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RESEARCH ARTICLE

Jasminum sambac (L.) Alleviates Rheumatoid Arthritis: Synergistic or Complementary Action? A Phytochemical and Pharmacological Investigation

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Abstract

Background: *Jasminum sambac* (L.) Aiton (JS) has promising anti-arthritic activity and is traditionally considered an analgesic. Although JS has been reported to exhibit multiple therapeutic values, its role in Rheumatoid Arthritis (RA) is under extensive research. The biological effect of β-sitosterol was evident in crude extracts and isolated fractions for various inflammatory disorders. However, it is still unclear if β-sitosterol is the only chemical constituent that contributes most to the reported anti-arthritic activity of JS. **Objectives:** The current study was designed to ascertain the role of β-sitosterol present in the ethanol extract of JS on Complete Freund's Adjuvant (CFA) induced Adjuvant-Induced Arthritis (AIA) model in Wistar rats. **Methodology:** The rats were injected with CFA and treatment (days 0 to 28) with vehicle (control), ethanol extract of JS (JSE 400 mg/kg) and β- sitosterol (2 mg/kg). The estimated parameters were clinical signs, oxidative biomarkers, inflammatory markers, and ankle joint destruction, using the CT scan technique. **Results:** The chronic JSE treatment significantly decreased swelling and reduced the severity of arthritis. Myeloperoxidase activity, an inflammatory marker, decreased while the free radical scavenging activity was significantly elevated. However, β-sitosterol failed to alleviate inflammation and scavenge free radicals in arthritic rats. Similarly, extensive osteopenia and erosion were displayed in β-sitosterol treated rats whereas JSE treatment has marked improvement in bone structure restoration. **Conclusion:** The outcome demonstrates anti-arthritic activity of JSE but β-sitosterol failed to exhibit similar efficacy on its own. Interestingly, HPTLC analysis detected β-sitosterol in JSE but individual β-sitosterol lacked therapeutic outcome of JSE. It suggests that the potent activity of JS cannot be attributed to β-sitosterol alone but other vital chemical constituent/s may contribute to the observed alleviation of rheumatoid arthritis by JSE in rats.

Keywords: Adjuvant Arthritis, CT Scan, Histological Studies, HPTLC, Inflammation,

Abbreviations: AIA: Adjuvant induced arthritis; ANOVA: One Way Analysis of Variance; BS: β–sitosterol; BSA: Bovine Serum Albumin; CFA: Complete Fruend's Adjuvant; CMC: Carboxymethylcellulose; Hb: Hemoglobin; COX: Cyclooxygenase; ESR: Erythrocyte sedimentation rate;COX-2: Cyclooxygenase-2; CT: Computed tomography (CT); DTNB: 5, 5′-dithiobis-(2-nitrobenzoic acid); GSH: Reduced glutathione; TCA: trichloroacetic acid; *JS*: *Jasminum sambac*; HPTLC: High-performance thin-layer chromatography; JSE: Ethanol extract of *Jasminum sambac*; MDA: Melondialdehyde; MPO: Myeloperoxidase; RA: Rheumatoid arthritis; SOD: Superoxide dismutase

1. Introduction

Herbal drugs are major components of the traditional medicinal system for the treatment and management of multiple disorders[1-3]. Ancient and traditional texts highlighted the use of *Jasminum sambac* (L.) Aiton (JS) (Family: Oleaceae) is a potent analgesic and anti-inflammatory herb apart from its cosmetic and healing properties[1,4]. Although JS has been reported to exhibit multiple therapeutic values, its role in RA is under extensive research[5-8].

The prevalence of RA is around 0.3 and 1% of people in the world and severely affects the quality

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of life due to its chronic and debilitating pathology[9]. Numerous natural products are effective against RA and most of them are devoid of major side effects but the lack of scientific evidence on the exact mechanism of action poses a question mark on their therapeutic acceptability[2,3]. Although RA has a multicomponent etiological milieu, JS has been able to counter vital pathological mechanisms like COX activation, inflammatory exacerbation and free radical-induced cellular damage[5,7,8]. Our previous investigation explored the role of sitosterol, iso-quercetin and linalool on COX-2 enzyme using a virtual docking technique to highlight the role of multiple chemical constituents of JS in arthritic conditions where COX-2 overexpression exacerbated inflammatory and immunological reactivity[8]. The chemical constituents of JS contributing to this anti-arthritic activity could range from steroids, flavonoids, and phenols to essential oils, moreover, β-sitosterol also imparts modest inhibition of inflammatory mediators and cytokines[7,10]. The biological effect of β-sitosterol was evident in crude extracts as well as in isolated fractions for various inflammatory disorders[11-14]. However, it is still unclear if β-sitosterol is the only chemical constituent that contributes most to the reported anti-arthritic activity of JS. Does this beneficial outcome result from synergistic or complementary actions of multiple constituents of JS?

The current study was designed to ascertain the role of β-sitosterol present in the ethanol extract of JS on CFA CFA-induced Adjuvant-Induced Arthritis (AIA) model in Wistar rats. The phytochemical characterization and quantification of β-sitosterol was undertaken to validate the extent of its contribution to the pharmacological outcomes of the study.

2. Materials and Methods

2.1 Materials

The Complete Fruend's Adjuvant (heat-killed *Mycobacterium butyricum;* Sigma-Aldrich, USA) and β–sitosterol (BS) (Acros organics, India) were procured. Pyrogallol (Sigma Aldrich, USA), 5, 5′-dithiobis-(2-nitrobenzoic acid (DTNB) (Merck Ltd., India) and thiobarbituric acid (Merck Ltd., India) were procured. Bovine Serum Albumin (BSA), hexadecyl trimethyl ammonium bromide, and ortho-dianisidine dihydrochloride were procured from Himedia Laboratories, India. Other chemicals of analytical grade only were used for the study.

2.2 Extract Preparation

Fresh JS leaves were collected from the medicinal garden of the institute, which was identified (specimen 116/Saif/Sci:/Clg/Bpl.) and authenticated. Shade-dried leaves were pulverized into a coarse powder (500 g) and macerated for 3 days with 95% ethanol and the extract was filtered. The extract was concentrated in a rotary evaporator (Buchi, India) under reduced pressure and stored (desiccator). The ethanol extract of JS leaf (JSE) was made into a suspension with carboxymethylcellulose (CMC, 0.1%) for oral administration to rats at 400 mg/kg of body weight.

2.3 Protein Denaturation Using Bovine Serum Albumin (BSA)

Inhibition of protein denaturation was evaluated using BSA. The reaction mixture consists of 100 µL of plant extract/β-sitosterol and 500 µL of 1% bovine serum albumin. The mixture was heated at 51°C for 20 minutes after allowing it to stand 10 minutes at room temperature. The cooled resultant solution absorbance was measured at 660 nm. Diclofenac sodium was used as positive control[15,16]. Triplicate experiments were carried out and percent inhibition for protein denaturation was determined.

$$\%\text{Inhibition} = 100 - \frac{(A1 - A2)}{A0} X100$$

where, A0, A1, and A2 are the absorbance of positive control, sample, and product control respectively.

2.4 Effect on Protein Denaturation using Egg Albumin

Inhibition of protein denaturation was estimated by adding 2 mL of different concentrations (50–500 µg mL^{-1}) of diclofenac sodium and JSE/β-sitosterol to the reaction mixture. The estimation was carried out spectrophotometrically at 660 nm[15,16] and % inhibition of protein denaturation was determined.

$$\%\text{inhibition} = 1 - \frac{AS}{AC} X100$$

where, AS and AC are the absorbances of the sample and control, respectively.

2.5 Membrane Stabilization/Heat-induced Hemolysis

Red blood cell (10% v/v) suspension was prepared in an isosaline solution and an estimate was carried out. The sample mixture contained 1 mL of different extract concentrations (50–500 µg mL^{-1}) and the control mixture contained 2 mL of distilled water. Diclofenac sodium was used in the standard mixture. The final supernatant of all mixtures was estimated at 560 nm[16,17] and the percentage membrane stabilization was determined.

$$\%\text{protection} = 1 - \frac{\text{ODS}}{\text{ODC}} \text{X}100$$

where, ODS and ODC are the optical densities of the sample and control, respectively.

2.6 Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of JSE and BS was carried out by standard methods. Different concentrations of JSE and β-sitosterol (50 to 500 µg ml^{-1}) in ethanol (1 ml) were used. Absorbance was measured at 230 nm against blank and the percentage was determined.

$$\text{Hydrogen peroxide activity}\% = \frac{(\text{AC} - \text{AS})}{\text{AC}} \text{X}100$$

where AC and AS are the absorbances of the control and sample respectively.

2.7 Experimental Animals

Young adult Wistar rats (200–250 g) were obtained from the Central Animal Facility and housed at standard conditions (light and dark cycle (12/12 h); temperature (22±2 °C) and humidity (40–70%)). Each cage housed two rats and they had free access to food and water *ad libitum*. The experimental protocol (PH/IAEC/VNS/2K21/02) was approved by the Institutional Animal Ethics Committee before experimentation.

