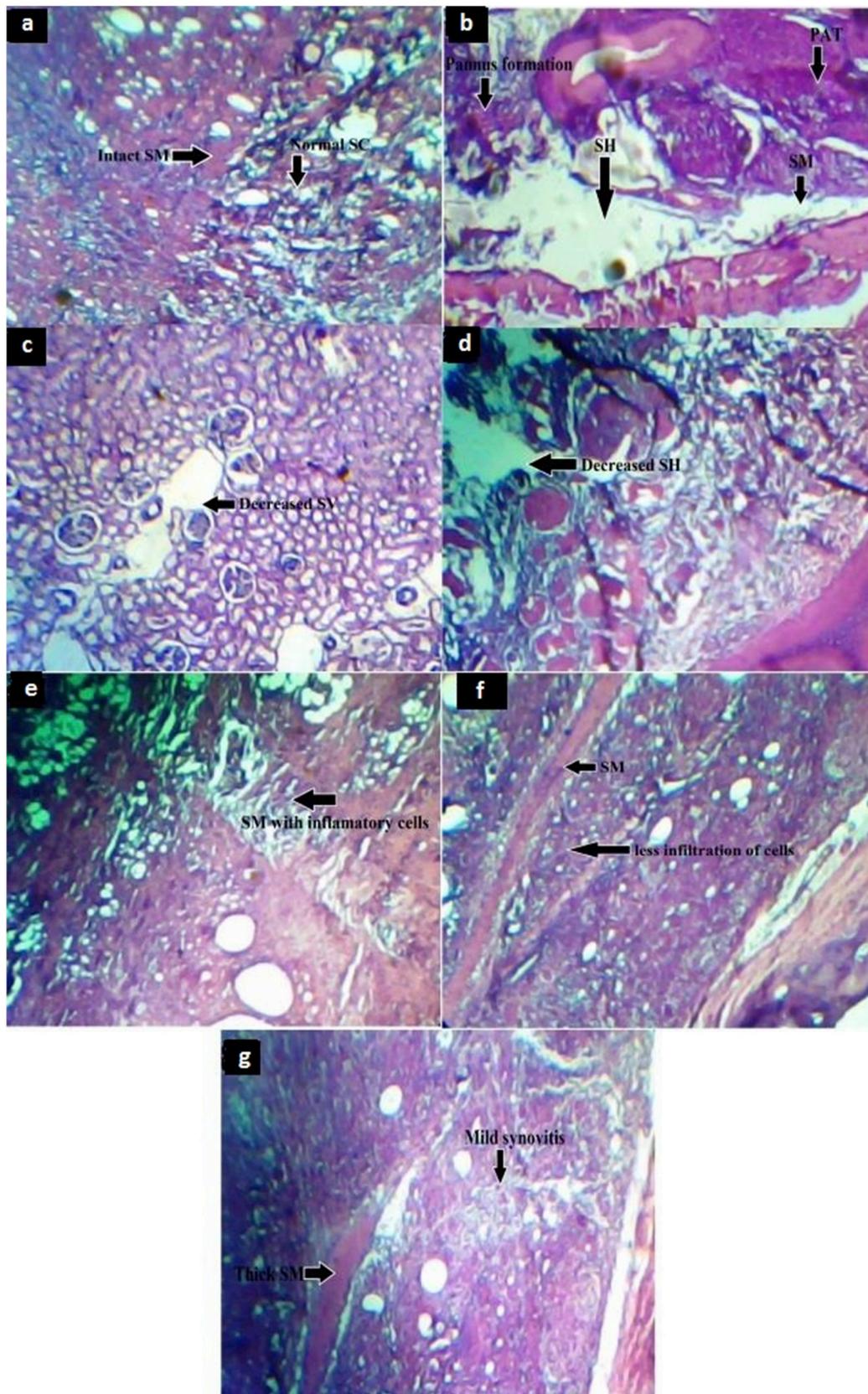


Fig. 7. Histopathological analysis of hind paw (a) intact synovial membrane with normal synovial cavity (SC) (b) Synovial membrane (SM), periarticular tissues (PAT), pannus formation and presence of synovial hyperplasia (SH) (c) Decreased influx of inflammatory cells, decreased synovial vascularity (SV), no pannus formation, no fibrin deposit with less proliferation (d) Decreased synovial hyperplasia (e) Decreased inflammatory cells with synovial space (f) Intact synovial membrane, normal nuclei and less infiltration of inflammatory cells (g) thickened synovial membrane with mild synovitis.

