ORIGINAL ARTICLE

Evaluation of Anti-inflammatory, Anti-arthritic and Antioxidant Activities of *Bauhinia variegata, Boerhavia diffusa* and *Limonia acidissima* Extracts in Complete Freund's Adjuvant–Induced Arthritic Rats

Chandrakala Aruga[1], Saravanan Ravindran[2], R. Karthikeyan[3]

[1]Department of Pharmacology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India, [2]Department of Pharmaceutics, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India, [3]Department of Pharmacognosy, School of Pharmacy, Sri Balaji Vidyapeeth, Pillayarkuppam, Puducherry, India

Abstract

Objective: This study aimed to evaluate the anti-inflammatory, anti-arthritic, and antioxidant activities of ethanolic extracts of *Bauhinia variegata, Boerhavia diffusa*, and *Limonia acidissima.* **Methods:** Anti-arthritic activity was assessed using Complete Freund's Adjuvant (CFA)-induced arthritis in Wistar rats. Animals were treated orally with plant extracts (100, 200, and 400 mg/kg) for 14 days, while diclofenac sodium (10 mg/kg) served as the standard. Parameters evaluated included paw volume, paw diameter, arthritis score, body weight, hematological indices (RBC, WBC, Hb, ESR), biochemical markers (AST, ALT, ALP, CRP, RF), pro-inflammatory cytokines (TNF-α, IL-1β), antioxidant enzymes (SOD, GPx, GRx), and histopathological and radiological changes. **Results:** The extracts significantly reduced paw edema, paw diameter, and arthritis scores in a dose-dependent manner. Hematological alterations were normalized, with increased RBC and hemoglobin levels and decreased WBC and ESR. Elevated liver enzymes, CRP, and rheumatoid factor levels were reduced. Pro-inflammatory cytokines were suppressed, while antioxidant enzyme levels were enhanced. Histopathological and radiological findings confirmed reduced inflammation and joint damage. The highest dose (400 mg/kg) showed effects comparable to diclofenac sodium. **Conclusion:** The studied plant extracts exhibit significant anti-inflammatory, anti-arthritic, and antioxidant activities, indicating their potential as safer therapeutic agents for managing inflammatory arthritis.

Key words: Anti-arthritic activity, complete Freund's Adjuvant, medicinal plants, oxidative stress, pro-inflammatory cytokines

INTRODUCTION

Arthritis and its related musculoskeletal imbalances are prevalent conditions that impact millions, significantly limiting their everyday activities. The phrase commonly employed by medical professionals refers to the progressive inflammatory responses affecting one or more joint structures due to numerous etiologies, including traumatic, rheumatic, and degenerative factors, leading to muscular stiffness and limited physical mobility. Moreover, arthritis impacts individuals across all age demographics, ethnicities, genders, and geographical locations, encompassing over 100 distinct varieties, including juvenile arthritis, rheumatoid arthritis, ankylosing spondylitis, gout, psoriatic arthritis, and osteoarthritis, the latter being degenerative in nature.[1] The clinical manifestations in afflicted individuals may differ

Address for correspondence:
Saravanan Ravindran, Department of Pharmaceutics, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
Mobile: +91-9700784826.
E-mail: rssrmcp@gmail.com

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among patients and encompass a spectrum from minor pain and edema to severe conditions such as full or partial joint immobility, muscle atrophy, and contractures. Subsequently, the pharmacological regimen involves the administration of non-steroidal anti-inflammatory drugs (NSAIDs) as the primary treatment; however, prolonged use has resulted in potential adverse effects, including gastroduodenal disorders and renal insufficiency, likely caused by the inhibition of cyclo-oxygenase, which reduces prostaglandin levels.[2] Despite the availability of numerous therapies, such as NSAIDs, corticosteroids, and disease-modifying antirheumatic drugs, these primarily address symptomatic issues and do not target the underlying pathological origins, such as membrane stabilization and protein denaturation. Furthermore, the aforementioned therapeutic modalities may result in significant hepatic injury, gastrointestinal hemorrhage, hospitalization, and mortality.[3]

Consequently, to overcome these challenges and identify a safer yet as effective therapeutic alternative, researchers are exploring plants as a medicinal resource. Initially, these plant-based medical systems have been the foundation of traditional or ethnomedicines, practiced in India and many regions such as China, Africa, and South America. A significant portion of this indigenous knowledge was subsequently codified, recorded, and ultimately integrated into structured medical systems such as Ayurveda, Unani, and Siddha. Historically, tribal people across India utilized plants for several therapeutic purposes.[4] The present work concentrated on its *Bauhinia variegata*, *Boerhavia diffusa*, And *Limonia acidissima in vitro* anti-inflammatory activity and *in vivo* arthritic efficacy utilizing the complete Freund's adjuvant (CFA) produced arthritis model.

MATERIALS AND METHODS

Induction of arthritis

CFA is used for the induction of arthritis in rats and is currently considered as a model for reactive arthritis. In 1956, Pearson found that rats immunized with CFA containing *Mycobacterium tuberculosis* developed arthritis. Wistar-Lewis, Sprague-Dawley, Wistar, and Buffalo rat strains are known as high responders to adjuvant-induced arthritis (AIA).

Wistar rats (150–200 g) were taken and divided into six groups, each group contains six animals. The animals were fasted overnight before the experiment. On 1st day, they were injected into the subplantar region of the right hind paw with 100 µL/mL of CFA. This consists of 5 mg *M. tuberculosis* (Difco) being suspended in heavy paraffin oil (Merck) by thoroughly grinding with a mortar and pestle to give a concentration of 5 mg/ml. The paw volume of all the animal groups was measured by plethysmograph on 0, 7, 14, 21, and 28th days after the injection of CFA.

Experimental design

The animals were dosing with the test compounds, and the standard was started on the same day and continued for 14 days. Paw volumes of injected sides and body weights were recorded on the day of injection, whereby paw volume was measured plethysmographically with equipment as described in the paw edema tests. On days 7, 14, 21, and 28th, the volumes of the injected paw were measured, indicating the primary lesion and the influence of therapeutic agents on this phase. From day 14th to 28th, the animals were not dosed with the test compound or the standard. On the 28th day, the body weights were determined, and the severities of the primary lesions were evaluated.

S. No.	Groups	Induction and treatment for 28 days
1	Group I (Normal control)	Normal control group rats were received normal saline 5 mL/kg b.wt for 14 days.
2	Group II (Arthritic control)	Arthritic control rats or negative control rats (CFA induction then given normal saline 5.0 mL/kg/day/p.o., from day 1 to until day 14.
3	Group III (Standard)	CFA+Standard drug (Diclofenac sodium 10 mg/kg p.o., for 14 days)
4	Group IV (Test-1)	CFA+100 mg/kg b. wt of BVEE/ BDEE/LAEE p.o., for 14 days.
5	Group V (Test-2)	CFA+200 mg/kg b. wt of BVEE/ BDEE/LAEE p.o., for 14 days.
6	Group VI (Test-3)	CFA+400 mg/kg b. wt of BVEE/ BDEE/LAEE p.o., for 14 days.

CFA: Complete Freund's Adjuvant. BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

All test extracts were suspended in 1% carboxymethylcellulose (CMC) and administered orally once daily for 14 consecutive days. Ten days after injection, secondary inflamed lesions were detected on the left hind paw, which began to increase in thickness and also in the fore-paws, ears, and tail.

Primary lesions of paw volume

The choice of the animal strain has been found to be very important for the performance of this activity. Wistar-rats have been proven to be very suitable in contrast to other sub-strains. Male rats with an initial body weight of 150–200 g were used. On day 1, they were injected into the subplantar region surface of the right hind paw with 0.1 mL of CFA. This consists of 5 mg *M. tuberculosis* (Difco) being suspended in heavy paraffin oil (Merck) by thoroughly grinding with a mortar and pestle to give a concentration of 5 mg/mL dosing. Drugs were administered orally once a day, from the day of injection of CFA and continued up to day 14. The change in the inflammatory reaction was measured using mercury plethysmograph on 0, 7, 14, 21 and 28th day from the day

of CFA injection. The animals were weighed, using digital weighing balance, on 0, 7, 14, 21, and 28th day from the day of CFA injection, % edema and % of edema inhibition were measured. At the end of the 28th day, rats were anaesthetized with diethyl ether, and blood was withdrawn by puncture of retro orbital plexus, centrifuged, and biochemical estimations were done with serum. Paws were measured, indicating the primary lesion and the influence of therapeutic agents on this phase.

$$\% \text{ Edema} = N' \times 100/N$$

$$\% \text{ Edema inhibition} = (N-N')/N \times 100$$

Where N=Is the edema value of the control group.
N'=Is the edema value of the experimental group.

Assessment of polyarthritis scores

The rats were assessed weekly for signs of arthritis between days 14–28th of CFA injection using a well-established, widely-used scoring system developed to evaluate the severity of AIA. Paws were examined and graded for the polyarthritis severity were graded on a scale of 0–4 (Yang *et al.*, 1999): 0 = No swelling; 1 = Isolated phalanx joint involvement; 2 = Involvement of phalanx joint and digits; 3 = Involvement of the entire region down to the ankle; 4 = Involvement of the entire paw, including ankle. The maximum joint score was 12, including 3 secondary arthritis paws for each rat.

Measurement of paw diameter

Paw diameter was measured at 1, 7, 14, 21, and 28th day by using a digital vernier caliper at the rat right hind paw.

Body weight

Body weight changes were observed during the experiment and measured using a digital balance once in 10 days, on days 1, 10, 20, an 30th day of the experiment using a digital weighing balance. Body weight changes were observed and measured using a digital balance. The percentage change in b.w was calculated using the following formula:[5]

$$\% \text{ Change in b.w} = \frac{\text{Body weight on } 1/7/14/21/28^{th} \text{ day} - \text{Initial weight}}{\text{Initial body weight}} \times 100$$

Biochemical estimations

On the 28th day, the blood (up to 5 mL) was collected by cardiac puncture under the influence of ether anesthesia. Some of the collected blood was used to perform hematological studies to estimate red blood cell (RBC), white blood cell (WBC), hemoglobin % (Hb%), and erythrocyte sedimentation rate (ESR). Remaining blood was used to centrifuge at 3000 rpm for 10 min at room temperature to perform the following biochemical studies. The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided with the biochemical using semi autoanalyzer.

Estimations of erythrocytes

Blood was taken up to 0.5 mark in the Thomas red cell diluting pipette and diluted up to 101 marks with Hayem's fluid, thus achieving a 1:200 dilution of the blood sample. The diluted sample was filled in the counting chamber and counted with the aid of the light microscope.

RBC = Cells counted × 5 (1/5 sq. cm counted) × 10 (depth) × 200 (dilution factor)

Values are expressed as cells per cubic ml of blood.

Estimations of WBC

The WBC pipette was filled to 0.5 mark with whole blood and diluted to the 11 mark with 1.5% HCl, resulting in a 1:20 dilution of the blood sample. The hemocytometer was filled with the diluted blood, and leukocytes present were counted.

$$\text{WBC (Per cu.mm)} = \frac{\text{Cells counted} \times 10 \text{ (depth)} \times 20 \text{ (dilution factor)}}{4 \text{ (sq.mm counted)}}$$

Values are expressed as the number of leukocytes present per cubic millimeters of the blood.

Estimations of Hb%

The hemoglobin pipette was filled to the 20 c mm mark with whole blood, and the blood was expelled into the Sahli tube containing the Hcl solution up to 3 g mark. Contents were mixed and keep the tube in the comparator and wait for 10 min. After 10 min, distilled water was added drop by drop into the tube till the color matches with the standard color of the comparator. The hemoglobin levels were expressed as g/100 mL.

Estimations of ESR

This is the rate at which erythrocytes sediment on their own weight when anti-coagulated blood is held in a vertical column, it is expressed as the fall of RBC's in mm at the end of 1st h (starting point-when the tube or pipette was filled with blood). Westergren pipette (open at both ends) is about 30 cm long with a bore diameter of about 2.5 mm. The lower 20 cm are marked from 0 (top) to 200 (bottom). Anticoagulant used is 3.8% trisodium citrate solution. One part of the anticoagulant is added to four parts of blood. The pipette accepts about 1 ml of blood. Fill the pipette by sucking till the 0 mark and clamp it vertically in the Westergren rack. Read the upper level of red cells exactly after 1 h. This is a better method than Wintrobe's since the reading obtained is magnified as the column is lengthier.