2.8 Induction of Arthritis in Wistar Rats

Adjuvant arthritis was induced in male Wistar rats by a single intradermal injection of 0.1 mL CFA at the base of the tail under the influence of light ether anaesthesia. The day of induction was considered as day 0 and the treatment to various groups (n=8) was administered by oral route. A group of rats received an injection of mineral oil on day 0 and subsequently administered with vehicle considered as vehicle control[18,19].

2.9 Treatment Schedule

The treatment schedule was initiated on day 0 of CFA induction. The rats were either orally administered with vehicle (CMC (0.1%) and tween 80 (0.5%)in deionized water) or with treatment (JSE, 400 mg kg^{-1}; β-sitosterol, 2mg kg^{-1}; diclofenac sodium, 2mg kg^{-1}) once daily. The dosing volume was 5 mL kg^{-1} of body weight and change in body weight was noted daily for 28 days. The groups for treatment were; Group I: control (Con) received vehicle; the rats injected with CFA divided into Group II: adjuvant-induced arthritic control (AIA) treated with vehicle; Group III: treated with diclofenac sodium (2 mg kg^{-1}), Group IV: received ethanol extract of JS (JSE, 400 mg kg^{-1}), Group V: treated with β-sitosterol (BS, 2 mg kg^{-1}). On the 29th day, all rats were sacrificed (inhalation of ether anaesthesia) to collect tissue and blood samples for the estimation of various parameters[8,19].

2.10 Assessment of Paw Edema and Clinical Arthritis Score

The severity of arthritis was observed on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day after induction of CFA in every rat to score (Grade 0 to 4). The total clinical arthritic score was obtained as the sum of grades from all four limbs of the rat[18]. In addition to clinical signs, hind paw edema was by calculating the change in displacement of volume on days 0, 14, 21 and 28 of the study.

2.11 Estimation of Erythrocyte Sedimentation Rate (ESR) and Haemoglobin (Hb)

Haematological parameters (ESR and Hb) affected by induction of AIA in rats were estimated on day 29 in blood samples withdrawn after retro-orbital puncture in heparinized Eppendorf tubes. ESR was estimated using standard reported methods[20]. The concentration of Hb in rats was estimated by using the cyanmethemoglobin method[21].

2.12 Organ Weight Ratio

The isolated thymus gland and spleen were weighed and the percentage (%) of wet weight against body weight was calculated in the control and all treatment groups.

2.13 Determination of Free Radical Scavenging Activity

The isolated liver (1 g) was homogenized (ice-cold 10% Trichloroacetic Acid (TCA)) and the activity of Superoxide Dismutase (SOD)[22], reduced Glutathione (GSH)[23] and Melondialdehyde (MDA) formed as the thiobarbituric acid-reactive substance[24] were determined.

2.14 Myeloperoxidase Activity

Inflammatory marker myeloperoxidase enzyme activity was determined using the method reported by Bradley *et al*[25]. The MPO activity was expressed as unit/mg of tissue protein[26].

2.15 Computed Tomography

On the 28th day, the rats were sacrificed; hind paws were extracted to expose knee and ankle joints to a Computed Tomography (CT) scan. All tissue samples were scanned and examined for bony erosions, soft tissue swelling, and narrowing of spaces between the joints using cross-sectional images[27].

2.16 Histological Studies

The hind limbs up to the knees were removed and fixed immediately in 10% formalin. It was decalcified with EDTA for 3 days and then dehydrated. The sections of sagittal slices (4 µm) of tissue were stained with hematoxylin and eosin (H and E) and studied for histological changes.

2.17 HPTLC Studies

JSE was subjected to HPTLC studies for the quantification of BS in a Camag TLC system with Linomat V, scanner 4 and visions CATS 3.1 integration software. Merck silica gel 60F_{254} TLC plates were used for the analysis. A calibration range of 500-3000 ng/spot was used for the standard ß-sitosterol. A volume of 4 µL JSE was applied onto the TLC plate and developed with 20 mL of the mobile phase toluene: chloroform: methanol (8:8:2, v/v/v) with a saturation time of 20 min. The post-chromatographic derivatization was carried out with anisaldehyde-sulphuric acid followed by heating in an oven for 5-10 min at 105°C. Densitometric scanning was performed in absorption-reflection mode at 527 nm. The amount of ß-sitosterol in JSE was calculated from the recorded peak areas.

2.18 Statistical Analysis

The data was expressed as mean±SEM and analyzed with One Way Analysis of Variance (ANOVA) followed by Tukey's test while *in-vitro* assay was analyzed with two-way ANOVA followed by Bonferroni multiple comparison post-hoc test. Arthritic scores were expressed as median values with the quartile range (25-75%) and analyzed with the non-parametric Kruskal-Wallis test. GraphPad Prism (GraphPad Software, USA) was used for statistical analysis and significant differences in values were considered as $p< 0.05$.

3. Results

3.1 Effects on Protein Denaturation

The protein destruction expressed as percentage change in the extent of protein degeneration was significantly inhibited by JSE and BS as compared with diclofenac treated but there was no marked between percentage inhibition exhibited by JSE and BS (Figures 1A and B). The JSE and BS showed concentration-dependent impediment of protein denaturation at the concentration range (50-500 µg mL^{-1}) evaluated in the BSA assay. However, the denaturation of protein in egg albumin assay demonstrated a significant difference in the extent of inhibition of protein denaturation by JSE and BS.

3.2 Effect of Treatment on Membrane Stabilization

The membrane stabilization using RBC was significantly increased in all treatment groups but the dose dependency to stabilize the membrane (38.33±0.33 %, 63.66±0.33 %, 77.33±0.33 % and 84.00±0.57 %) was observed only in JSE all the dose concentrations (50, 100, 250 and 500 µg mL^{-1}) (Figure 2A).

3.3 Hydrogen Peroxide Scavenging Activity

The outcomes of H_2O_2 scavenging activity failed to display any consistent pattern as well as a marked difference in the ability of JSE and BS to scavenge the free radicals generated in the reaction mixture after the addition of H_2O_2 (Figure 2B). Diclofenac sodium was taken as standard and showed excellent scavenging activity of H_2O_2 with percentage inhibition (63.33±7.90 %).

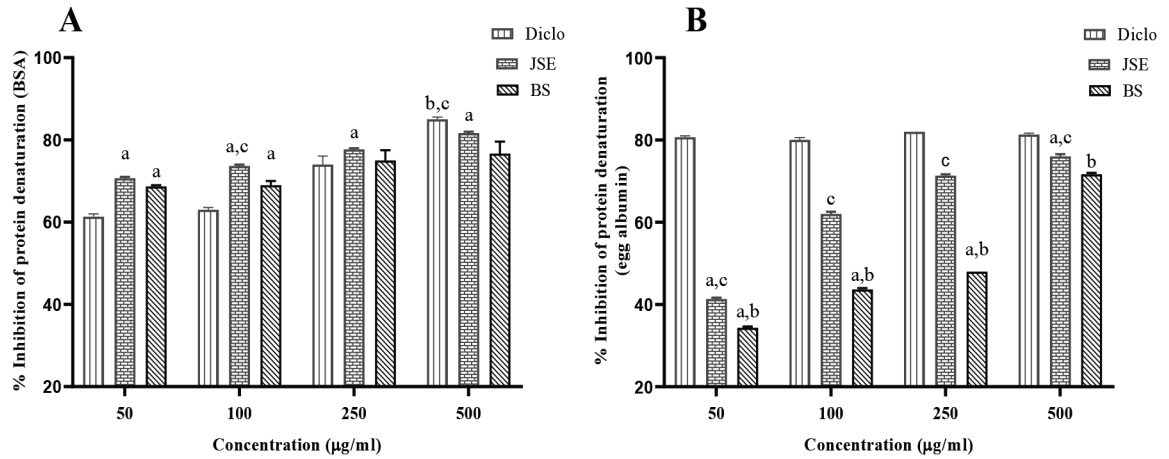


Figure 1. (A). Effect of JSE, BS and Diclo against protein denaturation using bovine serum albumin. (B) Effect of JSE, BS, and Diclo against protein denaturation using egg albumin. All the values are expressed as mean ± SEM (n = 3), using two-way ANOVA followed by Bonferroni test. [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS.

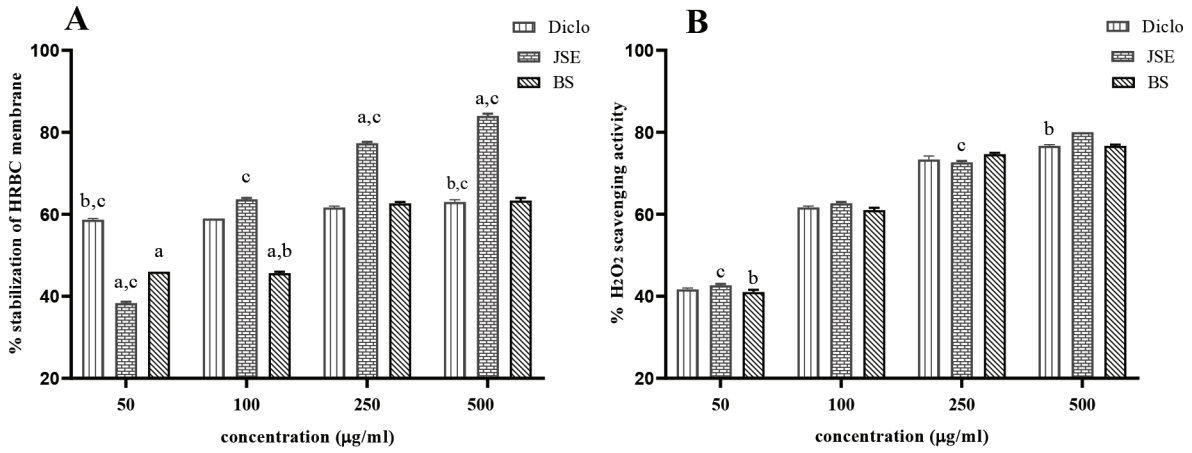


Figure 2. (A). Effect of JSE, BS, and diclofenac sodium on red blood cell membrane lysis (HRBC). (B) Effect on H_2O_2 scavenging activities. All the values are expressed as mean ± SEM (n = 3), using two-way ANOVA followed by Bonferroni test. [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS.

