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# ANTIARTHRITIC ACTIVITY OF CRUDE EXTRACT OF CLERODENDRUM PHLOMIDIS (L.) LEAVES IN FCA INDUCED ARTHRITIS IN RATS

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## ABSTRACT

The present study was carried out to evaluate the antiarthritic activity of crude extract of *Clerodendrum phlomidis* leaves on FCA induced arthritis in rats. The petroleum ether, ethyl acetate and alcoholic extract was administered orally at a dose 100, 200 and 400 mg/kg, body weight from day 13 to day 21. Arthritis was assessed by various parameters such as body weight, arthritic score, paw volume and ankle diameter on day 0, 3, 6, 9, 12, 15, 18 and 21. At the end of study, animals were anesthetised and blood was collected for the estimation of various haematological parameters such as haemoglobin content, total RBA, WBC, ESR count, CRP level and TNF alpha level. There was a significant increase in body weight, reduction in arthritic score, paw volume and ankle diameter in PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) treated animals. Above findings were confirmed by haematological results as, significant improvement in the levels of Hb and RBC count, and suppressed WBC count, ESR count, CRP and TNF alpha levels found in PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) administered arthritic group. Our finding showed a significant antiarthritic activity of *Clerodendrum phlomidis* leaves against FCA induced arthritis in rats.

**KEYWORDS:** *Clerodendrum phlomidis*, Rheumatoid arthritis, FCA.

## 1. INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common chronic, autoimmune, progressive, systemic inflammatory polyarticular joint disease characterized by symmetric, synovitis and

also may be extraarticular involvement.[1] Currently available treatments in the market are used to modify the progression of disease.[2-3] but these treatments are associated with several severe side effects.[4-5] Despite extensive use of current treatment, most RA patients are suffering from declined functional ability because of deformity and disability. Now a day, researchers have taken efforts to investigate newer agents with minimum side effects. Medicinal plants play important role in the development of plant therapeutic agents.

*Clerodendrum phlomidis* is commonly known as Ami, belongs to the family Lamiaceae. It is locally called Agnimantha (Sanskrit), Arani (Marathi), Arni (Hindi), Takkari (Tamil), Nelli (Telugu), Munja (Malayalam), Arni (Bengali), Aranimula (Gujarati) and Taggi (Kannada). *Clerodendrum phlomidis* is extensibly used in Ayurveda, Unani and Homeopathic medicine. In traditional systems of medicine, leaves, stem, aerial parts and root of the plant are used in *Shotha* (inflammation), *Prameha* (glycosuria), *Jwara* (coryza), *Upadamsha* (gonorrhea), *Sthaulya* (obesity) etc.[6] The plants has been reported to possess Anti-inflammatory activity[7-8] Analgesic activity,[9] Antiarthritic activity,[10] Antimicrobial activity,[11-12] Antiobesity activity,[13] Antihepatotoxic activity,[14] Antifertility,[15] Anti-amnesic activity,[16] Anti-asthmatic activity,[17] Antioxidant activity,[18] Antidiarrhoeal activity,[19] Hypoglycemic activity[20] and Immunomodulatory activity.[21]

*Clerodendrum phlomidis* plant have traditional claim for use in arthritic disorder. No pharmacological study has been carried out on evaluation of its antiarthritic activity. So present study was carried out to evaluate antiarthritic effect of crude extract of *Clerodendrum phlomidis* leaves in freund's complete adjuvent (FCA) induced arthritis in rat.

## 2. MATERIAL AND METHODS

### 2.1 Collection of plant material

Fresh leaves of *Clerodendrum phlomidis* were collected from local area of Aravalli district, Gujarat, India in the months of July-October. This plant was identified and authenticated to Botanical Survey of India, Pune.

### 2.2 Animals

Adult male Wistar albino rats, weighing between 180 - 220 g and albino mice (25-30 g) were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pelleted rats chow and water *ad libitum* as a

standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with CPCSEA.