treatment levels by Group VII, the levels of AST (120.00 ± 5.17 – 81.00 ± 2.16 , $p < 0.001$), ALT (150.73 ± 2.66 – 92.53 ± 3.89 , $p < 0.001$), ALP (483.56 ± 6.89 – 209.44 ± 1.98 , $p < 0.001$) also decreases except total proteins (4.93 ± 0.59 – 5.64 ± 0.98) and A/G ratio (0.68 ± 0.03 – 1.20 ± 0.08) and Group VI, the levels of AST (120.00 ± 5.17 – 90.75 ± 2.77 , $p < 0.001$), ALT (150.73 ± 2.66 – 106.89 ± 5.68 , $p < 0.001$), ALP (483.56 ± 6.89 – 255.12 ± 2.98 , $p < 0.001$) also decreases except total proteins (4.93 ± 0.59 – 5.89 ± 0.11) and A/G ratio (0.68 ± 0.03 – 1.16 ± 0.07).

3.8. Histopathological analysis

In histopathological illustrations, intact synovial lining with no changes in cellular infiltration was observed in Group I. The synovial cavity was observed as normal as in (Fig. 7a). Group II showed severe proliferation which is characterized by huge influx of inflammatory cells, chronic inflammation, pannus formation, increased synovial vascularity and synovial space with invasion of periarticular tissue (PAT) as in (Fig. 7b). Group III showed significant decreased influx of inflammatory cells, decreased synovial vascularity, no pannus formation, no fibrin deposit, less proliferation as in (Fig. 7c). Group IV and V showed presence of inflammatory cells, with synovial space, synovial hyperplasia, decreased fibrin deposit and moderate proliferation with mild synovial cell invasion of bone and tissues as in (Fig. 7d and e) respectively. Group VI showed moderate effect with little presence of inflammatory cells, mild proliferation and less synovial cell invasion as in (Fig. 7f), but Group VII showed significant protection with synovial space thickening, low fibrin deposit, less infiltration of cells and mild synovitis as in (Fig. 7g).

4. Discussion

Phytochemical studies inveterate the presence of various phytoconstituents in the ethanolic extract of the plant and its chloroform fraction. HPTLC analysis was performed for the quantitative estimation of β -sitosterol in CSCF. Previous studies Bouic et al. (1999), Navarro et al. (2001) explored that beta-sitosterol decreases the level of IL-6 suggesting lowered inflammatory response, paw edema and neutrophil infiltration in inflamed tissues, and the level of superoxide ions within systemic circulation. In this study CSCF signifies the effect of fraction on adjuvant arthritic model possibly by decreasing the level of interleukin.

Previous studies Singh et al. (2007) signified that in turpentine oil induced arthritic model, joint edema is related with successive liberation of mediators i.e. histamine, serotonin, kinin like substances and prostaglandin causes inflammation that sustained for few hours. In this study, CSCF at 200 mg/kg b. w dose showed significant decrease in joint edema possibly due to reduction in the level of mediators.

Formaldehyde induces chronic reaction gradually on repeated injection on 3rd day, accounts for edema at injected site Selye et al. (1949). Chloroform fraction treated rats at 200 mg/kg b. w. observed with significant reduction in swelling when compared with CSCF 100 mg/kg b. w treated rats.

As previous scientific report Ito et al. (2001) suggested that the aspirin blocks cyclooxygenase enzyme and inhibits prostaglandin production hence reduces pain; since increased levels of prostaglandins affect the mediators such as bradykinin or histamine which induces inflammation, the present study revealed in chronic arthritic model that CSCF treated rats showed reduced paw swelling and improved movement possibly due to reduction in level of prostaglandins. The presence of high circulating levels of interleukin 6 (IL6), a useful inflammatory marker in adjuvant arthritic rats, stimulates the hepatic production of an iron regulatory hormone, hepcidin which causes inhibition of iron release from macrophages hence sequesters the iron and reducing the iron supply to erythropoiesis causing anemia Hajar et al. (2015); CSCF at 200 mg/kg b. w significantly increased the level of Hb and RBC's probably due reduction in interleukin levels. As an increase in

neutrophils associated with presence of interleukin caused increased levels of WBC and acute phase proteins including CRP in adjuvant arthritis Milovanovic et al. (2004), CSCF at 200 mg/kg b. w showed decreased level of WBC may be due to decrease in neutrophil count and interleukin level. The fraction treated rats at 200 mg/kg b. w showed decreased CRP and RF factor, since serum Rheumatoid factor RF, an immunoglobulin molecule elicits immune response and is used as an inflammatory marker in RA count.