Estimation of liver enzymes

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by a colorimetric method

Estimations of AST

0.5 mL of buffered substrate was added to 0.1 mL of serum and placed in a water bath at 37°C. To the blank tubes, 0.1 mL of distilled water was added instead of serum. Exactly an hour later, 2 drops of aniline citrate reagent and 0.5 mL of DNPH reagent were added and kept at room temperature for 20 min. Finally, 5.0 mL of 0.4 N sodium hydroxide was added. A set of standards also treated in the same manner and read at 520 nm after 10 min. The results were expressed as IU/L of serum.

$$\text{AST activity}\left(\text{in}\frac{\text{IU}}{\text{L}}\right) = \frac{\text{Absorance of test} - \text{Absorbance of control}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \text{Conc.of Standard}$$

Estimations of ALT

Procedure was the same as that used for the assay of aspartate transaminase, except the incubation time, which was reduced to 30 min (60 min for AST). The results were expressed as IU/L of serum.

$$\text{ALT activity}\left(\text{in}\frac{\text{IU}}{\text{L}}\right) = \frac{\text{Absorance of test} - \text{Absorbance of control}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \text{Conc.of Standard}$$

Estimations of alkaline phosphatase (ALP)

Reagent	Blank (B)	Standard (S)	Control (C)	Test (T)
Working buffer	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Substrate purified water	1.5 mL	1.5 mL	1.5 mL	1.5 mL
Mix well and incubate at 37°C for 3 min				
Serum	-	-	-	0.05 ml
Reagent 3	-	0.05 ml	-	-
Mix well and incubate at 37°C for 15 min				
Reagent 2	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Serum	-	-	0.05 ml	-

Mix well after the addition of each reagent and measure the absorbance at 510 nm of blank (B), standard (S), control (C), and test (T) against purified water.

$$\text{Serum alkaline phosphate} = \frac{\text{O.D. Test} - \text{O.D. Control}}{\text{O.D. Standard} - \text{O.D. Blank}}$$

Estimation of C-reactive protein (CRP) and rheumatoid factor (RF))

CRP and RF were estimated in serum using kits according to manufactures instructions (Monzyme India Private Limited).

Determination of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) production in RAW 246.7 macrophages

Cell supernatants collected on ice and stored in a 70°C freezer were used for cytokine estimation. TNF-α and IL1-β were determined in the culture supernatant using a specific ELISA kit according to the manufacturer's instructions.[6]

Measurement of anti-oxidant enzymes

The experimental animals of the CFA-induced model from the respective groups were sacrificed. Liver was isolated, and whole tissue after rinsing in ice-cold normal saline was immediately placed in normal saline (double this volume) and homogenized at 4°C. The homogenate was centrifuged at 12000 rpm for 5 min, and the supernatant stored at 20°C was used for the assay of several antioxidant enzymes. The different biochemical parameters assessed were as follows.[7]

Assay of superoxide dismutase (SOD)

Liver tissue was homogenized with a polytron homogenizer in ice-cold Tris buffer to produce a 10% w/v homogenate. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Aliquot of 0.1 mL supernatant was added to 1.2 mL of 0.052 M sodium pyrophosphate buffer (pH 8.3), followed by the addition of 0.1 mL of 186 μM phenazine methosulphate, 0.3 mL of 300 μM nitroblue tetrazolium, and 0.2 mL of 780 μM NADH. The reaction mixture was incubated for 90 sec at 30°C, and the reaction was stopped by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml of *n*-butanol and centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer was measured at 560 nm. A control was prepared using 0.1 mL of distilled water devoid of 0.1 mL of homogenate. One unit of the enzyme activity is defined, as enzyme concentration required inhibiting the absorbance of chromogen production by 50% in the control sample under the assay conditions. The SOD level was expressed as Units/mg protein.[8]

Glutathione peroxidase (GPx)

0.2 ml each of ethylenediaminetetraacetic acid, sodium azide, glutathione (reduced), hydrogen peroxide, 0.4 mL of buffer, and 0.1 mL of homogenate were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 mL of supernatant, 4 mL of disodium hydrogen phosphate, and 0.5 mL of DTNB were added, and the color developed was read at 420 nm immediately. Standards were also treated

similarly. GPx activity was expressed as µg of glutathione utilized/min/mg protein at 37°C.[9]

Estimation of glutathione reductase activity (GR_x)

GR_x activity was assayed by the method of Carlberg and Mannervik, 1985. 50 µl of NADPH (2 mM) in 10 mM Tris buffer (pH 7.0) was added in cuvette containing 50 µl of oxidized glutathione (20 mM) in phosphate buffer. One hundred microliters of PMS were added to the NADPH-GSSG buffered solution, and was measured at 340 nm for 3 min. The enzyme activity measured at 340 nm was calculated as nmoles of NADPH oxidized/min/mg of protein using € of 6.22×10^3/M/cm.[10]

Histopathological findings with adjuvant arthritis in the rats

Histopathological examination was performed to determine the degree of infiltration of immune cells in the paw tissue upon adjuvant challenge. Tissues taken from the rat paws of all the different groups on Day 28th after fixing in 10% formalin were then dehydrated through a graded ethanol series, cleared in xylene, and processed for embedding in paraffin wax with routine protocols. A microtome was used to cut 4 µm-thick sections that were subsequently stained with hematoxylin and eosin (H and E) stain

Radiology score assessment

At the end of the experiment (day 28), rats were anaesthetized intramuscularly with 0.1 mL/100 g of rat body weight of Ketamine. Anesthetized rats were placed on a radiographic box at a distance of 107 cm from the X-ray source. Radiographic analysis of normal and arthritic hind paws was performed by using X-ray machine, with a 48 kVp exposure for 0.5 mAs. A blind and independent assessment of the radiological score was performed by two observers. The following radiological criteria were considered, normal: Score 0; no tissue swelling or bone damage: Score 1; tissue swelling and edema: Score 2; joint erosion: Score 3; bone erosion and osteophyte formation: Score 4. The total radiology scores were calculated from the sum of both hind paws, with a maximum possible score of 6 for each rat.

Statistical analysis

Results were expressed as mean ± standard error of the mean (n=6). Statistical analyses were performed with One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Instat Software (5.0). P value less than 0.05 was considered to be statistically significant. $^aP<0.05$, $^bP<0.01$, and $^cP<0.001$, when compared with the control and toxicant group as applicable.

RESULTS AND DISCUSSION

Results of antiarthritic activity

To determine the anti-arthritic effects of 70% ethanolic extracts of bark of *B. variegate* (BVEE), whole plant of *B. diffusa* (BDEE), and leaves of *L. acidissima* (LAEE) on CFA -induced arthritis, animals were treated with their different doses of 100, 200, and 400 mg/kg b.w over a period of 28 days. Different parameters were assessed to suggest the possible mechanism involved in their action.

Effect on hind paws edema: Primary lesions

The determination of paw edema is an apparently simple, sensitive, and quick procedure for evaluating the anti-arthritic activity and assessing the effectiveness of the treatment. To develop arthritis, 100 µL of CFA was injected in the subplantar region of the right hind paw of the rats. It was observed that initially acute inflammation developed, resulting in swelling in the hind paw of the rat within 24 h of the CFA injection. Slowly, the swelling on the hind paw subsided and again started appearing by day 7 and reached to its maximum by day 14 of the CFA injection, which was observed to persist till around day 28 of the CFA injection.

Edema in the injected paws (adjuvant-injected paw) was apparent from the day of CFA injection (day). In the uninfected paws of the same animal, where adjuvant was not injected, edema is developed on day 10 onwards, and this is used to determine secondary lesions**.** The different extracts demonstrated significant inhibition of swelling in the injected hind paw in weekly once for the entire period of study, as depicted in Table 1 and Figure 1.

Effect of BVEE

BVEE demonstrated significant inhibition of swelling in the injected hind paw in weekly once for the entire period of study. Maximal suppression of paw edema was observed in a dose-dependent manner. BVEE at the doses of 400 mg/kg b.wt with 76.43% inhibition on day 28th, which was statistically highly significant, which were compared with the standard drug diclofenac sodium 10 mg/kg b.wt.

Effect of BDEE

Paw volume measurements revealed that BDEE caused significant inhibition of injected paw edema as compared with arthritic control, and the suppressive effects were dose dependent. BDEE reduced the primary lesion was compared to the standard. Maximal suppression of paw edema was observed at a dose of 400 mg/kg b.wt with 70.10 % inhibition on day 28.

Effect of LAEE

LAEE produced significant dose-dependent inhibition of swelling in the injected paw, which was measured in weekly

Table 1: Effect of graded doses BVEE/BDEE/LAEE on primary lesions (injected paw edema) in CFA-induced arthritic and treated rats over 28 days

a. Effect of graded doses of BVEE on primary lesions of paw edema

Groups	Dose (mg/kg)	0 Day	7 Day	14 Day	21 Day	28 Day
I (Normal control)	-	0.37±0.05	0.45±0.04	0.46±0.05	0.45±0.08	0.47±0.04
II (Arthritic control)	-	0.39±0.02	0.98±0.05	1.41±0.09	1.76±0.12	1.91±0.16
III (Standard)	10	0.35±0.06	0.92±0.04	1.23±0.05	0.82±0.06^{c}	0.41±0.08^{c}
IV (Test-1)	BVEE 100	0.4±0.04	0.86±0.03	1.21±0.12	0.7±0.09^{b}	0.50±0.04^{b}
V (Test-2)	BVEE 200	0.38±0.03	0.95±0.10	1.18±0.05	0.73±0.08^{c}	0.4±0.03^{c}
VI (Test-3)	BVEE 400	0.3±0.04	0.88±0.15	1.25±0.06	0.83±0.05^{c}	0.45±0.02^{c}

b. Effect of graded doses of BDEE on primary lesions of paw edema

Groups	Dose (mg/kg)	0 Day	7 Day	14 Day	21 Day	28 Day
I (Normal control)	-	0.39±0.05	0.40±0.03	0.41±0.05	0.40±0.04	0.42±0.03
II (Arthritic control)	-	0.36±0.04	0.95±0.06	1.510±0.12	1.74±0.16	1.94±0.18
III (Standard)	10	0.39±0.06	0.86±0.04	1.09±0.11	0.69±0.07^{c}	0.48±0.03^{c}
IV (Test-1)	BDEE 100	0.40±0.05	0.91±0.05	1.22±0.09^{a}	0.83±0.04^{c}	0.62±0.05^{c}
V (Test-2)	BDEE 200	0.37±0.04	0.93±0.04	1.20±0.06	0.87±0.03^{c}	0.60±0.06^{c}
VI (Test-3)	BDEE 400	0.35±0.06	0.96±0.03	1.15±0.15	0. 5±0.04^{c}	0.55±0.05^{c}

c. Effect of graded doses of LAEE on primary lesions of paw edema

Groups	Dose (mg/kg))	0 Day	7 Day	14 Day	21 Day	28 Day
I (Normal control)	-	0.40±0.05	0.41±0.04	0.4±0.06	0.44±0.04	0.45±0.09
II (Arthritic control)	-	0.38±0.04	0.99±0.06	1.44±0.17	1.82±0.17	1.89±0.14
III (Standard)	10	0.37±0.06	0.90±0.05	1.21±0.12	0.87±0.04^{c}	0.45±0.04^{c}
IV (Test-1)	LAEE 100	0.35±0.04	0.88±0.08	1.16±0.11	0.81±0.04^{c}	0.55±0.03^{c}
V (Test-2)	LAEE 200	0.39±0.03	0.86±0.03	1.28±0.08	0.79±0.03^{c}	0.52±0.04^{c}
VI (Test-3)	LAEE 400	0.36±0.02	0.98±0.04	1.24±0.15	0.85±0.04^{c}	0.48±0.03^{c}

Data represent the mean±SEM, (n=6). Statistical significance: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

once through the entire period of study, as depicted in Table 1C. Maximal suppression of paw edema was observed at a dose of 400 mg/kg b.wt of LAEE, i.e., 74.60%inhibition on day 28, which was statistically significant.

Poly arthritis index

Purposely, the rats were induced arthritis with CFA. The severity of the induced arthritis disease is followed by measurement of the injected (Primary lesions) and non-injected paw (secondary lesions) with a digital plethysmometer. Edema in the injected paws (adjuvant-injected paw) was apparent from the day of CFA injection (day 1). In the uninjected paws of the same animal, where adjuvant was not injected, edema was developed on day 10 onwards, and this is used to determine secondary lesions. The treatment was started with graded doses 100, 200, and 400 mg/kg b.wt of BVEE/BDEE/LAEE, standard drug, and continued for 14 days. From day 15 to 28th, the animals were not dosed with the test compound or the standard. On day 28th, the score of the arthritis index was evaluated visually and graded (Yang *et al*., 1990), which was given in Table 2 and Figure 2.

Effect of BVEE

The polyarthritic index was seen in arthritic induction. The maximum polyarthritic index was seen in 21th day of arthritic induction. The arthritic index in the arthritic control was 9.8. BVEE produces a significantly dose-dependent inhibition of the polyarthritis index. BVEE (400 mg/kg) body weight showed a statistically significant 55.43% inhibition on day 28. These were compared with the standard drug diclofenac sodium with 56.52%.

Effect of BDEE

IBEE produces a significantly dose-dependent inhibition of polyarthritis index. The arthritic index in the arthritic control was 8.9. BDEE (400 mg/kg) body weight showed a statistically significant 41.02% inhibition on day 28. These were compared with the standard drug diclofenac sodium, with 46.15%.