3.4 Paw Edema and Inflammation

The paw edema measured on the 28th of CFA injection in the AIA group showed a significant ($p<0.05$) increase in (6.64±0.14 mL) as compared with the rats in the control group (2.86±0.06 mL). The increment in paw volume of arthritic rats was observed from day 14 which was maximum at day 28. However, on the 28th day, a significant ($p<0.05$) reduction in inflammation was observed in rats treated with diclofenac sodium (2.15±0.07 mL) and JSE (2.73±0.14 mL). The reduction in swelling was also observed with BS treatment (5.25±0.03 mL) but the change in paw volume was not significant compared to vehicle-treated AIA rats (Figure 3).

3.5 Arthritis Score

The consistent increase in the arthritic score of AIA rats continued till the end of the observation period on day 28. The treatment with JSE has a significant reduction in clinical signs of arthritis from day 14 onwards till day 28 while treatment with BS failed to show any marked restriction of progression in arthritis (Figure 4).

3.6 Effect on Body Weight

Loss of body weight is associated with disease progression and chronic body weight reduction indicates cachexia. The decline in the body weight of AIA rats during the late phase of disease progression suggests muscle mass wasting and reduced locomotor

activity (↓ 13%). However, the treatment with JSE stalled the decrease in body weight and posted recovery (↑ 7%) on day 28 but treatment with BS continued to exhibit weight loss (↓ 15%) in treated rats (Figure 5).

3.7 Effect on Hematological Parameters

Induction of arthritis resulted in a significant reduction in the Hb level of AIA rats (9.43±0.23 mg dL^{-1}) as compared to the control group (12.93±0.09 mg dL^{-1}).

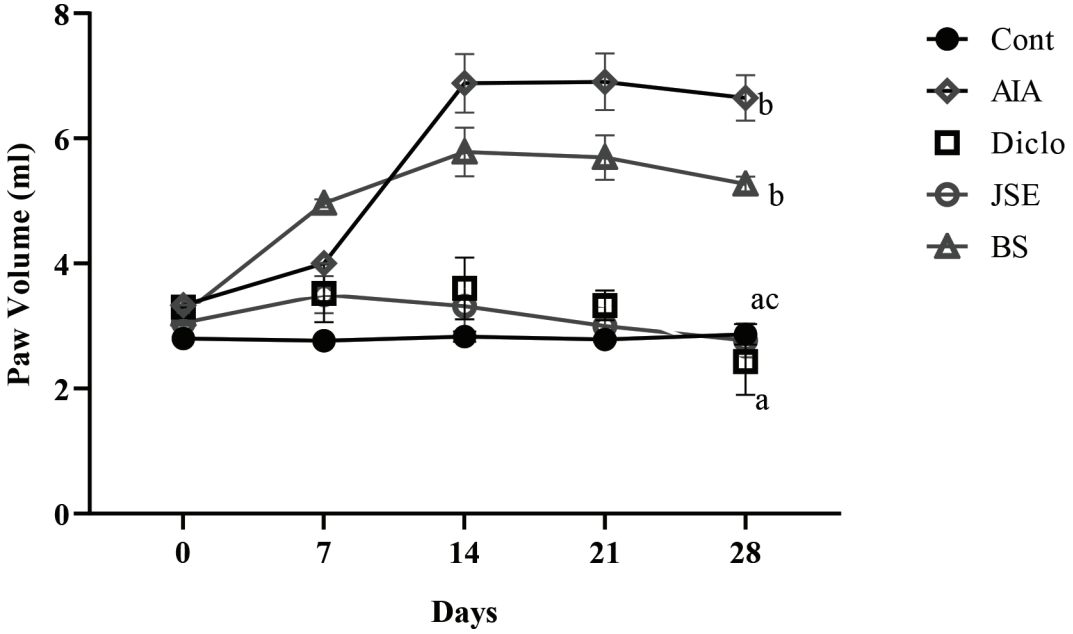


Figure 3. Effect on hind paws volume in CFA-induced arthritis. All data were expressed as Mean ± SEM. Statistical differences were determined by One- way ANOVA followed by Tukey's Test. [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS.

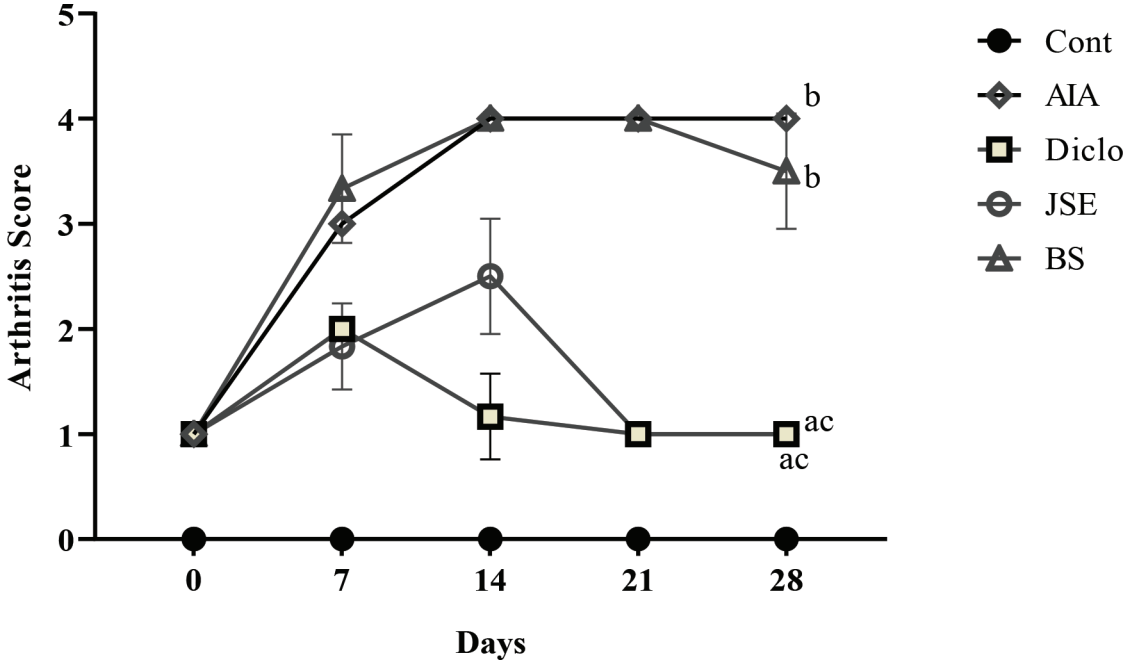


Figure 4. Effect on arthritis index. All data were expressed as median values. Statistical difference was determined by One-way ANOVA (non-parametric Kruskal- Wallis test). [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS. The values represented as median of scores and respective interquartile range of 28th day (n = 8).

The pain threshold reduction leads to enhanced discomfort that could reflect articular damage and disability in RA patients whose Hb levels had a marked decline during disease development. Treatment with diclofenac (12.18±0.10 mg dL^{-1}), JSE (10.55±0.38 mg dL^{-1}) and BS (10.83±0.18 mg dL^{-1}) showed a significant increase in Hb on 28th day. Similarly, the sedimentation rate of erythrocytes was markedly increased in the AIA group (6.83±0.30 mm) while treatment with JSE and BS had reduced the sedimentation (3.50±0.22 mm & 5.16±0.30 mm) respectively as compared to AIA but this change among the groups was not significant with BS (Table 1).

3.8 Thymus and Spleen Index

The AIA group had significantly higher spleen weight (296.2±16.37 vs 191.5±1.86 mg 100 g^{-1}) and decreased thymus weight (85.89±1.61mg 100g^{-1} vs 139.2±6.82 mg 100g^{-1}) as compared to a normal control group. The JSE-treated group significantly prevented the increase in spleen weight (233.2±10.19 mg 100g^{-1}) while there was a decrease in thymus weight in JSE-treated rats (116.3±3.48 mg 100g^{-1}) (Table 1).