### 2.3 Preparation of leaf extract

The leaves were collected and dried in shade and ground. Coarsely powdered leaves were used for the study. Coarsely powdered leaves material (1000 g) was subjected to successive extraction with different solvents (petroleum ether, ethyl acetate and alcohol) (60 – 80ºC) in a soxhlet extractor at a temperature of 45-50ºC to 45 cycles per batch for 2 batches. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish.[22] The yield of petroleum ether, ethyl acetate and alcohol extract of leaves of *Clerodendrum phlomidis* was 9.8, 6.3 and 5.9 g/100 g respectively.

### 2.4 Chemicals and Drugs

Suspension of petroleum ether (PECP), ethyl acetate (EACP) and alcohol (ACP) extract of leaves of *Clerodendrum phlomidis* was prepared in sodium carboxy methyl cellulose (CMC, 0.3 %) using distilled water. All the extracts was accomplished via oral gavage.

### 2.5 Test animals

Wistar rats (180-220 g) and Male Swiss albino mice (25-30 g) were divided into eleven groups containing six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Standard (10 mg/kg, p.o.), Group III: PECP (100 mg/kg, p.o.), Group IV: PECP (200 mg/kg, p.o.), Group V: PECP (400 mg/kg, p.o.), Group VI: EACP (100 mg/kg, p.o.), Group VII: EACP (200 mg/kg, p.o.), Group VIII: EACP (400 mg/kg, p.o. Group IX: ACP (100 mg/kg, p.o.), Group X: ACP (200 mg/kg, p.o.), Group XI: ACP (400 mg/kg, p.o.).

### 2.6 Preliminary phytochemical studies

Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the petroleum ether, ethyl acetate and alcohol extract of leaves of *Clerodendrum phlomidis* has been carried out.[23]

### 2.7 Acute oral toxicity of the extract

Adult Albino mice (25-30 g) were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Different group of animals received with different doses of petroleum ether, ethyl acetate and alcohol extract of leaves of *Clerodendrum phlomidis* i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality.[24-25]

### 2.8 Induction of arthritis

***Freund's complete adjuvant induced arthritis***

RA was induced by a single intradermal injection using 15 guage needle of 0.1 mL of freund's complete adjuvant (FCA, Sigma) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into sub planter region of the left hind paw of rats.[25] FCA produced a pronounced local oedema after a few hours with a progressive increase reaching its maximum on 13$^{th}$ day of FCA injection.

Treatment with PECP, EACP and ACP and dexamethasone were started from day 13 to day 21. After the injection of FCA, the body weight, arthritic score, paw volume and ankle diameter for all animal groups were measured at 0, 3, 6, 9, 12, 15, 18 and 21 day. Body weight was measured regularly by using digital balance. Animals were scored regularly by two investigators who were blind to the treatment. Each paw was graded according to the severity, extent of erythema, swelling of periarticular soft tissues, and the enlargement and distortion of the joints. Clinical score ranged from 0 (no sign) to 4 (severe lesions), yielding a maximum score of 16 per animal.[26] Paw volume and ankle diameter was measured by using Plethysmometer (7140-UGO Basile, Italy)[27] and vernier caliper[28] respectively.

At the end of experiment, blood was withdrawn from ratino bulbar venous plexus under light anesthesia (pentobarbitone sodium at a dose 40 mg/kg of body weight of animals, ip) with the help of a glass capillary. The serum separated from the blood was collected for further biochemical assays. Haemoglobin content was estimated by the method of Drabkin and Austin. Red blood cell (RBC) and white blood cell counts (WBC) were estimated according to the method of Chesbrough and Mc Arthur in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was followed by the method of Westergren. C-reactive protein (CRP) level was estimated using the ELISA kit obtained from Alpha Diagnostics Intl., USA.[29]