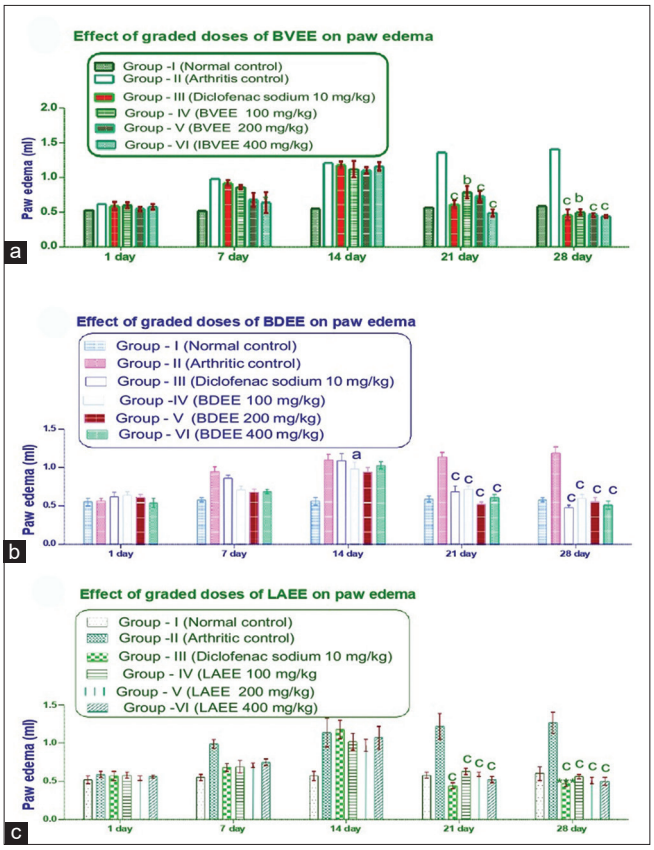

Figure 1: Graphical representation of the effect of graded doses of (a) BVEE/(b) BDEE/(c) LAEE on primary lesions (injected paw edema) in adjuvant-induced arthritic and treated animals over 28 days. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

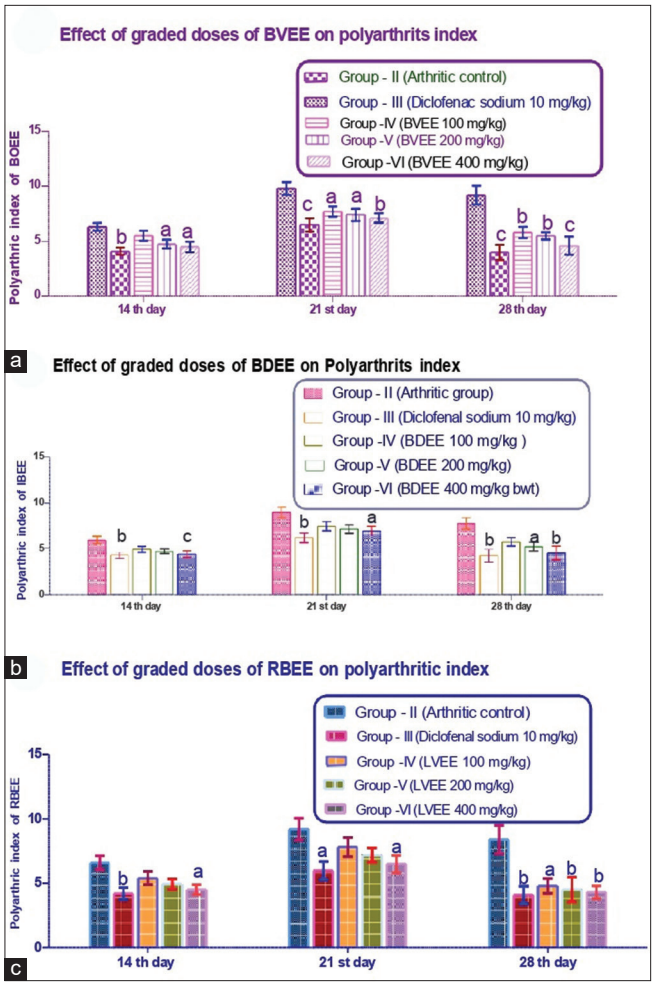

Figure 2: Graphical representation of effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on polyarthritis index in complete Freund's Adjuvant induced arthritis, standard and treated animals. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Effect of LAEE

The maximum polyarthritic index was seen in 21st day of arthritic induction. The arthritic index in the arthritic control was 9.2. LAEE produces a significantly dose-dependent inhibition of poly-arthritic-indexes. LAEE at the doses of 400 mg/kg b.wt with 51.19% inhibition on day 28th, which was statistically significant, was compared with the standard drug diclofenac sodium with 53.65%.

Effect on paw diameter

To determine the paw diameter, the effects of 70% BVEE, BDEE and LAEE on CFA-induced arthritis animals were treated with their graded doses of 100, 200 and 400 mg/kg b.wt over a period of 28 days.

Dose-dependent inhibition of paw swelling (paw diameter) in the injected paw was measured with vernier caliphs and given in Table 2 and Figure 2.

Effect of BVEE

BVEE demonstrated significant dose-dependent inhibition of paw diameter swelling in the injected paw in weekly once through the entire period of study. Maximal suppression of paw edema was observed at a dose of 400 mg/kg b.wt with 49.61% inhibition on day 28th, which was statistically significant and compared with 51.39% Inhibition shown by diclofenac sodium on day 28th.

Effect of BDEE

Paw diameter measurements revealed that BDEE caused significant inhibition of the injected paw in weekly once through the entire period of study. It is noteworthy that at the dose of 400 mg/kg b.wt, BDEE produced the 32.35% inhibition of paw diameter, which was comparable to

Table 2: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on arthritic index in CFA-induced arthritis treated rats

a. Polyarthritis index of BVEE				
Groups	**Dose (mg/kg)**	**14th day**	**21st day**	**28th day**
II	Arthritic control	6.3±1.2	9.8±1.4	9.2±1.4
III	10	4.8±0.7[b]	8.9±1.0[c]	4.0±0.7[c] (56.52%)
IV	BVEE 100	4.6±0.6	8.7±1.4[a]	4.8±1.0[b] (47.82%)
V	BVEE 200	4.8±0.9[a]	8.2±1.5[a]	4.4±0.9[b] (52.17%)
VI	BVEE 400	4.6±0.8[a]	8.6±1.3[b]	4.1±0.8[c] (55.43%)
b. Polyarthritis index of BDEE				
Groups	**Dose (mg/kg).**	**14th day**	**21st day**	**28thday**
II	Arthritic control	5.9±1.2	8.9±1.4	7.8±1.5
III	Standard 10	4.6±0.9[b]	7.9±1.6[b]	4.2±0.6[b] (46.15%)
IV	BDEE 100	4.3±0.8	8.4±1.2	5.7±1.0 (26.92%)
V	BDEE 200	4.6±0.5	7.9±1.1	5.2±0.7[a] (33.33%)
VI	BDEE 400	4.5±0.7[c]	7.4±1.4[a]	4.6±0.5[b] (41.02%)
c. Polyarthritis index of LAEE				
Groups	**Dose (mg/kg)**	**14th day**	**21st day**	**28thday**
II	Arthritic control	5.6±1.2	9.2±1.7	8.4±1.6
III	Standard 10	4.2±0.9[b]	8.5±1.2[a]	4.0±0.7[b] (53.65%)
IV	LAEE 100	4.8±0.8	7.4±1.4	4.8±1.0[a] (42.85%)
V	LAEE 200	4.5±0.7	6.7±1.3	4.2±0.8[b] (50%)
VI	LAEE 400	4.9±0.9[a]	7.8±1.1[a]	4.1±0.5[b] (51.19%)

Data represent the mean±SEM, (n=6). Statistical significance: [a]P<0.05, [b]P<0.01, [c]P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

that of diclofenac sodium weekly once through the entire period of study, with a maximal inhibitory effect of 38.61% on day 28th.

Effect of LAEE

LAEE demonstrated significant dose-dependent inhibition of paw diameter in the injected hind paw in weekly once through the entire period of study. Maximal suppression of paw diameter was observed at a dose of 400 mg/kg b.wt with 37.90% inhibition on day 28th, which was statistically significant which was compared with inhibition shown by diclofenac sodium on day 28th was 38.90%.

Effect on body weight changes

Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on b.wt changes in CFA-induced arthritic, standard, and extract-treated animals are depicted in Table 4 and Figure 4.

The development of arthritis was accompanied by a decrease in the b.wt of the individual rats; hence, it could serve as one of the preliminary parameters to be monitored during the treatment of arthritis. Immediately after the injection of CFA in the sub-plantar region of the hind paw of the rat, there was no major change observed in the weight of the animals. Next, we started noticing the changes in b.wt of CFA-injected rats slow and gradient decrease in the b.wt of the rats from day 7th of the injection. As shown in Figure 4, the decline in the b.wt of the arthritic control rats continued till day 28. Thus, this indicates the correlation of the development of arthritis and the change in b.w given in Table 4 and Figure 4.

Effect of BVEE

The challenges in b.wt with CFA-induced arthritis showed a significant decrease in the average b.wt in arthritis control as compared to the normal control. Under similar conditions, diclofenac sodium and the graded doses of 100, 200, and 400 mg/kg b.wt of BVEE treated groups showed average gain in b.wt. The reduction of mean change in b.wt of arthritic control after 28days was (-) 26.66%, whereas the weight gain of mean change in body weight of standard, 100, 200, and 400 mg/kg b.wt of BVEE treated groups were (+) 12.76, (+) 8.96, (+) 10.64, and (+) 11.86.

Effect of BDEE

The graded doses of BDEE were found to improve the b.wt gain in arthritis treated rats. These effects were dose-dependent and maximally marked at the tested dose of 400 mg/kg b.wt of BDEE.

Table 3: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on paw diameter in CFA-induced arthritis treated rats

Groups	Dose (mg/kg b.w)	a. Effect of graded doses of BVEE paw diameter (mm)				
		1st day	7th day	14th day	21st day	28th day
I (Normal control)	-	8.42±0.71	8.68±0.79	8.72±0.62	8.91±0.70	9.16±0.76
II (Arthritic control)	-	8.29±0.89	18.75±1.16	17.08±1.64	18.61±1.24	17.69±1.84
III (Standard)	50 mg/kg	7.7±0.84	17.90±1.14	13.51±1.07	10.04±1.07[c]	8.70±0.75[c] (51.39)
IV (Test-1)	BVEE 100	7.92±0.77	15.57±1.18	14.15±1.18	12.43±1.14[b]	11.86±1.24[b] (23.82)
V (Test-2)	BVEE 200	8.74±0.79	15.33±1.11	13.09±1.15	11.86±1.16[c]	10.12±0.84[c] (33.98)
VI (Test-3)	BVEE 400	8.12±0.62	17.98±1.13	14.49±1.07	11.02±1.22[c]	9.04±0.46[c] (49.61)
Groups	**Dose (mg/kgb.w)**	**b. Effect of graded doses of BDEE on Paw Diameter (mm)**				
		1st day	7thday	14thday	21stday	28thday
I (Normal control)	-	8.16±0.84	8.36±0.11	8.69±0.09	8.87±0.15	9.27±0.58
II (Arthritic control)	-	8.14±0.93	18.91±0.15	20.39±0.13	20.74±1.22	(19.54±1.73
III (Standard)	10	9.22±089	16.60±0.12[b]	14.12±0.11[c]	12.75±1.08[c]	10.19±0.85[c] (38.61)
IV (Test-1)	BDEE 100	8.96±0.71	16.87±0.09[a]	14.36±0.18[c]	13.86±1.12	12.53±1.06[c] (25.72)
V (Test-2)	BDEE 200	8.49±0.64	15.79±0.14[c]	13.15±0.09[c]	12.72±1.14	11.05±0.92[c] (30.01)
VI (Test-3)	BDEE 400	8.86±0.78	14.96±0.10[c]	12.13±0.13[c]	11.92±1.13	10.12±0.15[c] (32.35)
Groups	**Dose (mg/kg)**	**c. Effect of graded doses of LAEE on Paw diameter (mm)**				
		1st day	7thday	14thday	21stday	28thday
I (Normal control)	-	8.12±0.11	8.23±0.11	8.41±0.86	8.69±0.74	8.96±0.78
II (Arthritic control)	-	8.37±0.92	17.85±1.06	18.10±1.63	19.53±1.79	20.89±1.74
III (Standard)	10	9.12±0.78	16.64±1.09	13.11±1.13[a]	11.10±1.12[c]	10.16±1.10[c] (38.90)
IV (Test-1)	LAEE 100	8.92±0.69	15.87±1.32	13.89±1.08	12.16±1.16[c]	11.15±1.13[b] (29.74)
V (Test-2)	LAEE 200	8.71±0.78	16.78±1.13	14.12±1.13	12.97±1.11[b]	11.06±1.11[b] (34.08)
VI (Test-3)	LAEE 400	8.62±0.61	16.91±1.08	12.58±1.09[a]	11.10±1.16[c]	10.50±1.23[c]

Data represent the mean±SEM, (n=6). Statistical significance: [a]P<0.05, [b]P<0.01, [c]P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Effect of LAEE

Arthritic control rats exhibited a significant reduction in mean body weight compared to the normal control group. The treatment with standard drug diclofenac sodium and graded doses of 100, 200, and 400 mg/kg b. w of LAEE showed an average gain in body weights. The results are depicted in Table 4 and Figure 4.