3.9 Alleviation of Oxidative Injury

A significant increase in the level of lipid peroxidation in the liver was noted in arthritic rats (3.08±0.10 µM mg^{-1} of TP). On the contrary,

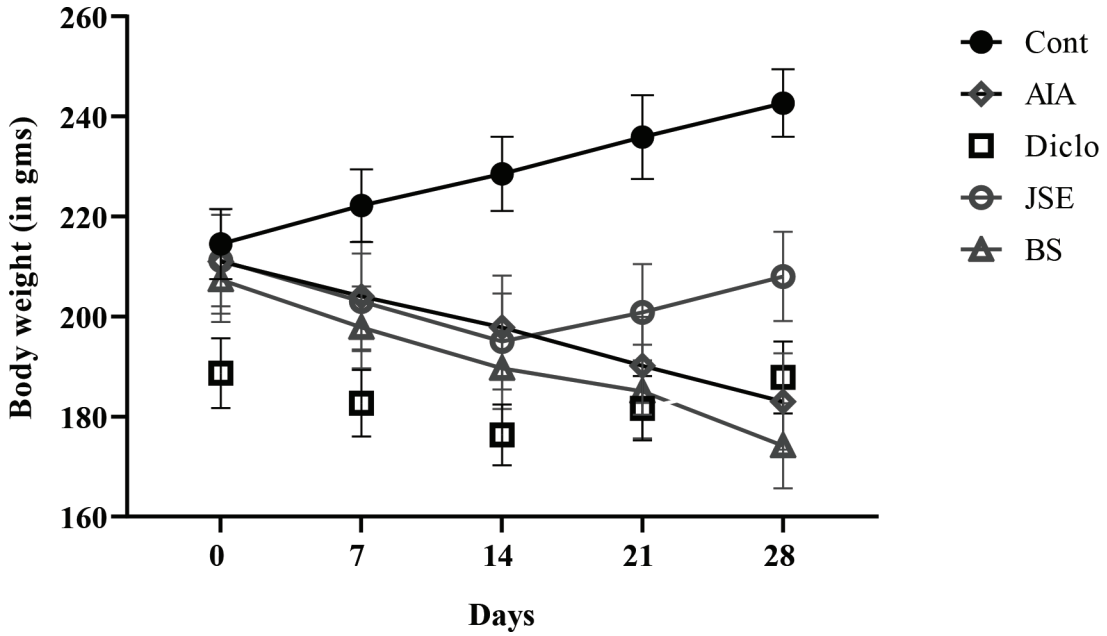


Figure 5. Effect on body weight of CFA-induced arthritic and treated. All data were expressed as Mean ± SEM. The statistical difference was determined by One- way ANOVA followed by Tukey's Test. [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS.

Table 1. Tissue weight and hematological parameters.

Groups	Thymus weight (mg 100 g-1 of body weight)	Spleen weight (mg 100 g-1 of body weight)	Hb (g dL-1)	ESR (mm)
Con	139.2 ± 6.82a	191.5 ± 1.86a	12.93 ± 0.09	2.50 ± 0.34a
AIA	85.89 ± 1.61	296.2 ± 16.37	9.43 ± 0.23b	6.83 ± 0.30b
Diclo	124.5 ± 12.05a	216.8 ± 7.88a	12.18 ± 0.10	2.66 ± 0.33a
JSE	116.3 ± 3.48a	233.2 ± 10.19a	10.55 ± 0.38	3.50 ± 0.22a
BS	106.8 ± 2.02	265.1 ± 13.71b	10.83 ± 0.18	5.16 ± 0.30a

Values presented as mean ± SEM (n = 8) and statistical differences were determined by One- way ANOVA followed by Tukey's Test 29th day. [a]*p* against AIA Control (< 0.05); [b]*p* against Vehicle Control (< 0.05).

decreased level of SOD (2.14±0.04 IU mg^{-1} protein) and GSH (1.46±0.07 µg mg^{-1}) was observed in the AIA group as compared to the control group SOD (3.37±0.07 IU mg^{-1} protein), GSH (3.51±0.07 µg mg^{-1}). Treatment with JSE significantly restored SOD (2.83±0.04 IU mg^{-1} protein) and GSH (3.06±0.07µg mg^{-1}) levels. However, JSE-treated rats showed a marked decrease in the level of lipid peroxidation (Figures 6A, 6B, and 6C).

3.10 Myeloperoxidase Activity

Myeloperoxidase activity is one of the vital parameters for inflammation. JSE-treated rats significantly reduced MPO activity but the BS-treated treated reduced MPO activity (Figure 6D).

3.11 Photographic Observation of Paw

AIA rats showed edema after injection of CFA as compared to the control group, whereas in the diclofenac-treated group significant decrease in edema was observed. On the 28th day rats treated with JSE resolved paw swelling with redness by suppressing the spread of edema more as compared to the BS-treated rats (Figure 7).

3.12 Computed Tomography (CT Scan)

Soft tissue swelling is the early developmental sign which continues throughout the disease state, whereas

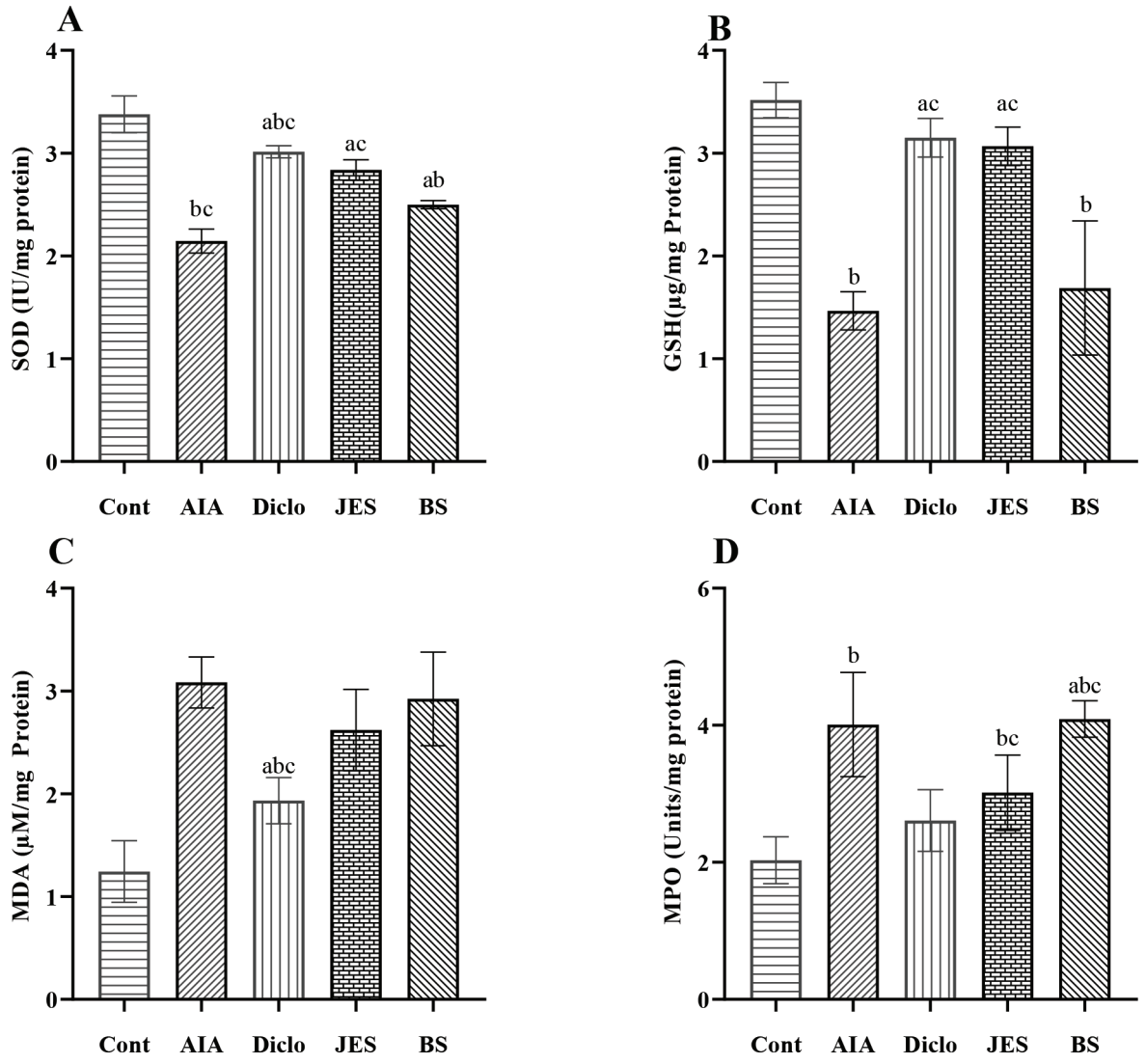


Figure 6. Effect of JSE and BS on oxidative stress biomarkers (A) SOD levels in the liver (B) GSH levels in the liver (C) MDA levels in the liver (D) Effect on Myeloperoxidase activity. All data were expressed as Mean ± SEM. The statistical difference was determined by One- way ANOVA followed by Tukey's Test. [a]p< 0.05 against Diclo; [b]p<0.05 against JSE; [c]p<0.05 against BS.

prominent changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis. The soft tissue swelling in the hind paws of the rats immediately after sacrifice and AIA rats had marked differences (Figure 8I). The bone erosion was evident in the form of erosion in the tibia and fibula bones of the arthritic rats (Figure 8IIB) while prominent cessation of bone resorption was observed in JSE treatment animals. However, treatment with BS failed to restrict or recover the bone resorption at the end of 28 days. Similarly, ankle joint erosion was also displayed as the destruction of tarsal bone in arthritic rats (Figure 8III) but treatment with JSE restricted the damage to a minimum (Figures 8IIIB and 8IIID). The ankle joint and tarsal bone in BS-treated rats showed signs of marked erosion resembling the damage caused by arthritis (Figure 8IIIE).