Plasma tumour necrosis factor-*α (TNF-α)* concentration was determined with an ELISA commercial kit (Rat TNF α ELISA kit, Sigma Aldrich, St. Louis, USA). At the end of the experiment, samples of blood (0.5 mL) were drawn from a tail vessel. The blood was collected in polyethylene tubes having 25 µL of heparin solution (4000 IU). The plasma samples obtained after centrifugation for 10 min at 3000 *g* and 4ºC were frozen at –80ºC until assay. In brief, 100 µL of standard, sample and control were added to each well of the coated microplate. After 3 h of incubation at 24ºC the microplate was decanted and the liquid discarded. Then, 100 µL of biotinylated anti-TNF-α antibody was added to each well. After 45 min of incubation at 24°C and a further elimination of the liquid from the wells, 100 µL of Streptavidin– horseradish peroxidase conjugate was added. After incubation for a further 45 min and a washing of the wells, 100 µL of chromogen was added. The absorbance of each well was read spectrophotometrically at 450 nm. TNF-α values were expressed as pg/mL.

### 2.9 Statistical analysis

All the values were expressed as mean ± SE. Statistical evaluation of the data was done by one-way ANOVA (between control and drug treatments) followed by Dunnett's t-test for multiple comparisons and two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at P <0.001 using Graph-Pad Prism 5, San Diego, CA software.

## 3. RESULTS AND DISCUSSION

Rheumatoid arthritis is a autoimmune chronic inflammatory joint disease and it affects several parts of joints including cartilage, synovium, tendon and muscles.[30-31] In the present study, Arthritis was induced in Wistar rats because rats develop a chronic swelling in multiple joints. *Clerodendrum phlomidis* significantly reduces the signs of pain, inflammation and other symptoms of RA. Therefore further the antiarthritic activity of *Clerodendrum phlomidis* was evaluated by FCA induced arthritis in rats.

The petroleum ether extract of *Clerodendrum phlomidis* (L.) showed the presence of alkaloids, terpenoids, and flavonoids; ethyl acetate extract of *Clerodendrum phlomidis* (L.) showed the presence of tannins, alkaloids, terpenoids, saponins and glycosides; and alcoholic extract of *Clerodendrum phlomidis* (L.) showed the presence of sterols and flavonoids. While administering of all extracts orally, there was no mortality found up to the dose of 4000 mg/kg and to be safe at all doses used. By the results, we have taken 400 mg/kg as the

therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

Physical observations such as body weight, arthritic score, paw volume, ankle diameter and haematological parameters were recorded after the induction of arthritis by FCA. FCA injection on the rat hind paw causes pronounced swelling and hyperalgesia without involvement of contra lateral paw which is associated with the accumulation of leukocytes in the arthritic joint fluid. The mediators of chronic inflammation are responsible for pain, severe destruction of bone and cartilage that can lead to severe disability. FCA treated rats found progressive decrease in body weight till day 12, and no marked differences were seen between them. This weight loss was significantly ($p<0.001$) reduced from day 15 to day 21 while receiving with PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) in FCA induced arthritis in rats (Figure 1). In arthritic animals, the loss of body weight could be due to reduced absorption of glucose and leucine in rat intestine in arthritic condition.[32] After inoculation of FCA, animals begun to show progression of clinical inflammation from day 3. The time for the development and progression of disease was assessed by mean arthritic severity score. The mean arthritic severity score in FCA treated animal was progressive from day 12 to 18 and achieved values of about 10. Administration of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) attenuated mean arthritic severity score significantly ($p<0.001$) from day 15 to day 21 as compared with vehicle treated animals in FCA induced arthritis in rats (Figure 2). Paw swelling is one of the major factors for determination of quick, simple, sensitive and therapeutic effects of drugs.[33] The initial inflammatory response was developed within 3 to 5 days considered as primary lesion. Secondary lesions occur after 11 to 12 days after inoculation of FCA. Oral administration of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) significantly ($p<0.001$) reduced the paw swelling from day 15 to day 21 as compared with vehicle treated animals in FCA induced arthritis in rats (Figure 3). The change in paw volume has been found to associate with an increase in granulocyte and monocytes. Paw thickness (ankle diameter) are also used for assessment of RA. Ankle diameter significantly ($p<0.001$) decreased with the treatment of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) as compared to vehicle treated rats in FCA induced arthritis in rats (Figure 4).