Effects on hematological parameters

Effect of BVEE

The changes in hematological parameters as monitored on day 28th in all treated groups with adjuvant-induced arthritic rats. In CFA-induced untreated group, there was a significant decrease in RBC count and Hb%, and also a marked increase in WBC count and ESR when compared to the normal control. However, treatment with graded doses of 100, 200, and 400 mg/kg b.wt BVEE reversed these altered hematological parameters, and the effects of BVEE were found to be dose dependent. At 400 mg/kg b.wt, the levels of Hb% was up regulated from 11.64 ± 1.04 to 15.08 ± 1.38 and of RBC count from 4.19 ± 0.47 to 6.62 ± 0.73, as compared to untreated arthritic controls in each case. The WBC count was reduced from 9.84 ± 0.74 to 4.89 ± 0.42 and ESR from 16.34 ±1.79 to 11.08 ± 1.12 mm/h; results were given in Table 5 and Figure 5.

Effect of BDEE

BDEE significantly restored all the CFA-induced hematological perturbations. In CFA-induced untreated group, there was a significant decrease in RBC count, Hb%, and also a marked increase in WBC count and ESR when compared to the arthritic control. However, treatment with graded doses of 100, 200, and 400 mg/kg b.wt of BDEE reversed these altered hematological parameters, and the effects of BDEE were found to be dose dependent. At the dose of 400 mg/kg b.wt, the levels of Hb% was up regulated from 10.37 ± 1.57 to 13.05 ± 0.98 and of RBC count from 6.81± 0.45 to 10.04 ± 0.89, as compared to untreated arthritic control in each case. The WBC count also reduced from 10.15 ± 0.55 to 7.14 ±0.44 and ESR from 10.82 ±1.47 to 8.14 ± 0.68 mm/h.

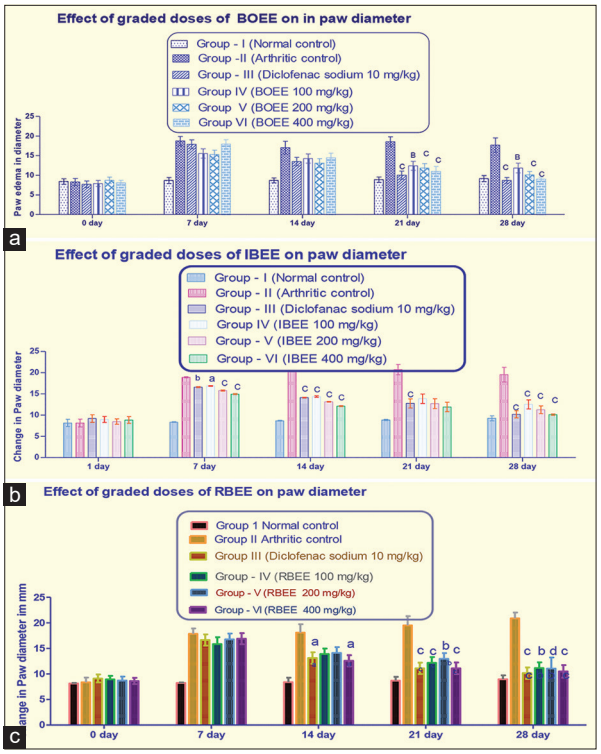

Figure 3: Graphical representation of the effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on paw diameter in complete Freund's Adjuvant-induced arthritic and treated animals. Data represent the mean ± standard error of the mean, (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

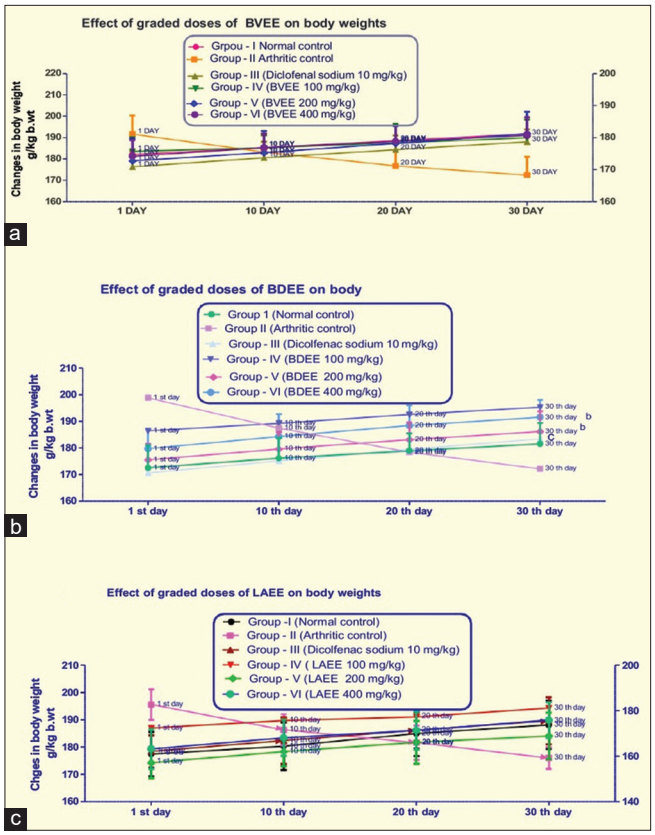

Figure 4: Graphical representation of the effect of graded doses of BVEE (a), BDEE (b) and LAEE (c) on body weight changes in complete Freund's Adjuvant-induced arthritic and treated animals. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Effect of LAEE

LAEE significantly restored all the CFA-induced hematological perturbations. The effects of LAEE were dose-dependent, and LAEE at 400 mg/kg b.wt produced maximal effects.

All three extracts at graded doses of 100, 200, and 400 mg/kg b.wt of BVEE, BDEE, and LAEE produced dose-dependent action. The dose of 400 mg/kg b.wt of BVEE, BDEE, and LAEE produced a more significant protective effect on hematological changes than the 100 and 200 mg/kg b.wt of BVEE, BDEE, and LAEE. BVEE at the dose of 400 mg/kg b.wt maximally altered the hematological changes then the 400 mg/kg b.wt of BDEE and LAEE.

Effect of graded doses of different plant extracts on AST, ALT and ALP

Rats treated with CFA developed a significant hepatic damage observed as elevated serum levels of hepatic-specific enzymes like AST, ALT, and ALP compared to the normal group. Treatment with at different doses of 100, 200, and 400 mg/kg b.wt of BVEE, BDEE, and LAEE, and 10 mg/kg b.w tof diclofenac sodium had showed good protection against CFA-induced arthritis in rats. These indicate a significant reduction in elevated serum enzyme levels with extract-treated animals compared to toxic control animals, which were given in Table 6 and Figure 6.

Effect of BVEE

In CFA-induced untreated group, there were significant increases in AST, ALT, and ALP. However, treatment with graded doses of BOEE reversed these altered enzyme effects, which were found to be dose dependent. The % of reduction of AST, ALT, and ALP in standard and graded doses of BVEE-treated groups, AST were 28.69, 16.88, 21.02, 22.66%, ALT were: 34.26, 22.15, 26.81, 30.90%; ALP were: 37.16, 25.47, 27.16, and 32.74%.

Effect of BDEE

Diclofenac sodium and graded doses of BDEE significantly reduced all the CFA-induced liver enzymes when compared

Table 4: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on body weights changes in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE

Groups	Dose (mg/kg)	Mean change in body weights (g)					
		0 Day	7 Day	14 Day	21 Day	28 Day	Mean change
I (Normal control)	-	172.56±8.56	174.12±6.63	176.13±7.83	178.93±6.49	181.58±7.84	-
II (arthritic control)	-	198.84±6.47	191.42±8.52	187.49±8.59	178.42±4.63	172.18±6.48	(-) 26.66
III (Standard)	10	170.63±5.49	173.32±6.74[b]	175.16±5.41[a]	179.51±6.49	183.39±8.42	(+) 12.76
IV (Test-1)	BVEE 100	186.38±6.73	188.50±9.43	189.26±7.49	192.55±8.52[a]	195.34±6.90[c]	(+) 8.96
V (Test-2)	BVEE 200	175.54±5.98	177.25±6.57	179.62±6.31	183.13±6.48	186.18±7.62[a]	(+) 10.64
VI (Test-3)	BVEE 400	179.71±7.56	182.14±6.94	184.25±8.53	188.41±5.53	191.57±6.52[c]	(+) 11.86

b. Effect of graded doses of BDEE

Groups	Dose (mg/kg)	Mean change in body weights (g)					
		0 Day	7 Day	14 Day	21 Day	28 Day	Mean change
I (Normal control)	-	182.14±9.23	184.60±9.16	185.34±8.69	188.52±9.18	190.84±7.42	-
II (Arthritic control)	-	191.69±8.69	183.12±7.90	179.23±7.89	176.79±6.89	172.45±8.56	(-) 19.24
III (Standard)	10	176.34±5.54	181.94±8.57	183.54±7.53	186.34±6.74	188.05±5.83[b]	(+) 11.71
IV (Test-1)	BDEE 100	183.5±6.59	185.50±6.58	187.1±5.99	188.53±8.97	189.92±8.50	(+) 6.34
V (Test-2)	BDEE 200	172.75±6.91	175.88±9.13	176.33±6.69	179.16±5.47	181.23±6.84[b]	(+) 8.48
VI (Test-3)	BDEE 400	181.29±7.52	186.31±7.28	188.27±6.64	190.21±7.41	192.04±8.48[c]	(+) 9.75

c. Effect of graded doses of LAEE

Groups	Dose (mg/kg)	Mean change in body weights (g)					
		0 Day	7 Day	14 Day	21 Da	28 Day	Mean change
I (Normal control)	-	177.43±8.13	180.43±8. 69	183.32±8.76	186.98±8.28	183.12±8.76	-
II (Arthritic control)	-	182.72±6.74	174.18±5.81	169.58±6.84	165.9±7.57	159.23±4.75	(-) 23.49
III (Standard)	10	178.38±5.90	181.1±6.72	183.39±8.58[a]	186.14±6.58[c]	189.62±8.63[c]	(+) 11.24
IV (Test -1)	LAEE 100	186.93±7.56	187.22±7.32	189.73±7.56	191.1±8.58[c]	194.3±5.89[c]	(+) 7.39
V (Test -2)	LAEE 200	174.26±5.67	178.23±5.11	181.36±5.39	182.72±7.84[b]	184.09±8.53[c]	(+) 9.83
VI (Test -3)	LAEE 400	179.32±7.26	182.44±6.34	184.4±6.69	186.10±6.90[c]	189.9±6.58[c]	(+) 10.59

Data represent the mean±SEM, (n=6). Statistical significance: [a]P<0.05, [b]P<0.01, [c]P<0.001, versus with respect to arthritic control groups (One way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

to CFA-induced arthritic group. The effects of BDEE on liver enzymes were dose-dependent, and BDEE at 400 mg/kg b.wt depicted maximal effects. The effect of diclofenac sodium and graded doses of BDEE on AST, ALT, and ALP were in, AST: 49.75, 25.60, 33.13, 39.83%; ALT: 59.09, 34.38, 42.61, 46.65%; ALP: 55.69, 43.63, 47.47, and 52.55%.

Effect of LAEE

In CFA-induced untreated group, there was a significant increase in AST, ALT, and ALP. However, treatment with graded doses of LAEE reversed these altered enzymes, and the effects for LAEE were found to be dose dependent. At 400 mg/kg b.wt, the levels of enzymes were downregulated and compared to the untreated arthritic control in each case. The % of reduction of AST, ALT, and ALP in standard and graded doses of LAEE-treated groups AST were 57.11, 45.03, 49.13, 53.14%, ALT: 58.78, 39.12, 41.46, 51.52%; ALP: 55.70, 47.78, 49.42, and 52.55%.

Effect of different doses of 100, 200, and 400 mg/kg b.wt of extracts of BVEE, BDEE, and LAEE on CRP and RF in CFA-induced arthritis treated rats

The serum CRP and RF are markers of systemic inflammation and antibody production, respectively, against the CFA. CFA treatment has considerably increased the CRP and RF levels in serum. Treatment with at different doses of 100, 200, and 400 mg/kg b.wt of BVEE/BDEE/LAEE had showed significantly decreases CRF and RF levels as compared

Table 5: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on hematological alterations in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE on hematological changes

Groups	Dose (mg/kg)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb %	ESR mm/h
I (Normal control)	-	7.89±0.58	3.83±0.3	16.76±1.38	9.64±0.86
II (Arthritic control)	-	4.19±0.47	9.84±0.74	11.64±1.04	16.34±1.79
III (Standard)	10	7.13±0.64^c	4.10±0.48^c	15.94±1.85^c	10.26±1.35^c
IV (Test-1)	BVEE 100	5.26±0.68^a	5.98±0.64	13.56±1.26	13.15±1.96^b
V (Test-2)	BVEE 200	5.83±0.22^c	5.11±0.51^b	14.55±1.36^a	12.48±1.25^c
VI (Test-3)	BVEE 400	6.62±0.73^c	4.89±0.42^c	15.08±1.38^b	11.08±1.12^c

b. Effect of graded doses of BDEE on hematological changes

Groups	Dose (mg/kg)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb %	ESR mm/h
I (Normal Control)	-	10.92±0.94	6.18±0.56	14.54±1.23	6.84±0.47
II (Arthritic control)	-	6.81±0.45	10.15±0.55	10.37±1.57	10.82±1.47
III (Standard)	10	10.63±0.86^c	6.96±0.32^c	13.81±1.68^c	7.39±0.42^c
IV (Test-1)	BDEE 100	8.76±0.47^c	8.82±0.64^b	11.76±1.08	9.93±0.85
V (Test-2)	BDEE 200	9.11±0.74^c	7.73±0.76^c	12.54±1.52^a	8.74±0.56^c
VI (Test-3)	BDEE 400	10.04±0.89^c	7.14±0.44^c	13.05±0.98^b	8.14±0.68^c

c. Effect of graded doses of LAEE on hematological changes

Groups	Dose (mg/kg)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb%	ESR mm/h
I (Normal Control)	-	9.17±0.84	3.42±0.14	15.95±1.79	10.96±1.12
II (Arthritic control)	-	5.28±0.46	10.17±0.86	11.18±1.36	16.59±2.84
III (Standard)	10	8.69±0.7^c	4.21±0.74^c	15.22±1.68^c	11.12±1.21^c
IV (Test-1)	LAEE 100	6.91±0.6^c	6.38±0.89^c	13.12±1.84	13.39±1.73^a
V (Test-2)	LAEE 200	7.04±0.4^c	5.91±0.41^c	13.90±0.85^a	12.65±1.46^b
VI (Test-3)	LAEE 400	7.81±0.6^c	5.18±0.36^c	14.42±0.58^b	11.82±1.26^c

Data represent the mean±SEM, (n=6). Statistical significance: ^aP<0.05, ^bP<0.01, ^cP<0.001, versus with respect to arthritic control groups (One way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, RBC: Red blood cell, WBC: White blood cell, Hb%: Hemoglobin %, ESR: Erythrocyte sedimentation rate, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

to toxicant group rats, which were given in Table 7 and Figure 7.