The knee joint space visible in normal animals was narrowed down in AIA animals. Although JSE treatment did display relatively larger joint space than AIA rats it is narrower when compared with control rats. The smooth texture of knee joints observed in control animals is markedly altered in AIA rats, however, closer images would have given conclusive observation in other treatment groups (Figure 8IV).

3.13 Histopathology of Ankle Joints

No histological abnormalities were demonstrated in the joints and cartilage of normal rats (Figure 9A). Meanwhile, the joints of arthritic rats (CFA) showed destructive arthritis. In addition, pannus formation indicated massive synovial inflammation and synovial hyperplasia with intense leukocyte infiltration. (Figure 9B). On the other hand, the JSE-treated group revealed significant improvement in synovial membrane integrity reduced infiltrated inflammatory cells and restriction of bone marrow destruction. (Figure 9D). However, treatment with BS for 28 days displayed marked synovial

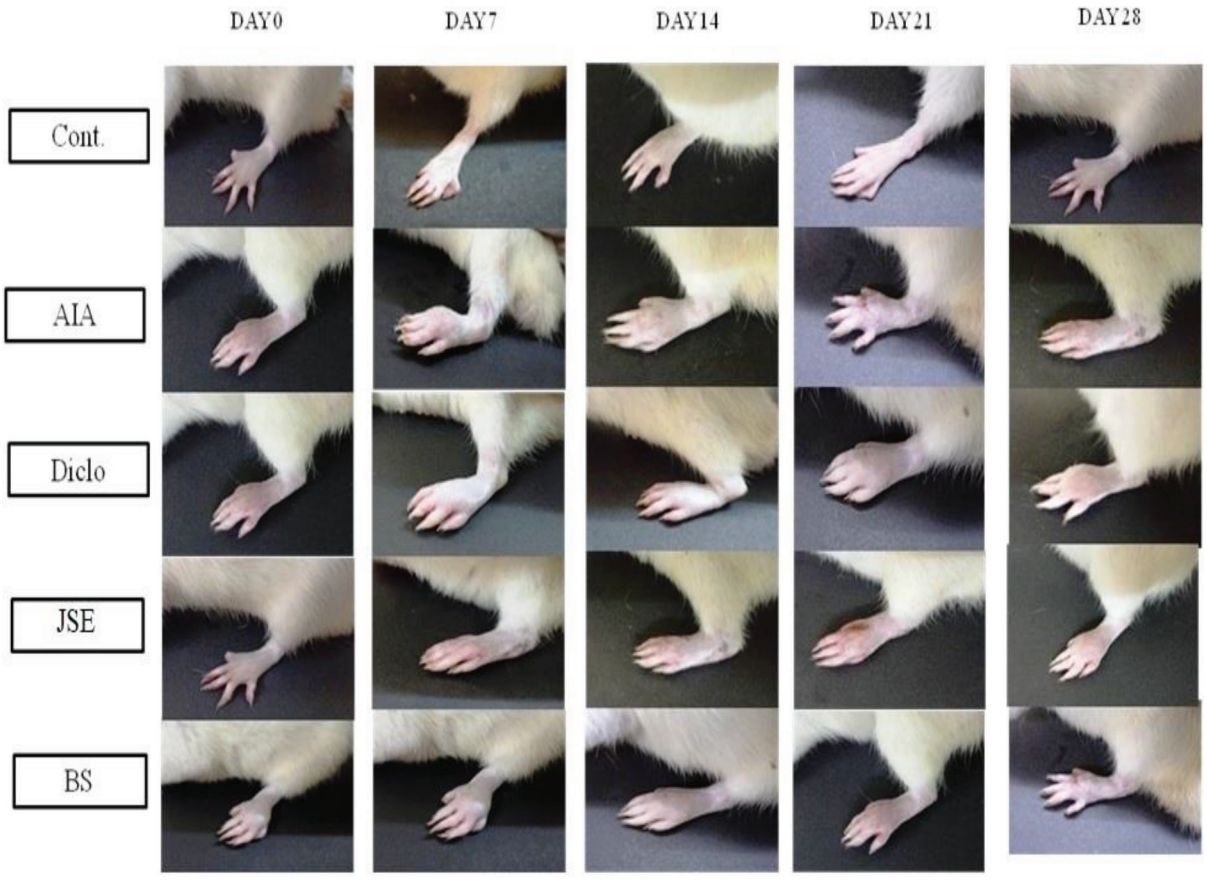
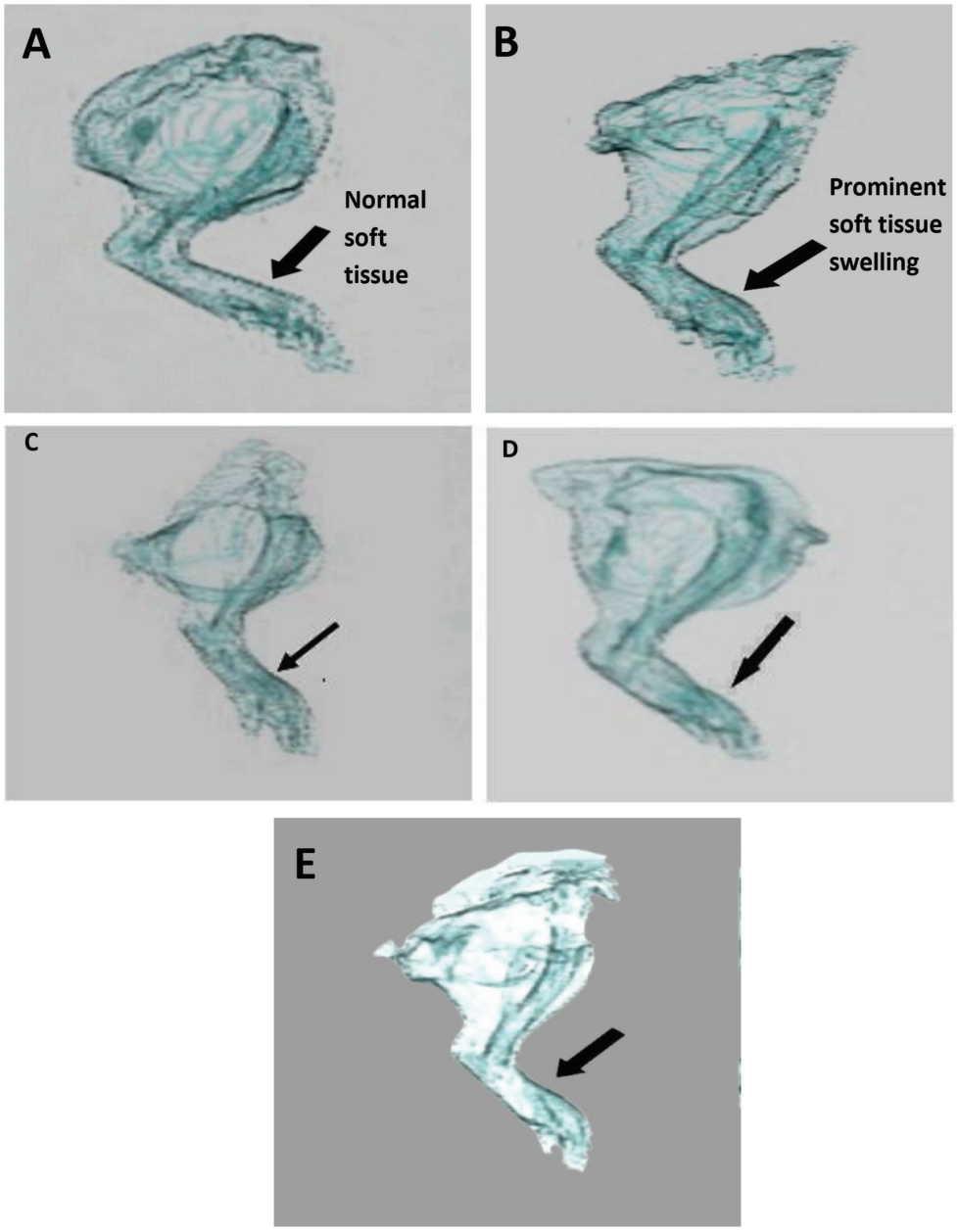


Figure 7. Photographic Representation of rat paws treated with JSE and BS on different days in CFA-induced arthritis i.e, Day 0, 7, 14, 21 & 28.