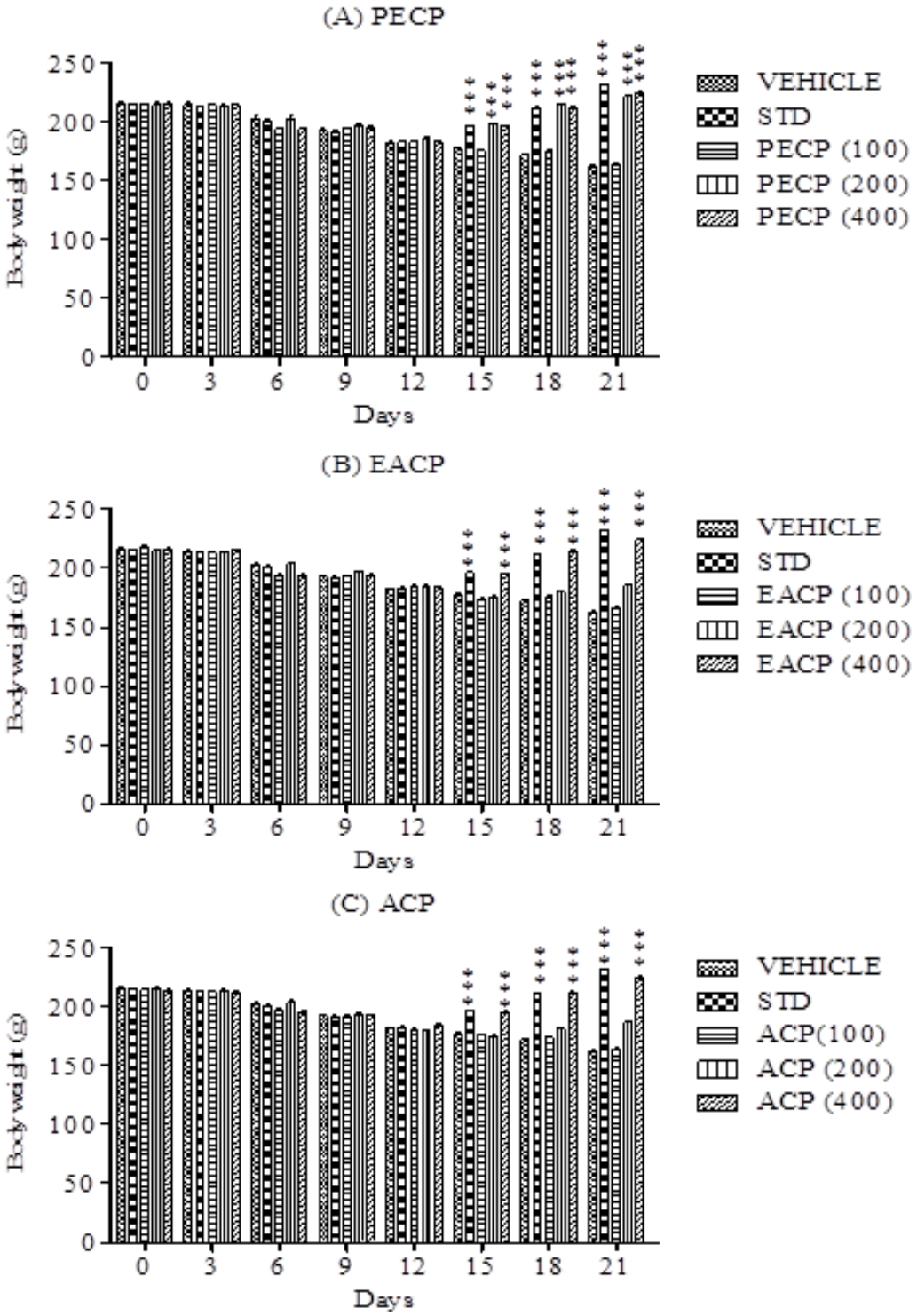


**Figure 1: Effect of (A) Petroleum ether, (B) Ethyl acetate, (C) Alcohol extract of *Clerodendrum phlomidis* on change in body weight in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