Since the permeability of vascular tissues to albumin was increased due to the release of mediators including histamine, bradykinin and prostaglandins which leads to reduction in level of albumin in serum Kahn et al. (1976), a significant reduction in the total protein level and A/G ratio was found in arthritic control group. However upon the treatment with CSCF 200 mg/kg b. w. dose, there were significant rise in total protein and A/G ratio but decrease in platelet count which instigates a restorative effect in arthritis.

Impairment in liver function was ascertained by increased levels of serum enzyme biomarkers including ALP, AST and ALT in the serum. These enzymes are responsible for the release of active constituents hence induces inflammatory response Mbiantcha et al. (2017). The CSCF 200 mg/kg b. w fraction treated group significantly decreases the elevated levels of biochemical parameters hence reduces the levels of these enzymes in the serum.

Histopathological analysis evidently decreased joint inflammation and improved cell proliferation indicates antiarthritic potential of *Cleodendrum serratum* (Linn.) Moon.

5. Conclusion

In the view of above study, it is postulated that *C. serratum* (L.) Moon has been observed to exert significant anti-arthritic effect in experimental studies. Furthermore, chloroform fraction has been avowed to be most potent and its beneficial effects on RA could possibly be correlated with the presence of β -sitosterol reported previously by other researchers and identified in leaves of this plant. In summary, this contemporary research lends pharmacological support to reported folkloric usage of *C. serratum* in the treatment and management of painful arthritic inflammatory conditions.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

None.

Acknowledgement

Authors acknowledges the contribution of United Diagnostic Centre, Allahabad for serving their practical hands for estimating the serum parameters and histopathology and United Institute of Pharmacy, Allahabad for providing the animal house facility to carry out in vivo experiments.

References

- Bouic, P.J., Clark, A., Lamprecht, J., Freestone, M., Pool, E.J., Liebenberg, R.W., Kotze, D., Van Jaarsveld, P.P., 1999. The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: inhibition of post marathon immune suppression and inflammation. *Int. J. Sports Med.* 20, 258–262.
- Chaudhary, M., Kumar, V., Gupta, P.K., Singh, S., 2014. Anti-arthritic activity of *Barleria prionitis* Linn. Leaves in acute and chronic models in Sprague Dawley rats. *Bull. Fac. Pharm. Cairo Univ.* 52, 199–209.