(I) CT Scan Images of Left Hind Paw on 29th Day; showing soft tissue swelling. (A) Cont, (B) AIA, (C) Diclofenac, (D) JSE and (E) BS.
(II) CT Scan Images of Right Hind Paw on 29th Day where shows degeneration of bone. (A) Cont, (B) AIA, (C) Diclofenac, (D) JSE and (E) BS CT Scan image of Hind paw is shown - Increased number of degenerative uptake were observed in (A) AIA control group as compared to vehicle control group whereas less degenerative uptake was observed in the (B) Standard group followed by JSE and BS group as compared to AIA control Group. Bone marrow shows patches of low signal intensity and high signal intensity on animals, with focal areas of bone erosion. The soft tissue swelling and edema have spread to the ankle joint.
(III) Shows CT image shows more extensive osteopenia and endosteal erosion. (A) Cont, (B) AIA, (C) Diclofenac, (D) JSE and (E) BS. The CT Scan features of the rat's joints in the AIA model are shown in (B) AIA control group, soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The standard drug (C) Diclofenac sodium treated group has prevented this bony destruction and also there is no swelling of the joint. Similar to histopathological studies, (D) JSE have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces, when compared to the BS, treated group.
(IV) CT Scan Images of Ankle joints on 29th Day where arrow showing joint space narrowing (A) Cont, (B) AIA, (C) Diclofenac, (D) JSE, and (E) BS.

Figure 8. Computed Tomography (CT scan).

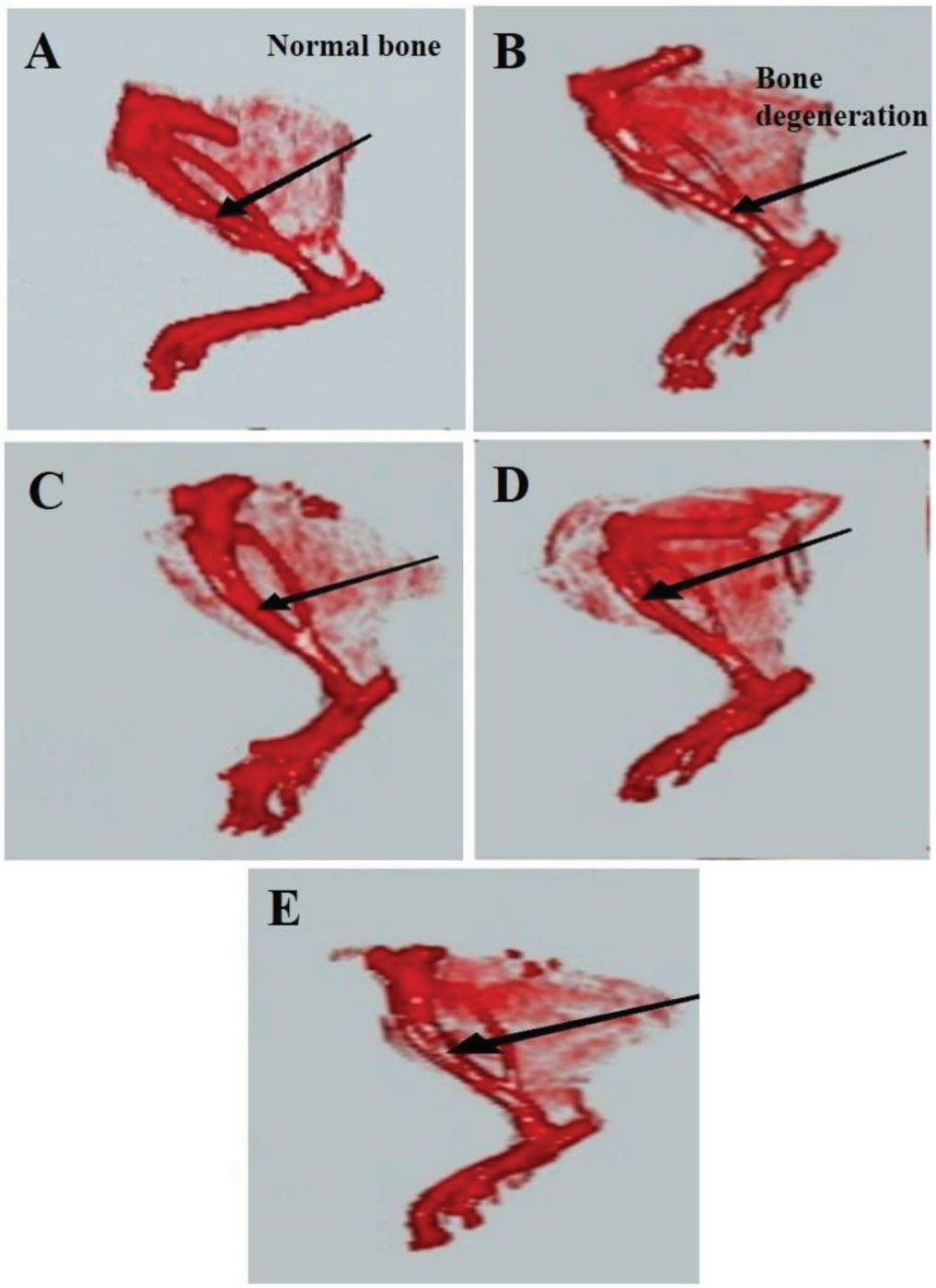


Figure 8. Continued...

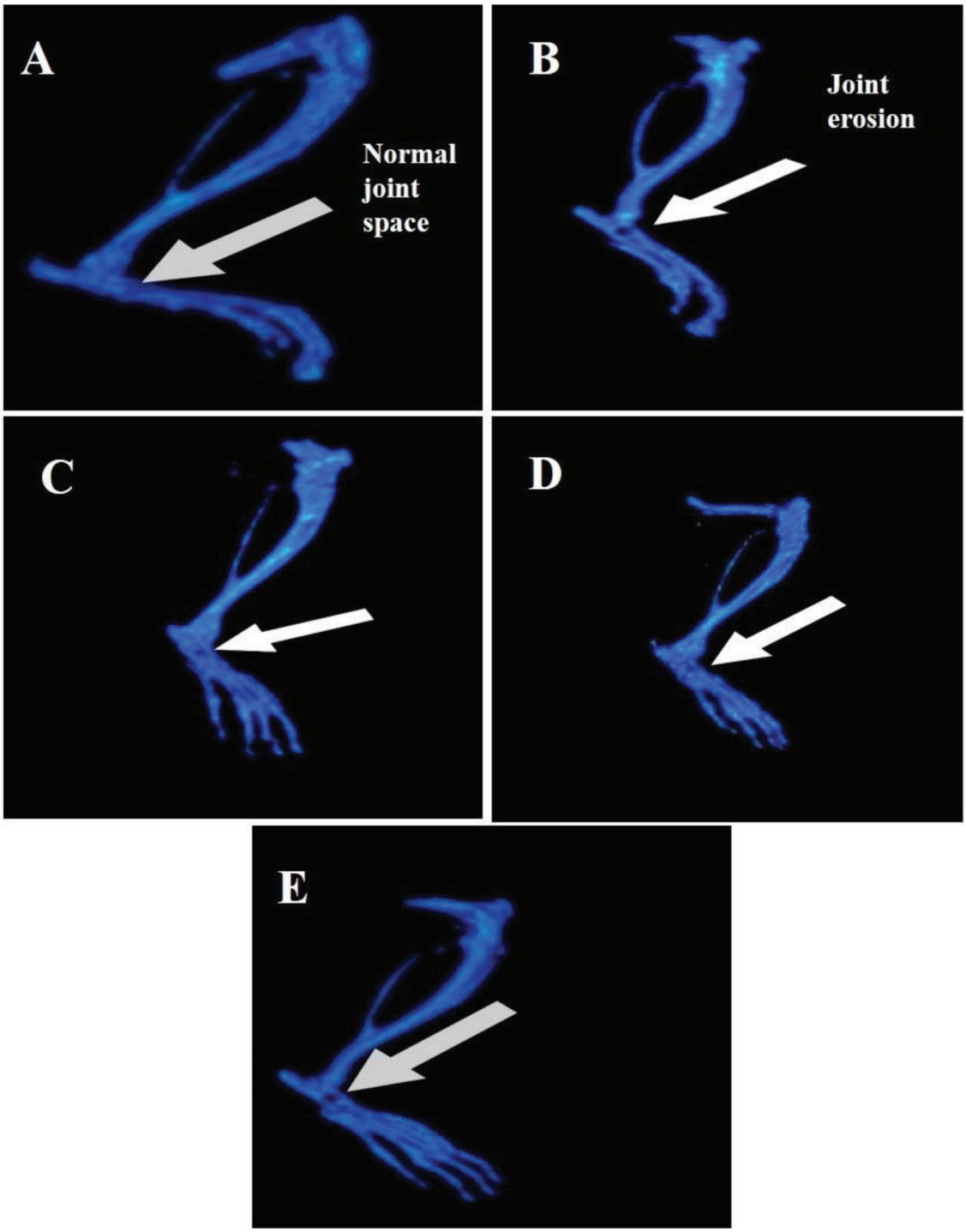


Figure 8. Continued...