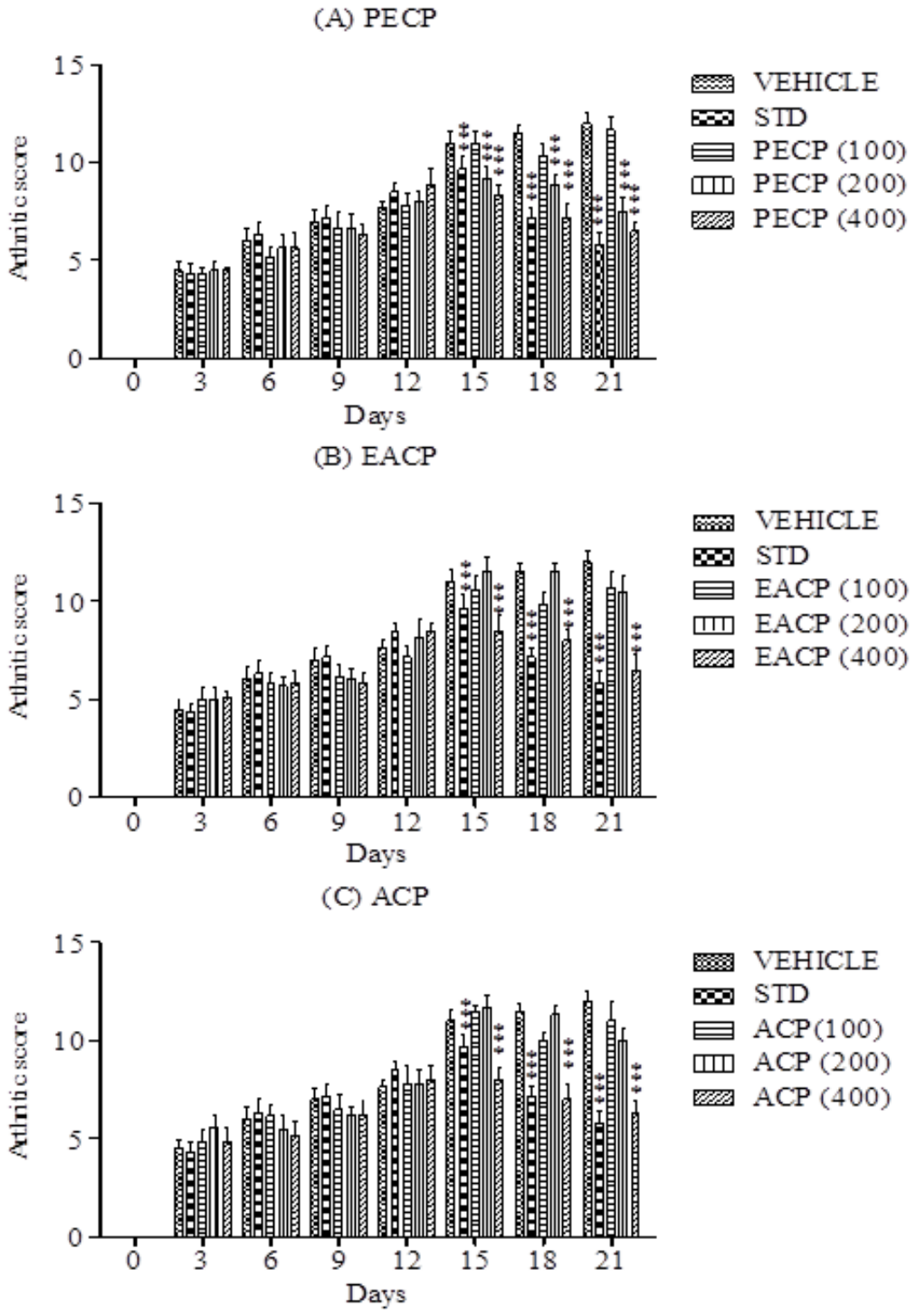


**Figure 2: Effect of (A) Petroleum ether, (B) Ethyl acetate, (C) Alcohol extract of *Clerodendrum phlomidis* on change in arthritic score in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