Effect of BVEE

High levels of serum CRP (10.96 ± 1.16 mg/dL) and RF (81.65 ± 4.74 IU/mL) were observed in the CFA control group rats when compared to the normal control group. BVEE at the doses of 100, 200, 400 mg/kg b.wt and 10 mg/kg b.wt of diclofenac sodium treated were observed to reduce the increased levels of both CRP and RF in the serum. The % reduction of CRP was: 74.08, 38.68, 62, 50, 78.01%, and RF was 73.81, 63.72, 67.58 and 73.36%.

Effect of BDEE

Significant decrease in the levels of CRP and RF in serum by treated with 10 mg/kg b.wt of diclofenac sodium, BDEE at doses of 100,200 and 400 mg/kg b.wt as compared to the arthritic group. High levels of serum CRP (8.64 ± 1.32 mg/dL) and RF (75.38 ± 6.38 IU/mL) were observed in the CFA control group rats. These effects were significantly dose-dependent. The % reduction of CRP was: 66.55, 50.34, 52.31, 59.83 %, and RF was: 72.63, 53.34, 58.49, 64.27%.

Effect of LAEE

High levels of serum CRP (9.52 ± 1.16 mg/dL) and RF (77.74 ± 5.64 IU/mL) were observed in the arthritic control rats. Table 4C shows a significant decrease in the levels of CRP and RF in serum with 10 mg/kg b.wt of diclofenac sodium and graded doses of LAEE-treated groups as compared to the arthritic control group. The % reduction of CRP was 72.26, 50.0, 55.04, 64.49 %; RF was 72.35, 55.90, 61.16, and 69.95%. These effects were significantly dose-dependent.

Effect of different doses of 100, 200, and 400 mg/kg b.wt of extracts of BVEE, BDEE, and LAEE on IL-1β and TNF–α in CFA-induced arthritis treated rats

The most abundant cytokines promoting inflammatory responses are TNF-α and IL-1β. IL-1β is considered to be the

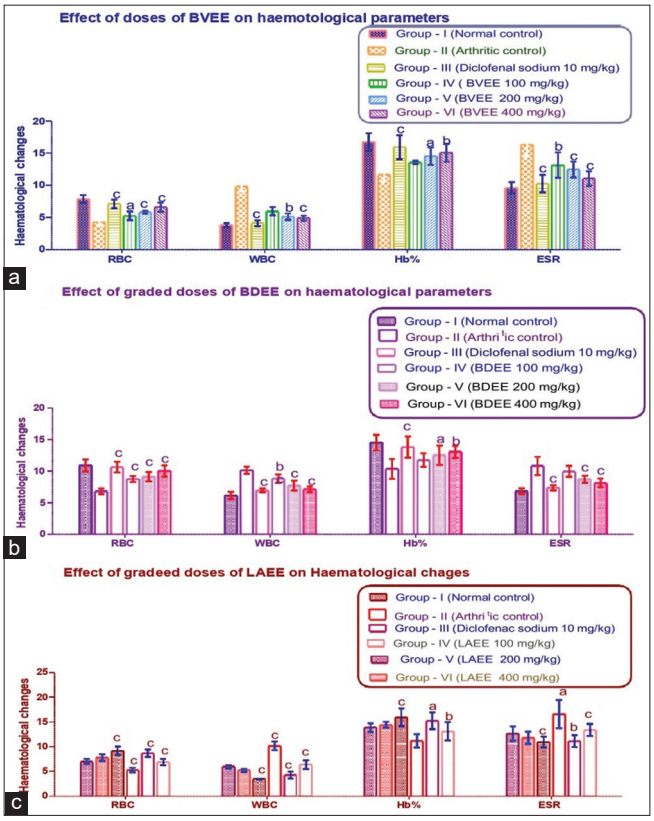

Figure 5: Graphical representation of effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on hematological alterations in complete Freund's Adjuvant-induced arthritis and treated animals. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

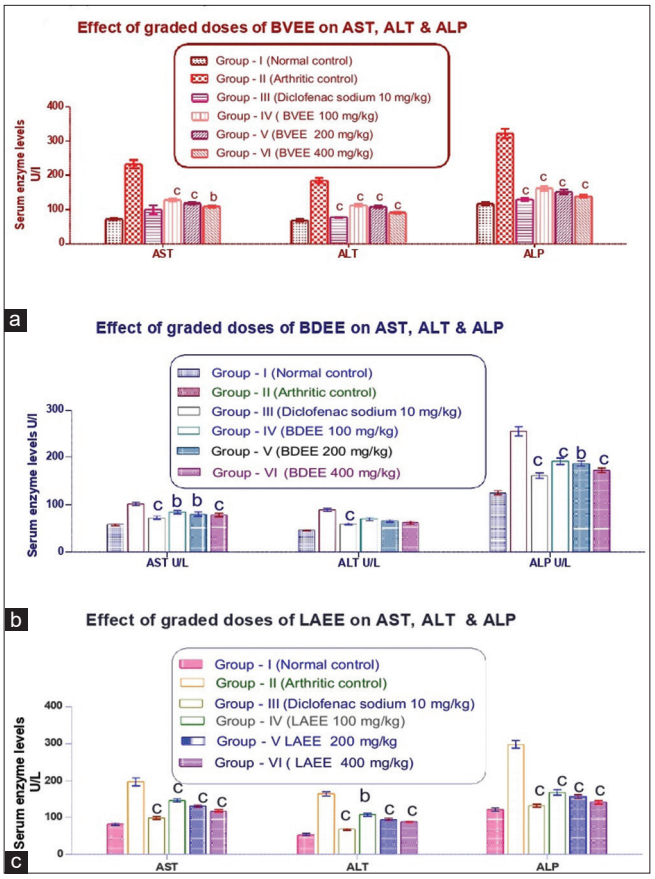

Figure 6: Graphical representation of effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on liver enzymes in complete Freund's Adjuvant-induced arthritis and treated animals. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

key mediator with regard to cartilage and bone destruction TNF-α is considered to be a hallmark cytokine in the pathogenesis of RA were given in Table 8 and Figure 8.

Effect of BVEE

Treatment with BVEE for 28 days suppressed the levels of all the evaluated cytokines in a dose-dependent manner in serum. The cytokines (IL-1β and TNF-α) and the inhibitory effects exhibited by BVEE were most significant and potent at the dose of 400 mg/kg b.wt. In serum, the levels of IL-1β and TNF-α were reduced by BVEE in a dose-dependent manner, with maximal downregulation in comparison to the arthritic control group at a dose of 400 mg/kg were IL1β 61.32% and TNF-α: 63.24%.

Effect of BDEE

Treatment with BDEE for 28 days suppressed the levels of all the evaluated cytokines in a dose-dependent manner in serum. The cytokines (IL-1β and TNF-α) and the inhibitory effects exhibited by BDEE were most significant and potent at the dose of 400 mg/kg b.wt. In serum, the levels of IL-1β and TNF-α were reduced by BDEE in a dose-dependent manner, with maximal downregulation in comparison to the arthritic control group at a dose of 400 mg/kg were IL 1β:61.32% and TNF-α: 63.24%.

Effect of LAEE

Treatment with LAEE for 28 days suppressed the levels of all the evaluated cytokines in a dose-dependent manner in serum. For the cytokines (IL-1β and TNF-α) and the inhibitory effects exhibited by LAEE were most significant and potent at the dose of 400 mg/kg b.wt. In serum, the levels of IL-1β and TNF-α were reduced by LAEE in a dose-dependent manner, with maximal downregulation in comparison to the arthritic control group at a dose of 400 mg/kg were IL1β 61.32% and TNF-α: 63.24%.

Table 6: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on liver enzymes in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE on liver enzymes

Groups	Dose (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I (Normal control)	-	72.19±3.09	68.52±3.80	117.31±4.43
II Arthritic	-	232.6±11.78	184.85±7.59	321.79±13.76
III Standard	10	99.76±12.58	76.19±2.13^{c}	129.18±4.51^{c}
IV Test-1	BVEE 100	127.86±4.53^{c}	112.53±4.15^{c}	162.17±6.68^{c}
V Test-2	BVEE 200	118.32±3.98^{c}	108.21±3.59^{c}	151.53±6.49^{c}
VI Test-3	BVEE 400	109±3.93^{b}	89.60±3.89^{c}	138.24±4.79^{c}

b. Effect of graded doses of BDEE on liver enzymes

Groups	Dose (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I (Normal Control)	-	57.67±2.86	45.67±1.91	125.33±4.31
II (Arthritic control)	-	101.67±3.74	89.50±3.49	256.50±9.59
III (Standard)	10	72.50±3.59^{c}	58.83±2.58^{c}	161.16±5.37^{c}
IV (Test-1)	BDEE 100	84.50±3.69^{b}	69.67±3.61	191.16±6.63^{c}
V (Test-2)	BDEE 200	80.29±4.41^{b}	65.50±3.12	186.83±5.42^{b}
VI Test-3	BDEE 400	78.63±3.38^{c}	61.84±2.94	172.52±4.98^{b}

c. Effect of graded doses of LAEE on liver enzymes

Groups	Dose (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I (Normal Control)	-	81.12±3.31	53.56±2.95	121.45±4.46
II (Arthritic control)	-	196.48±10.47	164.13±15.86	298.38±10.69
III (Standard)	10	98.73±3.98^{c}	67.14±7.89^{c}	132.19±4.52^{c}
IV (Test-1)	LAEE 100	146.18±4.12^{c}	107.69±10.10^{b}	168.19±7.40^{c}
V (Test-2)	LAEE 200	131.38±3.09^{c}	94.18±9.19^{c}	156.71±5.37^{c}
VI (Test-3)	LAEE 400	118.22±3.38^{c}	87.559±7.49^{c}	141.58±4.31^{c}

Data represent the mean±SEM, (n=6). Statistical significance: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Effect of graded doses of 100, 200, and 400 mg/kg b. w of different extracts of BVEE, BDEE, and LAEE on the levels of anti-oxidant enzymes

The CFA-induced inflammatory response has also been strongly linked to the release of neutrophil-derived mediators like H_2O_2, superoxide, and OH radicals. Reactive oxygen species produced for the neutrophils and macrophages contribute to macromolecular-based tissue injuries during inflammatory reactions. *In vivo* models it has been revealed that many of the anti-inflammatory agents mediate their effects through the inhibition of free radical generation by activated neutrophils. The liver tissues of the animals used for the study were analyzed for anti-oxidant enzyme activities (SOD, $GP_{X,}$ and GR_X) were given in Table 9 and Figure 9.

Effect of BVEE

As shown in Table 4 in the arthritic control group, the levels of antioxidant enzymes (SOD, $GP_{X,}$ and GR_X) were markedly reduced. Treatment with 10 mg/kg b.wt of diclofenac sodium and 100, 200, and 400 mg/kg b.wt of BVEE significantly upregulated the levels of SOD, $GP_{X,}$ and GR_X in comparison to the arthritic control group. The effects of BVEE were dose-dependent, and the maximum effect was found at 400 mg/kg b.wt.

Effect of BDEE

SOD, $GP_{X,}$ and GR_X levels were significantly reduced in Group II animals when compared with Group I. Diclofenac sodium 10 mg/kg b.w and graded doses of BDEE-treated animals showed a significant ($P < 0.01$) increase when compared to Group II animals. The effects of graded doses of BDEE were dose dependent, and at 400 mg/kg b.wt, the levels of SOD, $GP_{X,}$ and GR_X were upregulated maximally.