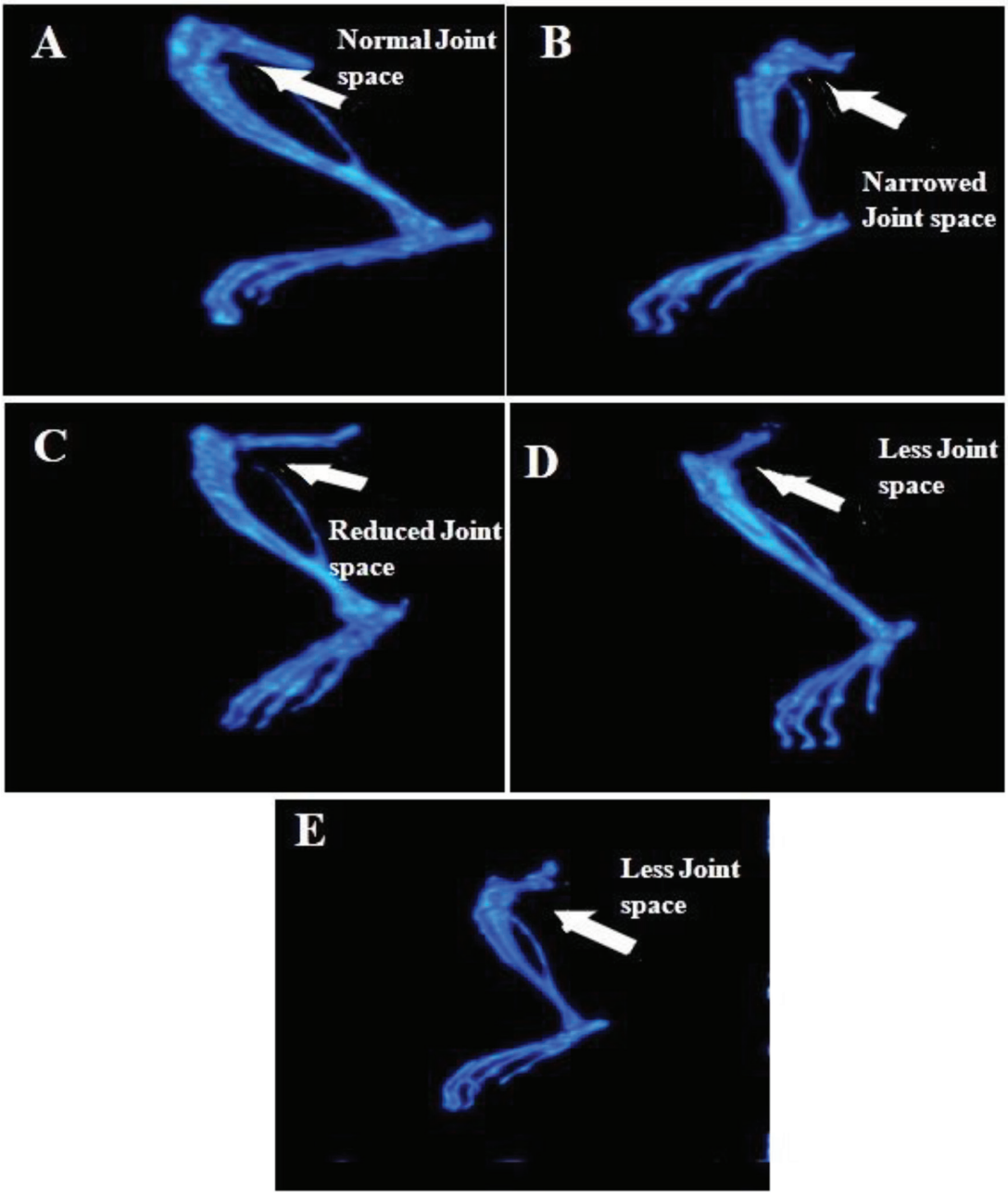
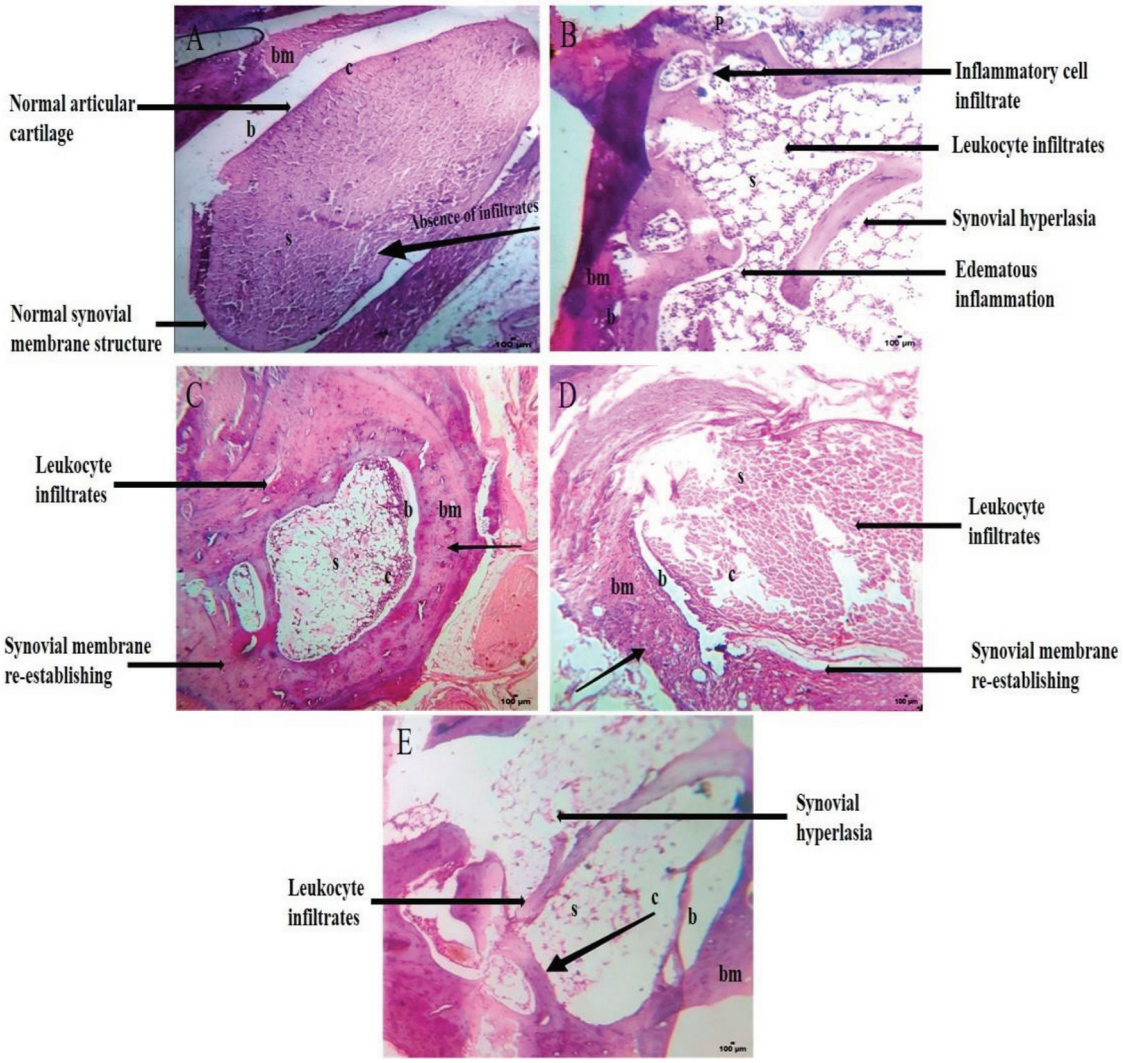


Figure 8. Continued...



Histopathology examination of arthritic joints in which (A) Control group- histology of normal ankle joint normal articular cartilage and absence of infiltrate in the synovium. ; (B) AIA control group- prominent abnormalities like edema formation, degeneration with partial erosion of the cartilage, destruction of bone marrow, and extensive infiltration of inflammatory exudates in the articular surface; (C) Standard group- normal bone marrow with less cellular infiltrates and reduced edema formation; (D) JSE group- reduced inflammatory sign, absence of edema and reformation of synovial membrane; (E) BS group- showed cellular infiltrates on the articular surface with less cartilage destruction and moderate recovery injury of the synovial membrane was observed. b- Bone; bm- Bone marrow; c- Cartilage; P- Synovial Pannus; s- Synovium; Ankle joint synovial membrane structure in Normal control rat; Edematous inflammation; Synovial hyperplasia with increased vascularity; Inflammatory cell infiltrate (involve T-lymphocyte); Synovial membrane structure re-establishing with the less edema and inflammatory cell.

Figure 9. Histopathology of ankle joints.

hyperplasia, leukocyte infiltration, and incomplete restoration of bone marrow (Figure 9E).

3.14 HPTLC Studies

The developed TLC plate showed a BS band in JSE. A spectral overlay was carried out of the band with R_f of 0.67 in JSE corresponding to BS bands and it was confirmed as BS. The calibration range of standard BS for 500-3000 ng/spot was linear (R^2=0.997) (Figures 10-12). The quantity of BS was 0.7 mg/100 mg of JSE and the total BS in the administered JSE dose of 400 mg kg^{-1} was found to be 2.8 mg.