- Di Rosa, M., Giroud, J.P., Willoughby, D.A., 1971. Studies on the Mediators of the acute inflammatory response induced in rats in different sites by Carrageenan and Turpentine. *J. Pathol.* 104, 15–29.
- Foyet, H.S., Tsala, D.E., Zogo Essono Bodo, J.C., Carine, A.N., Heroyne, L.T., Oben, E.K., 2014. Anti-inflammatory and anti-arthritis activity of a methanol extract from *Vitellaria paradoxa* stem bark. *Pharmacogn. Res.* 7, 367–377.
- Hajar, T.L., Rostom, S., Hari, A., Lahlou, R., Bahiri, R., Abouqal, R., Hajjaj-Hassouni, N., 2015. Prevalence of anemia and its association with parameters of rheumatoid arthritis patients: a study from the Moroccan quest - RA data. *J. Palliat. Care Med.* 5, 221. <https://doi.org/10.4172/2165-7386.1000221>.
- Harborne, J.B., 1998. *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*, third ed. Chapman and Hall, London.
- Ito, S., Okuda-Ashitaka, E., Minami, T., 2001. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. *Neurosci. Res.* 41, 299–332.
- Kahn, A., Brachet, E., Conard, V., 1976. Correlation between permeability to albumin and levels of cyclic adenosine monophosphate in the incubated rat mesentery. *C. R. Seances Soc. Biol. Fil.* 170, 227–230.
- Kar, K.M., Swain, T., Mishra, S.K., 2014. Antidiabetic activity of *Clerodendrum serratum* (L.) moon leaves in streptozotocin-induced diabetic rats. *Asian J. Pharmaceut. Clin. Res.* 7, 260–263.
- Khader, S.Z.A., Ahmed, S.S.Z., Arunachalam, T., Nayaka, S., 2018. Radical scavenging potential, anti-inflammatory and antiarthritic activity of isolated isomer Methyl- γ -Orellinate and roccellatol from *Roccella montagnei* Bel. *Bull. Fac. Pharm. Cairo Univ.* 56, 39–45.
- Khare, C.P., 2007. *Indian Medicinal Plants an Illustrated Dictionary*, first ed. Springer Science + Business Media LLC, New York.
- Kirtikar, K.R., Basu, B.D., 2005. *Indian Medicinal Plants*, second ed. International book distributors, Dehradun.
- Mbiantcha, M., Almas, J., Shabana, S.U., Nida, D., Aisha, F., 2017. Anti-arthritis property of crude extracts of *Piptadeniastrum africanum* (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. *BMC Complement Altern. Med.* 17, 111.
- Milovanovic, M., Nilsson, E., Jaremo, P., 2004. Relationships between platelets and inflammatory markers in rheumatoid arthritis. *Clin. Chim. Acta* 343, 237–240.
- Navarro, A., De las Heras, B., Villar, A., 2001. Anti-inflammatory and immunomodulating properties of a sterol fraction from *Sideritis foetens* Clem. *Biol. Pharm. Bull.* 24, 470–473.
- Newbould, B.B., 1963. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. *Br. J. Pharmacol. Chemother.* 21, 127–136.
- OECD, 2001. Number 423 for the Testing of Chemicals: Revised Draft Guideline 423 (Acute Oral Toxicity), France.
- Pandey, S., 2010. Various techniques for the evaluation of antiarthritic activity in animals models. "J. Adv. Pharm. Technol. Research" " (JAPTR)" 1, 164–171.
- Patel, J.J., Acharya, S.R., Acharya, N.S., 2014. *Clerodendrum serratum* (L.) Moon. – a review on traditional uses, phytochemistry and pharmacological activities. *J. Ethnopharmacol.* 154, 268–285.
- Petkova, S.B., Konstantinov, K.N., Sproule, T.J., Lyons, B.A., Awwami, M.A., Roopenian, D.C., 2006. Human antibodies induce arthritis in mice deficient in the low-affinity inhibitory IgG receptor Fc gamma RIIB. *J. Exp. Med.* 203, 275–280.
- Pirmardvand Chegini, S., Varshosaz, J., Taymouri, S., 2018. Recent approaches for targeted drug delivery in rheumatoid arthritis diagnosis and treatment. *Artif. Cells. Nanomed. Biotechnol.* 16, 1–13.
- Selye, H., 1949. Further studies concerning the participation of the adrenal cortex in the pathogenesis of arthritis. *Br. Med. J.* 2, 1129–1135.
- Singh, S., Taneja, M., Majumdar, D.K., 2007. Biological activities of *Ocimum sanctum* L. fixed oil- an overview. *Indian J. Exp. Biol.* 45, 403–412.
- Tatiya, A.U., Saluja, A.K., Kalaskar, M.G., Surana, S.J., Patil, P.H., 2017. Evaluation of analgesic and anti-inflammatory activity of *Bridelia retusa* (Spreng) bark. *J. Tradit. Complement. Med.* 7, 441–451.
- Vazhayil, B.K., Rajagopal, S.S., Thangavelu, T., Swaminathan, G., Rajagounder, E., 2017. Neuroprotective effect of *Clerodendrum serratum* Linn. leaves extract against acute restraint stress-induced depressive-like behavioral symptoms in adult mice. *Indian J. Pharmacol.* 49, 34–41.
- Wang, J.H., Luan, F., He, X.D., Wang, Y., Li, M.X., 2018. Traditional uses and pharmacological properties of *Clerodendrum* phytochemicals. *J. Tradit. Complement. Med.* 8, 24–38.