Effect of LAEE

In the rats of CFA-induced arthritic group (Group II), the levels of antioxidant enzymes (SOD, $GP_{X,}$ and GR_X) were markedly reduced when compared with Group 1. Treatment with graded doses of LAEE significantly upregulated the levels of SOD, $GP_{X,}$ and GR_X and in a dose-dependent pattern. These effects were fairly comparable to that of diclofenac

Table 7: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on CRP and RF in CFA-induced arthritis treated rats

(a) BVEE

Groups	Dose mg/kg)	CRP (mg/dL)	RF (IU/mL)
I (Normal control)	-	1.91±0.6	18.42±1.82
II (Arthritic control)	-	10.96±1.16	81.65±4.74
III (Standard)	10	2.84±0.98[c]	21.38±2.62[c]
IV (Test-1)	BVEE 100	6.72±1.92	29.62±3.96[c]
V (Test-2)	BVEE 200	4.11±1.83[b]	26.47±2.88[b]
VI (Test-3)	BVEE 400	2.41±0.94[c]	21.75±3.81[c]

(b) BDEE

Groups	Dose mg/kg)	CRP (mg/dL)	RF (IU/ml)
I (Normal control)	-	1.72±0.6	17.74±1.69
II (Arthritic control)	-	8.64±1.32	75.38±6.38
III (Standard)	10	2.89±0.8[c]	20.63±1.85[c]
IV (Test-1)	BDEE 100	4.29±1.02[b]	35.17±3.69[b]
V (Test-2)	BDEE 200	4.12±0.92[b]	31.29±2.98[c]
VI (Test-3)	BDEE 400	3.47±0.7[b]	26.93±2.83[c]

(c) LAEE

Groups	Dose (mg/kg)	CRP (mg/dl)	RF (IU/ml)
I (Normal control)	-	1.86±0.8	19.12±1.91
II (Arthritic control)	-	9.52±1.16	77.74±5.64
III (Standard)	10	2.64±0.81[c]	21.49±2.58[c]
IV (Test-1)	LAEE 100	4.76±0.96[b]	34.28±3.79[c]
V (Test-2)	LAEE 200	4.28±0.89[b]	30.19±2.16[b]
VI (Test-3)	LAEE 400	3.38±0.76[c]	23.36±1.48[c]

Data represent the mean±SEM, (n=6). Statistical significance: [a]P<0.05, [b]P<0.01, [c]P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, CRP: C-reactive protein, RF: Rheumatoid factor, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Table 8: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on IL-1β and TNF-α in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE on IL -1β and TNF-α

Groups	Dose (mg/kg)	IL-1β (µg/mL)	TNF-α (µg/mL)
I (Normal control)	-	19.50±1.98	8.32±1.13
II (Arthritic control)	-	472.56±18.48	89.14±3.91
III (Standard)	10	175.17±8.62[c]	30.94±2.37[c]
IV (Test-1)	BVEE 100	214.63±10.32[c]	38.92±2.85
V (Test-2)	BVEE 200	202.34±9.43[c]	35.36±2.41[b]
VI (Test-3)	BVEE 400	182.75±7.12[c]	32.76±2.32[a]

b. Effect of graded doses of BDEE on IL -1β and TNF-α

Groups	Dose (mg/kg)	IL-1β (µg/mL)	TNF-α (µg/mL)
I (Normal control)	-	18.20±1.89	7.15±0.96
II (Arthritic control)	-	451.60±17.21	85.59±4.38
III (Standard)	10	178.59±9.13[c]	32.75±2.62[a]
IV (Test-1)	BDEE 100	261.42±12.45[c]	52.36±3.83[b]
V (Test-2)	BDEE 200	248.85±10.22[c]	45.72±3.36[c]
VI (Test-3)	BDEE 400	210.38±9.37[c]	41.38±4.24[a]

(Contd...)

Table 8: *(Cotinued)*

c. Effect of graded doses of LAEE on IL -1β and TNF-α

Groups	Dose (mg/kg)	IL-1β (µg/mL)	TNF-α (µg/mL)
I (Normal control)	-	16.72±1.62	6.96±0.82
II (Arthritic control)	-	439.25±16.89	93.62±4.72
III (Standard)	10	171.86±8.13[c]	36.19±3.74[b]
IV (Test -1)	LAEE 100	242.83±11.45[c]	49.71±3.11[a]
V (Test -2)	LAEE 200	230.74±9.78[c]	42.89±3.27[a]
VI (Test -3)	LAEE 400	193.16±7.56[c]	37.82±2.68[b]

Data represent the mean±SEM (n=6). Statistical significance: [a]P<0.05, [b]P<0.01, [c]P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

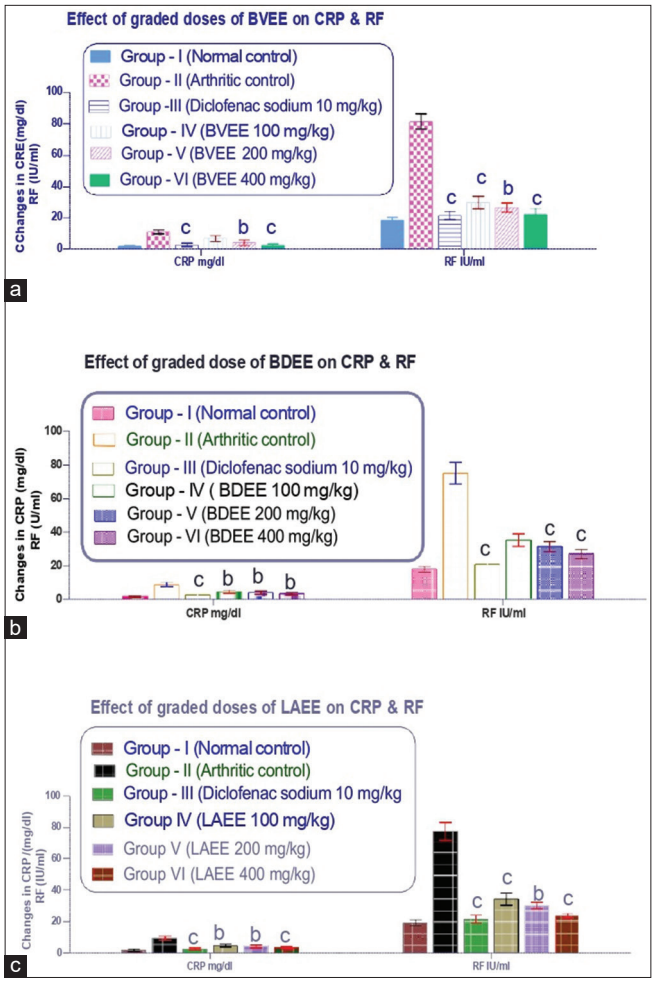

Figure 7: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on CRP and RF in complete Freund's Adjuvant induced arthritic and arthritic treated rats. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: [a]P < 0.05, [b]P < 0.01, [c]P < 0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

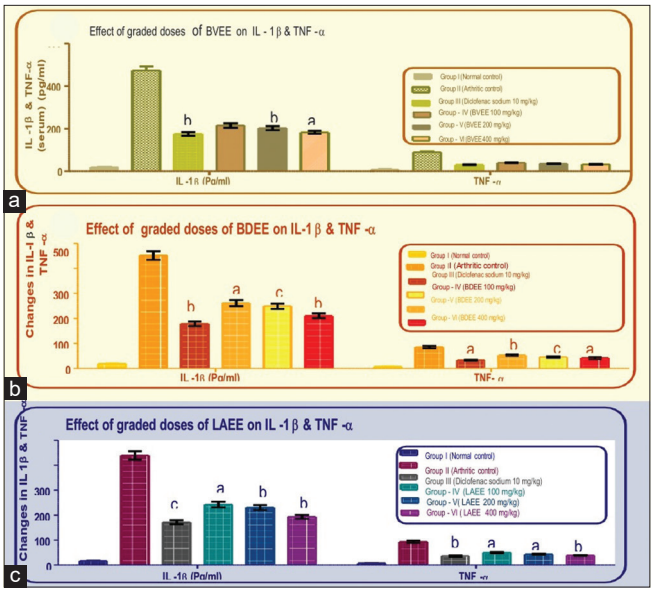

Figure 8: Effect of graded doses of BVEE (a), BDEE (b), and LAEE (c) on IL-1β and TNF-α in complete Freund's Adjuvant-induced arthritic and treated animals. Data represent the mean ± standard error of the mean (n = 6). Statistical significance: [a]P < 0.05, [b]P < 0.01, [c]P < 0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

sodium. The effects of LAEE at a dose of 400 mg/kg b.wt., the levels of SOD, $GP_{X,}$ and GR were upregulated maximally.

Effect on body weight and organ weight changes

The results are depicted in Table 10.

Effect of BVEE

The challenge with CFA showed a significant decrease in the average b.wt and an increase in the spleen and thymus weight in arthritic control as compared to the normal

Table 9: Effect of graded doses of BVEE/BDEE/LAEE on SOD, GPX and GR in in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE on SOD, GPX and GR

Groups	Dose (mg/kg)	SOD (U/mg protein)	GPX (U/mg protein)	GR U/mg protein)
I (Normal control)	-	42.74±3.07	37.52±2.49	29.38±1.83
II (Arthritic control)	-	10.56±1.02	3.94±0.10	03.18±0.08
III (Standard)	10	39.79±1.21^{c}	34.14±6.72^{c}	27.89±5.94^{c}
IV (Test-1)	BVEE 100	30.67±4.18^{a}	27.64±262^{b}	16.58±1.05^{a}
V (Test-2)	BVEE 200	33.93±2.61^{b}	29.48±232^{b}	20.23±4.05^{b}
VI (Test-3)	BVEE 400	36.11±7.36^{b}	32.82±6.763^{c}	24.04±1.72^{c}

b. Effect of graded doses of BDEE on SOD, GPX and GR

Groups	Dose	SOD	GPX	GR
I (Normal control)	-	49.26±2.03	34.40±0.12	31.51±2.26
II (Arthritic control)	-	13.49±1.12	5.90±0.10	02.98±0.06
III (Standard)	10	47.13±2.10^{c}	31.12±1.81^{c}	28.13±5.26^{c}
IV (Test-1)	BDEE 100	36.62±6.29^{b}	18.21±1.35^{c}	21.85±1.08^{a}
V (Test-2)	BDEE 200	40.42±3.62^{b}	26.94±1.43^{c}	26.43±1.02^{b}
VI (Test-3)	BDEE 400	44.90±5.90^{c}	28.20±1.31^{c}	28.42±7.04^{c}

c. Effect of graded doses of LAEE on SOD, GPX and GR

Groups	Dose	SOD	GPX	GR
I (Normal control)	-	46.37±3.74	36.60±2.12	32.43±1.06
II (Arthritic control)	-	11.82±1.23	4.26±0.16	03.97±0.18
III (Standard)	10	44.02±2.13^{c}	33.36±6.92^{c}	28.33±7.11^{c}
IV (Test-1)	LAEE 100	26.51±2.60^{a}	21.72±1.35^{a}	20.99±1.09^{a}
V (Test-2)	LAEE 200	32.86±2.96^{b}	26.55±1.21^{b}	24.66±1.06^{b}
VI (Test-3)	LAEE 400	39.71±6.99^{c}	29.41±7.41^{b}	27.85±5.09^{c}

Data represent the mean±SEM, (n=6). Statistical significance: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GR: Glutathione reductase, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

control. Under similar conditions, diclofenac sodium and graded doses of the treated groups showed an average gain in body weight.

Effect of BDEE

As shown in Table 9, BDEE was found to improve the b.wt gain and decreased spleen and thymus weights in arthritis treated rats. These effects were dose-dependent and maximally marked at the tested dose of 400 mg/kg b.wt of BDEE.

Effect of LAEE

As shown in Table 9, LAEE was found to improve the b.wt gain and decreased spleen and thymus weights in arthritis treated rats. These effects were dose-dependent and maximally marked at the tested dose of 400 mg/kg b.wt. of LAEE.

Effect of graded doses of 100, 200, and 400 mg/kg b.wt of BVEE (a)/BDEE (b)/LAEE (c) on radiology score assessment and radiographical evaluation was given in Figure 11 to 13

Bone destruction, which is a common feature of arthritis, was examined by radiological analysis as shown in Figures 10-13.