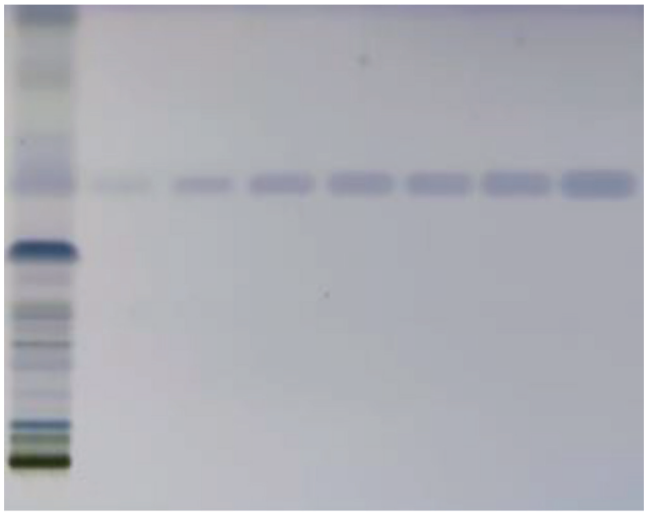


Figure 10. TLC plate showing ß-sitosterol in JSE.

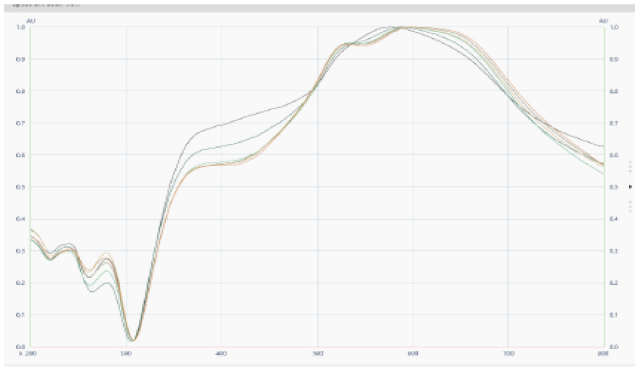


Figure 11. Spectral overlay of standard β-sitosterol bands and the corresponding band in JSE.

4. Discussion

Traditional herbal medicines are commonly used for the treatment of a wide range of diseases. Often, crude extracts are the traditional choice for therapeutic uses; however ancient as well as modern techniques facilitate the isolation of individual chemical constituents from the extract for its ethno-pharmacological use. Numerous isolated active constituents from plant sources (artemisinin from *Artemisia annua* as an antimalarial drug) have been developed as potent drugs but there are incidences where isolation of a single ingredient from extract failed to exhibit similar pharmacological activity as that of crude extract[28-30]. The current investigation was designed to ascertain the role of β-sitosterol in the previously reported anti-arthritic activity of *Jasminum sambac*. We contemplated the traditional Indian medicinal system principle of complementary and synergistic coexistence of multiple chemical constituents to promote therapeutic outcomes of herbal medicine.

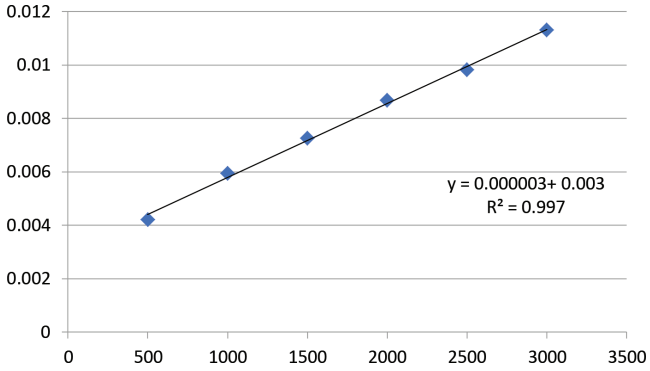


Figure 12. Linearity of BS for 500-3000 ng spot^{-1}.

Herein, we investigated the ethanol extract of *Jasminum sambac* and β-sitosterol for its effect on arthritis induced by CFA in rats. Our study demonstrated that the reported anti-arthritic activity of *Jasminum sambac* could be attributed to multiple chemical constituents, present in *Jasminum sambac*, and not solely attributed to the presence of β-sitosterol in the extract. Herein, we envisaged the synergistic activity of multicomponent extract of *Jasminum sambac* mediated through pharmacokinetic or physiochemical alterations brought by more than one active constituent. Another possibility could be the simultaneous trigger of multiple targets by chemical constituents to exhibit synergistic physiological activity[31].

The pharmacological activity was evaluated after 28 days of repeated dose administration of *Jasminum sambac* and β-sitosterol to respective groups. We substantiated the pharmacological outcome from *in vivo* studies with the observations of the *in vitro* experiments indicating the anti-arthritic activity of the test compounds[32]. Functional and structural damage characterized by arthritic patients is intricately associated with chronic inflammatory reactivity induced by the antigen[33]. Initial stages of disease progression are marked by acute inflammation and immunological exacerbation displayed in the form of cytokine release and Cyclooxygenase enzyme (COX) activation[34]. Tissue protein denaturation is linked to auto-antigen generation that leads to inflammation similarly, cell membrane destabilization due to

heat-induced RBCs lysis serves as an additional trigger for chronic inflammatory reactivity contributing to arthritic damage[35-37]. Herein, both treatments, *Jasminum sambac* and β-sitosterol, reduced protein denaturation and improved membrane stability but *Jasminum sambac* has a significant advantage over β-sitosterol (at 100 µg mL^{-1}) with both indicators of inflammatory reactivity.

It is previously known that the generation of free radical species induced oxidative stress, a hallmark of chronic inflammatory diseases, is cross-linked to inflammatory reactivity[8,38,39]. Exacerbation of oxidative stress culminates in the progression of arthritis demonstrated by neutrophil infiltration, pannus formation and structural damage[40]. The scavenging ability of *Jasminum sambac* restricted debilitating mechanisms of rheumatoid arthritis like cachexia, over-activated immunological response, inflammatory reactivity and functional tissue loss[7,41-43]. Oxidative biomarkers: SOD activity, GSH content and lipid peroxidation were positively modulated with ethanol extract of *Jasminum sambac as well as* with **β**-sitosterol. This anti-oxidant potential coupled with observed inhibition of inflammatory reactivity-MPO activity could contribute to the reduced rate of erythrocyte sedimentation and improved thymus-spleen ratio. The outcome of the current investigation resembles clinical evidence in rheumatoid arthritis patients[44]. Interestingly, the multi-component extract exhibited better (significant change in most parameters) activity over β-sitosterol treatment at the end of 28 days. A similar pattern of synergistic activity (although for different pharmacological action) of cannabis crude extract was observed when compared with isolated constituents from cannabis extract[30].

Herein, we employed the CT scan technique to explore structural changes especially bone erosion which was evident on the exterior surface of the bone and often associated with pannus formation[45]. The bone degradation was markedly reduced with *Jasminum sambac* treatment but the recovery was incomplete with **β**-sitosterol. Similarly, tissue swelling was also evident with the CT scan and the outcome with treatment of *Jasminum sambac* extract was better than the reduction effected by β-sitosterol administration to arthritic rats. Joint erosion and articular space were positively modulated by crude extract of *Jasminum sambac* than that of β-sitosterol suggesting synergistic or complementary action of multiple chemical constituents, in addition to β-sitosterol, present in ethanol extract of *Jasminum sambac*.

We further pondered upon the role of β-sitosterol in the multifaceted anti-arthritic activity of the extract of *Jasminum sambac* by quantifying the content of β-sitosterol in the extract. The standard β-sitosterol with R_f 0.67 matched with a similar band in JSE. The comparative spectral overlay of this band confirmed the presence of β-sitosterol in JSE. The calibration curve with a range of 500-3000 ng $spot^{-1}$ was found to be linear. The quantification data revealed that 2.8 mg of β-sitosterol was present in the administered experimental dose of 400 mg kg^{-1} of JSE. Although we employed β-sitosterol at 2 mg kg^{-1} for the treatment of arthritic rats the outcomes were better with JSE; it suggests that there are other chemical constituent/s in *Jasminum sambac* which would have contributed to potent synergistic anti-arthritic activity. Previous studies reported the presence of salicylic acid, sambacin, rutin and (+)-jasminoids could be responsible for anti-inflammatory actions[46-49]. Moreover, BS is also present in many plant species, if the observed effect is mainly due to BS, then all these plants will have to exhibit potent anti-arthritic activity, which is not so. Hence, it is evident that the exhibited anti-arthritic activity of JSE is due to synergistic effects only. Nevertheless, there are a few limitations of the study, especially associated with the dose range of β-sitosterol and estimation of other chemical constituent/s in *Jasminum sambac* for its anti-arthritic activity which we proposed as our objectives for future studies using advanced techniques. The multifaceted pathology of arthritis, especially RA, requires a multipronged approach to target immune and inflammatory components. It is imperative to further explore synergistic and complementary therapeutic tools to ameliorate the debilitating features of arthritis. *Jasminum sambac*, with its chemical constituents, could provide a way forward to advance a better understanding of the explanation of the actions of herbal products.

In conclusion, we proposed a multi-component contribution to the beneficial synergistic effect of *Jasminum sambac* on the experimental model of rheumatoid arthritis. The use of advanced tools like CT scans, employed in a murine model, can help to

ascertain specific effects on clinical features resembling arthritic patients. Further, phytochemical and DNA fingerprinting of *Jasminum sambac* could provide insight into the exact mechanisms involved in this synergistic or complementary therapeutic activity on rheumatoid arthritis.

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