Radiology score assessment

CFA-administered rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft-tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone, and extensive erosions produced narrowing or pseudo-widening of all joint spaces. In contrast, the rats treated with graded doses of 100, 200, and 400 mg/kg b.wt of BVEE (a)/BDEE (b)/LAEE (c) attenuate abnormalities

Table 10: Effect of graded doses of 100, 200, and 400 mg/kg b.wt of different extracts of BVEE, BDEE, and LAEE on b.wt and organ weight changes in CFA-induced arthritis treated rats

a. Effect of graded doses of BVEE b.wt (g), Thymus weights (mg), and spleen (g)

Groups	Dose (mg/kg)	0 Day	28 Day	Mean change	Thymus (mg)	Spleen (g)
I	-	172.56 ± 8.56	181.58 ± 7.84	-	119.15 ± 12.5	0.6 ± 0.005
II	-	198.84 ± 6.47	172.18 ± 6.48	(-) 26.66	212.38 ± 13.35	1.45 ± 0.12
III	10	170.63 ± 5.49	183.39 ± 8.42	(+) 12.76	135.16 ± 13.15^{b}	0.75 ± 0.09^{c}
1V	BVEE 100	186.38 ± 6.73	195.34 ± 6.90^{c}	(+) 8.96	165.70 ± 12.88	1.18 ± 0.05
V	BVEE 200	175.54 ± 5.98	186.18 ± 7.62^{a}	(+) 10.64	142.34 ± 13.22^{a}	1.010 ± 0.35
VI	BVEE 400	179.71 ± 7.56	191.57 ± 6.52^{c}	(+) 11.86	126.21 ± 12.91^{b}	0.80 ± 0.15^{c}

b. Effect of graded doses of BDEE on b.wt (g), Thymus weights (mg), and spleen (g)

Groups	Dose (mg/kg)	0 Day	28 Day	Mean change	Thymus (mg)	Spleen (g)
I	-	182.14 ± 9.23	190.84 ± 7.42	-	117.15 ± 12.5	0.7 ± 0.008
II	-	191.69 ± 8.69	172.45 ± 8.56	(-) 19.24	215.30 ± 13.35	1.45 ± 0.12
III	10	176.34 ± 5.54	188.05 ± 5.83^{b}	(+) 11.71	135.16 ± 13.15^{b}	0.85 ± 0.08^{c}
1V	BDEE 100	183.5 ± 6.59	189.92 ± 8.50	(+) 6.34	161.15 ± 11.75	1.16 ± 0.05
V	BDEE 200	172.75 ± 6.91	181.23 ± 6.84^{b}	(+) 8.48	149.34 ± 15.22^{a}	1.09 ± 0.15
V 1	BDEE 400	181.29 ± 7.52	192.04 ± 8.48^{c}	(+) 9.75	123.21 ± 13.91^{b}	0.70 ± 0.19^{c}

c. Effect of graded doses of LAEE on b.wt (g), Thymus weights (mg) and spleen (g)

Groups	Dose (mg/kg)	0 Day	28 Day	Mean change	Thymus (mg)	Spleen (g)
I	-	177.43 ± 8.13	183.12 ± 8.76		117.13 ± 14.19	0.60 ± 0.002
II	-	182.72 ± 6.74	159.23 ± 4.75	(-) 23.49	211.38 ± 12.15	1.75 ± 0.31
III	10	178.38 ± 5.90	189.62 ± 8.63^{c}	(+) 11.24	201.54 ± 13.21^{c}	0.95 ± 0.09^{c}
1V	LAEE 100	186.93 ± 7.56	194.35.89^{c}	(+) 7.39	186.31 ± 12.43	1.25 ± 0.03^{a}
V	LAEE 200	174.26 ± 5.67	184.09 ± 8.53^{c}	(+) 9.83	168.02 ± 12.89	1.14 ± 0.08^{c}
VI	LAEE 400	179.32 ± 7.26	189.9 ± 6.58^{c}	(+) 10.59	123.26 ± 10.76^{b}	0.90 ± 0.07^{b}

Data represent the mean ± SEM, (n = 6). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to CCl_4 groups (One-way analysis of variance followed by Dunnett's multiple comparison test). CFA: Complete Freund's Adjuvant, BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

consists of asymmetric soft-tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws.

The BVEE at doses of 100, 200, and 400 mg/kg b.wt produced better results than the 100, 200, and 400 mg/kg b.wt of BDEE and LAEE. At the dose of 400 mg/kg b.wt of BVEE showed better results than the 400 mg/kg b.wt of BDEE and LAEE.

Radiographic evaluation

Effect of graded doses of BVEE (a)/BDEE (b)/LAEE (c) on radiographical evaluation of joints.

Radiographic evaluation

Effect of graded doses of BVEE (a)/BDEE (b)/LAEE (c) on radiographical evaluation of joints. Effect of graded doses of BVEE (a)/BDEE (b)/ LAEE(c) on histopathology of hind paw in CFA-induced arthritis treated rats over 28 days. Results were shown in Figure 14 to 16.

Effect of graded doses of BVEE on histopathology of hind paws joints

Group -1: Normal control

Normal ankle joint intact, articular cartilage with normal joint space and synovial tissue.

Group – II: Arthritic control

Arthritic joint showed abnormal joint like pannus formation, degeneration with partial erosion of the cartilage, destruction of bone marrow, destruction of bone, and extensive infiltration of inflammatory exudates in the articular surface.

Group – III: Standard (diclofenac sodium)

Recovery of destruction of articular cartilage, suppressed inflammatory cells in synovial membrane and joint cavity. Recovery of bone erosion and vascularity formation.

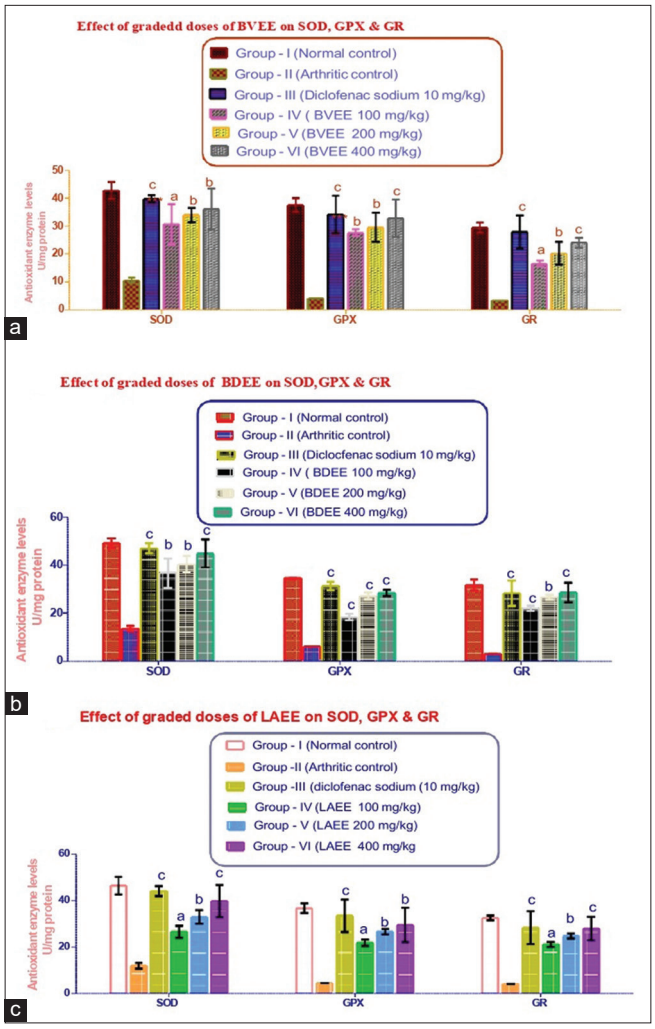

Figure 9: Graphical representations of the effect of graded doses of BVEE/BDEE/LAEE on SOD, GPX, and GR in complete Freund's Adjuvant-induced arthritis and treated rats. Data represent the mean ± standard error of the mean ($n = 6$). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GR: Glutathione reductase

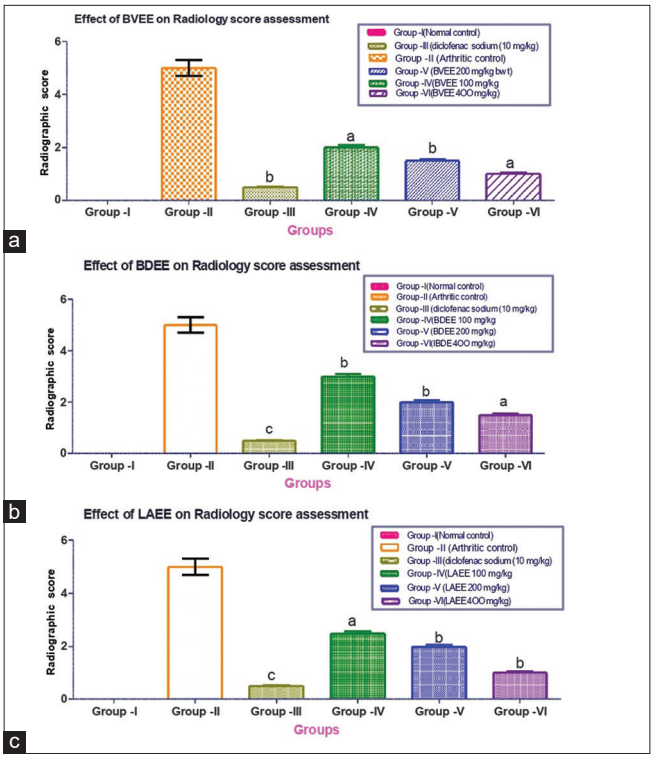

Figure 10: Effect of graded doses of 100, 200, and 400 mg/kg b.wt of extracts of (a) BVEE, (b) BDEE, and (c) LAEE on radiology score assessment in complete Freund's Adjuvant-induced rats. Data represent the mean ± standard error of the mean ($n = 6$). Statistical significance: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, versus with respect to arthritic control groups (One-way analysis of variance followed by Dunnett's multiple comparison test). BVEE: Ethanolic extracts from *Bauhinia variegata*, BDEE: Ethanolic extracts from *Boerhavia diffusa*, LAEE: Ethanolic extracts from *Limonia acidissima*

Group –IV: BVEE (100 mg/kg b.wt)

100 mg/kg b.wt of BVEE shown mild reduction in synovial lining, Recovery of bone erosion, suppressed inflammatory cells in synovial membrane and joint cavity.

Group – V: BVEE (200 mg/kg b.wt)

Light cartilage destruction. Recovery of bone erosion and vascularity formation

Group – VI: BVEE (400 mg/kg b.wt)

Recovery of bone erosion and vascularity formation. Recovery of articular cartilage, suppressed inflammatory cells in synovial membrane, joint cavity, and normal joint space.

Figure 14 shows the effect of graded doses of BVEE on histopathology of hind paw joints in CFA-induced arthritis treated rats over 28 days.

Effect of graded doses of BDEE on histopathology of hind paw joints

Group -1: Normal control

Normal ankle joint intact, bone and bone marrow, articular cartilage with normal joint space, and synovial tissue.

Group – II: Arthritic control

Arthritic joint showed pannus formation, degeneration with partial erosion of the cartilage, destruction of bone marrow, extensive infiltration of inflammatory exudates in the articular surface, and destruction of bone.

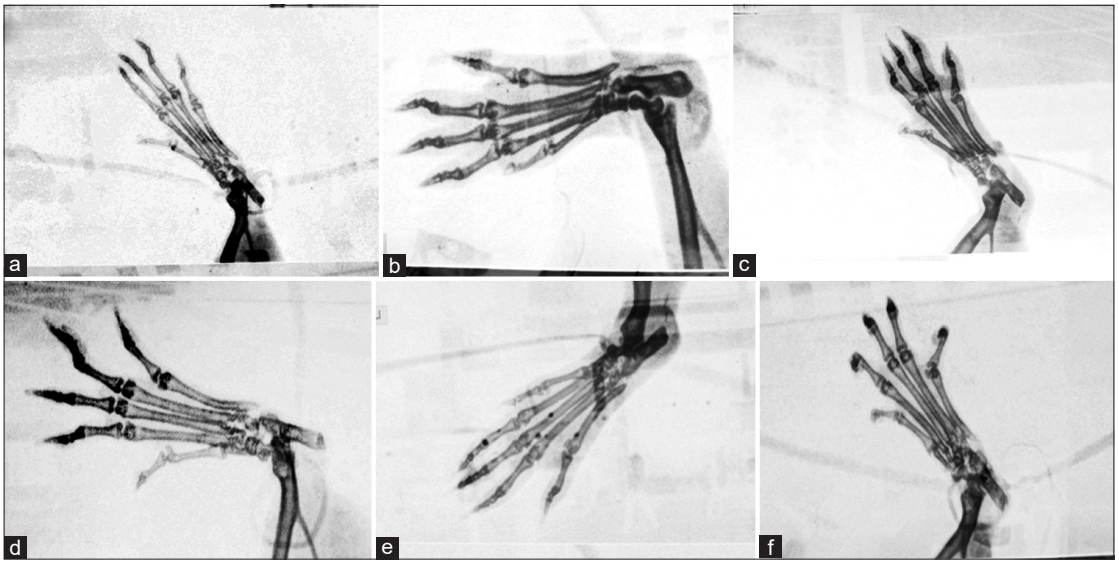

Figure 11: Effect of graded doses of BVEE on Radiographical evaluation of joints. A: Articular spaces, B: Bone, S: Synovium, IC: Intact cartilage, BM: Bone Marrow, CU: Cartilage ulceration, SM: Synovial membrane. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: BVEE 100 mg/kg. (e) Group –V: BVEE 200 mg/kg. (f) Group –VI: BVEE 400 mg/kg.

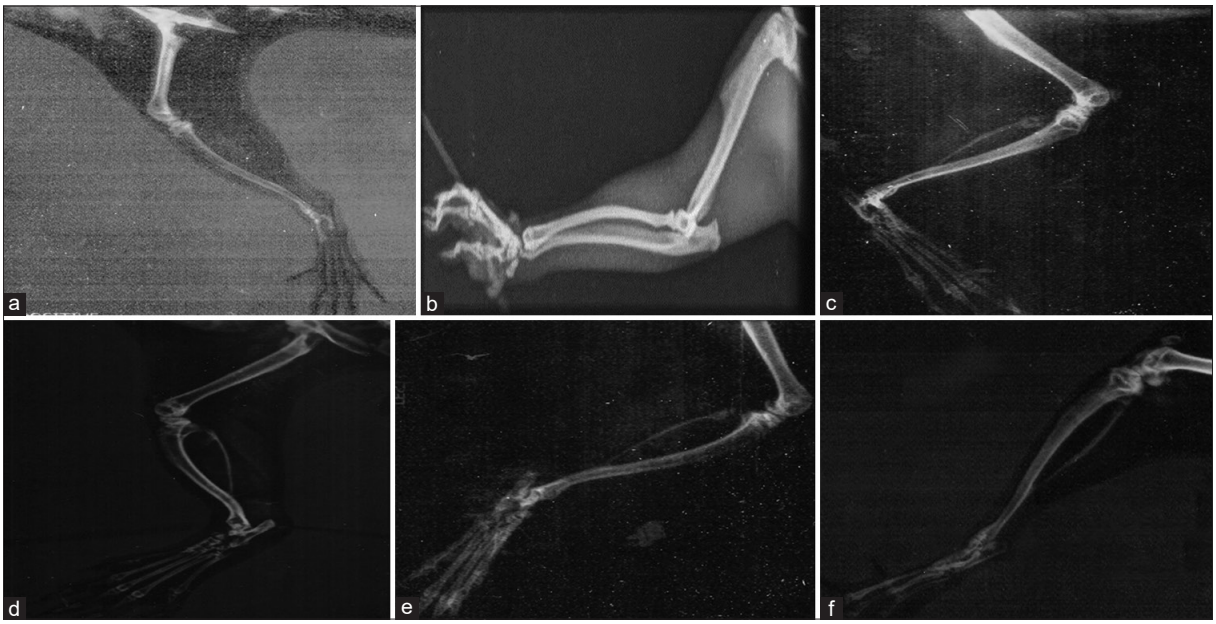

Figure 12: Effect of graded doses of BDEE on radiographical evaluation of joints. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: BDEE 100 mg/kg. (e) Group – V: BDEE 200 mg/kg. (f) Group –VI: BDEE 400 mg/kg.

Group – III: Standard (Diclofenac sodium)

Suppressed inflammatory cells in synovial membrane and joint cavity, Recovery of articular cartilage. Recovery of bone erosion and vascularity formation with normal joint space and synovial tissue.

Group –IV: BDEE (100 mg/kg b.wt)

100 mg/kg b.wt of BDEE shown moderate reduction in synovial lining, recovery of bone erosion, suppressed inflammatory cells in the synovial membrane and joint cavity.

Group – V: BDEE (200 mg/kg b.wt)

200 mg/kg b.wt of BDEE showed recovery of bone erosion, vascularity formation, and light cartilage destruction and bone erosion.

Group – V I: BDEE (400 mg/kg b.wt)

400 mg/kg b.wt of BDEE showed less inflammatory signs like scanty cellular infiltrate, absence of edema formation, normal bone marrow, and recovery of destruction of articular cartilage.

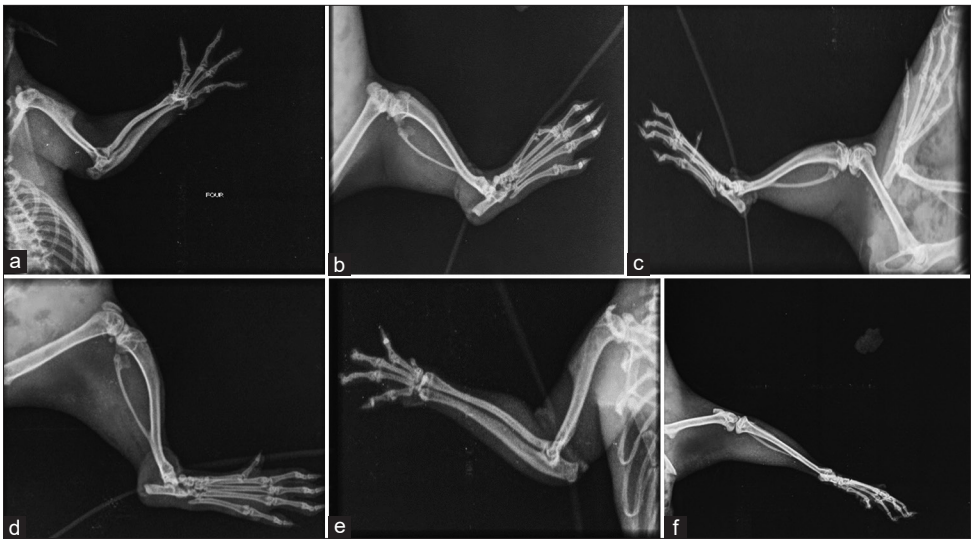

Figure 13: Effect of graded doses of LAEE on radiographical evaluation of joints. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: LAEE 100 mg/kg. (e) Group – V: LAEE 200 mg/kg. (f) Group – VI: LAEE 400 mg/kg.

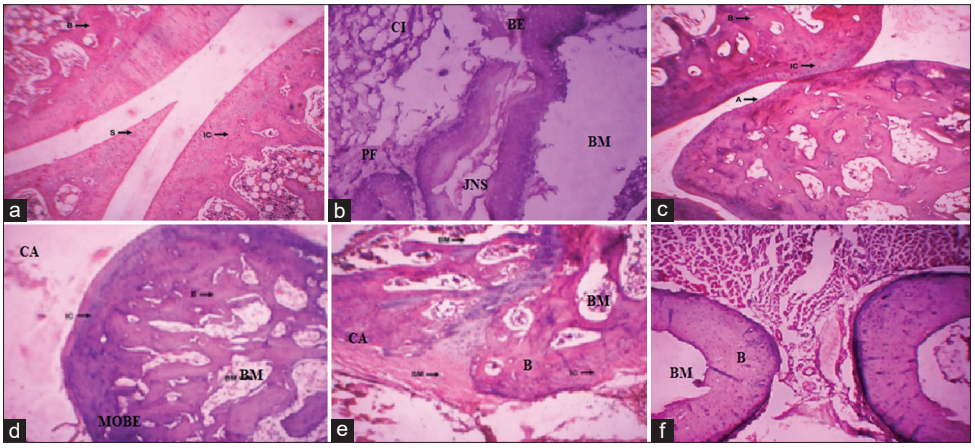

Figure 14: Histological observation on joint sections from complete Freund's Adjuvant-induced joints damage in rats with or without BVEE (Ethanolic extracts from *Bauhinia variegata*) treatment. Photomicrographs of histological changes of rat joints: B: Bone, BE: Bone erosion, CA: Cartilage, PF: Pannus formation, CI: Cellular infiltration, JNS: Joint narrow space, MIBE: Mild bone erosion, MOBE: Moderate bone erosion. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: BVEE 100 mg/kg. (e) Group – V: BVEE 200 mg/kg. (f) Group – VI: BVEE 400 mg/kg.

Figure 15 shows the effect of graded doses of 100, 200, and 400 mg/kg b.wt of BDEE on histopathology paw joints.

Effect of graded doses of 100, 200, and 400 mg/kg b.wt of LAEE on histopathology of hind paw joints

Group - 1: Normal control

Articular cartilage with normal joint space, synovial tissue, normal ankle joint intact, and normal bone and cartilage.

Group – II: Arthritic control

Soft-tissue swelling, massive influx of inflammatory cells, bone mineralization, pannus formation, cartilage erosion, and joint space narrowing were observed.

Group – III: Standard (Diclofenac sodium 10 kg/b.wt)

Normal joint space, Recovery of bone, cartilage destruction, suppressed inflammatory cells in the synovial membrane and joint cavity. Recovery from bone erosion and vascularity formation.

Group – IV: LAEE (100 mg/kg b.wt)

100 mg/kg b.wt of LAEE showed light bone erosion, suppressed inflammatory cells in the synovial membrane and joint cavity, mild reduction in synovial lining, and light pannus formation.

Group – V: LAEE (200 mg/kg b.wt)

Recovery of bone erosion, vascularity formation, light cartilage destruction, and joint narrow space.

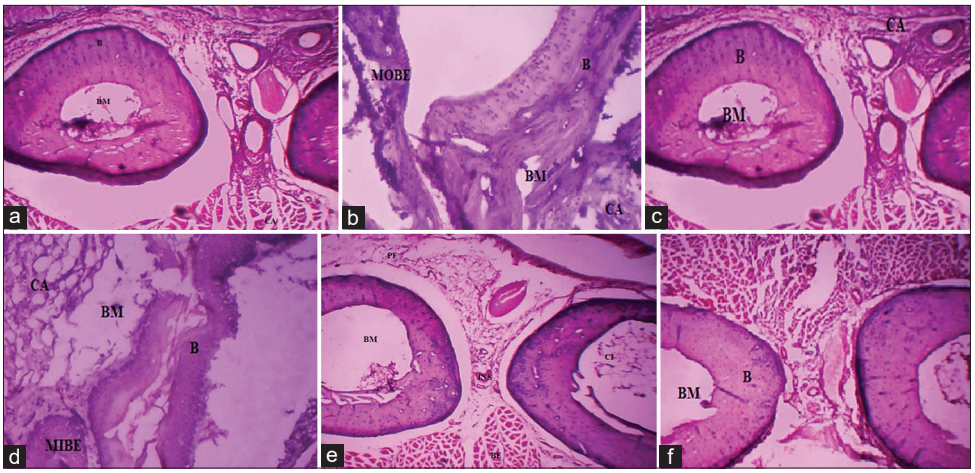

Figure 15: Histological observation on joint sections from complete Freund's Adjuvant-induced joints damage rats with or without BDEE (Ethanolic extracts from *Boerhavia diffusa*) treatment. Photomicrographs of histological changes of rat joints: B: Bone, BE: Bone erosion, CA: Cartilage, PF: Pannus formation, CI: Cellular infiltration, JNS: Joint narrow space, MIBE: Mild bone erosion, MOBE: Moderate bone. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: BDEE 100 mg/kg. (e) Group – V: BDEE 200 mg/kg. (f) Group – VI: BDEE 400 mg/kg.

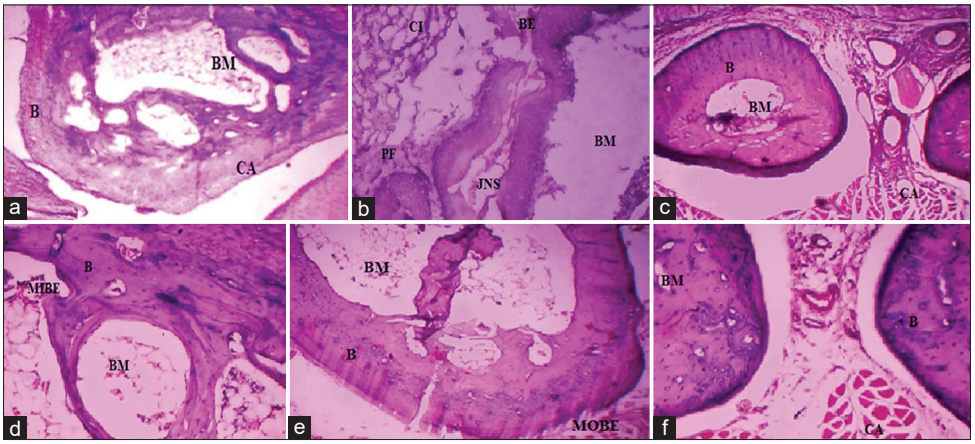

Figure 16: Histological observation on joint sections from complete Freund's Adjuvant-induced joint damage of rats with or without LAEE (Ethanolic extracts from *Limonia acidissima*) treatment. Photomicrographs of histological changes of rat joints: B: Bone, BE: Bone erosion, CA: Cartilage, PF: Pannus formation, CI: Cellular infiltration, JNS: Joint narrow space, MIBE: Mild bone erosion, MOBE: Moderate bone erosion. (a) Group – I: Normal control. (b) Group – II: Positive control. (c) Group – III: Standard (Diclofenac sodium 10 mg/kg). (d) Group – IV: LAEE 100 mg/kg. (e) Group – V: LAEE 200 mg/kg. (f) Group – VI: LAEE 400 mg/kg.

Group – VI: LAEE (400 mg/kg b.wt)

Recovery of destruction of articular cartilage, recovery of bone erosion, vascularity formation, normal joint space, suppressed inflammatory cells in the synovial membrane and joint cavity.

Figure 16 shows the effect of graded doses of 100, 200, and 400 mg/kg b.wt of LAEE on histopathology paw joints.

CONCLUSION

This study shows that *B. variegata, B. diffusa*, and *L. acidissima* ethanolic extracts effectively reduce arthritic symptoms in CFA-induced rats. Paw edema, joint inflammation, polyarthritis scores, radiological damage, body weight, and hematological parameters improved after treatment. Inflammatory mediators like TNF-α, IL-1β, CRP, and RF were reduced by the extracts, while antioxidant defenses like SOD, GPx, and glutathione reductase were restored. Further histopathological findings revealed lower inflammatory cell infiltration and joint architectural preservation. These findings indicate that the examined plant extracts' anti-inflammatory, immunomodulatory, and antioxidant capabilities may explain their anti-arthritic effects. Thus, these medicinal plants may be safer and more successful than standard anti-arthritic medicines, warranting molecular and clinical studies.

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