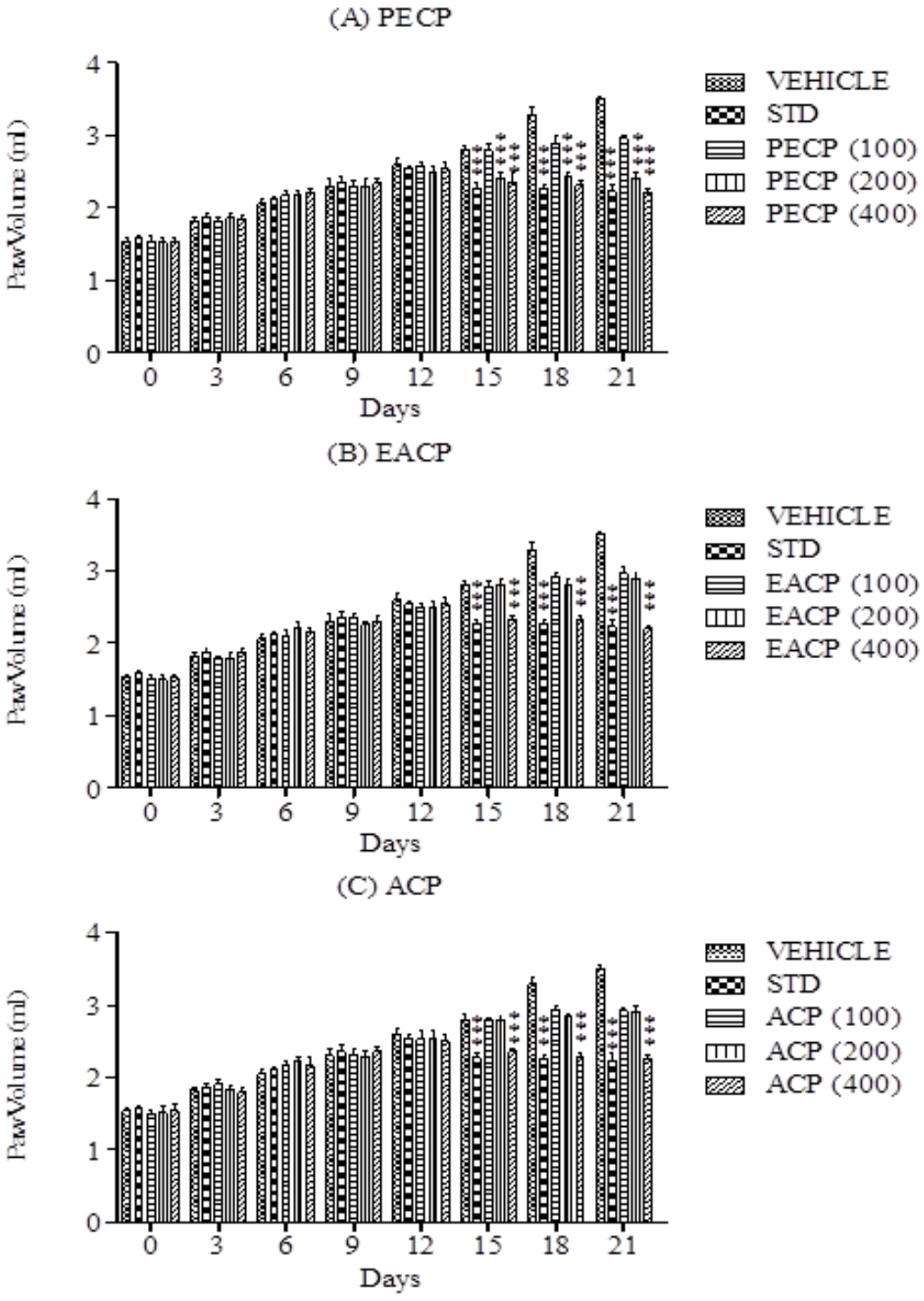


**Figure 3: Effect of (A) Petroleum ether, (B) Ethyl acetate, (C) Alcohol extract of *Clerodendrum phlomidis* on change in injected paw volume in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

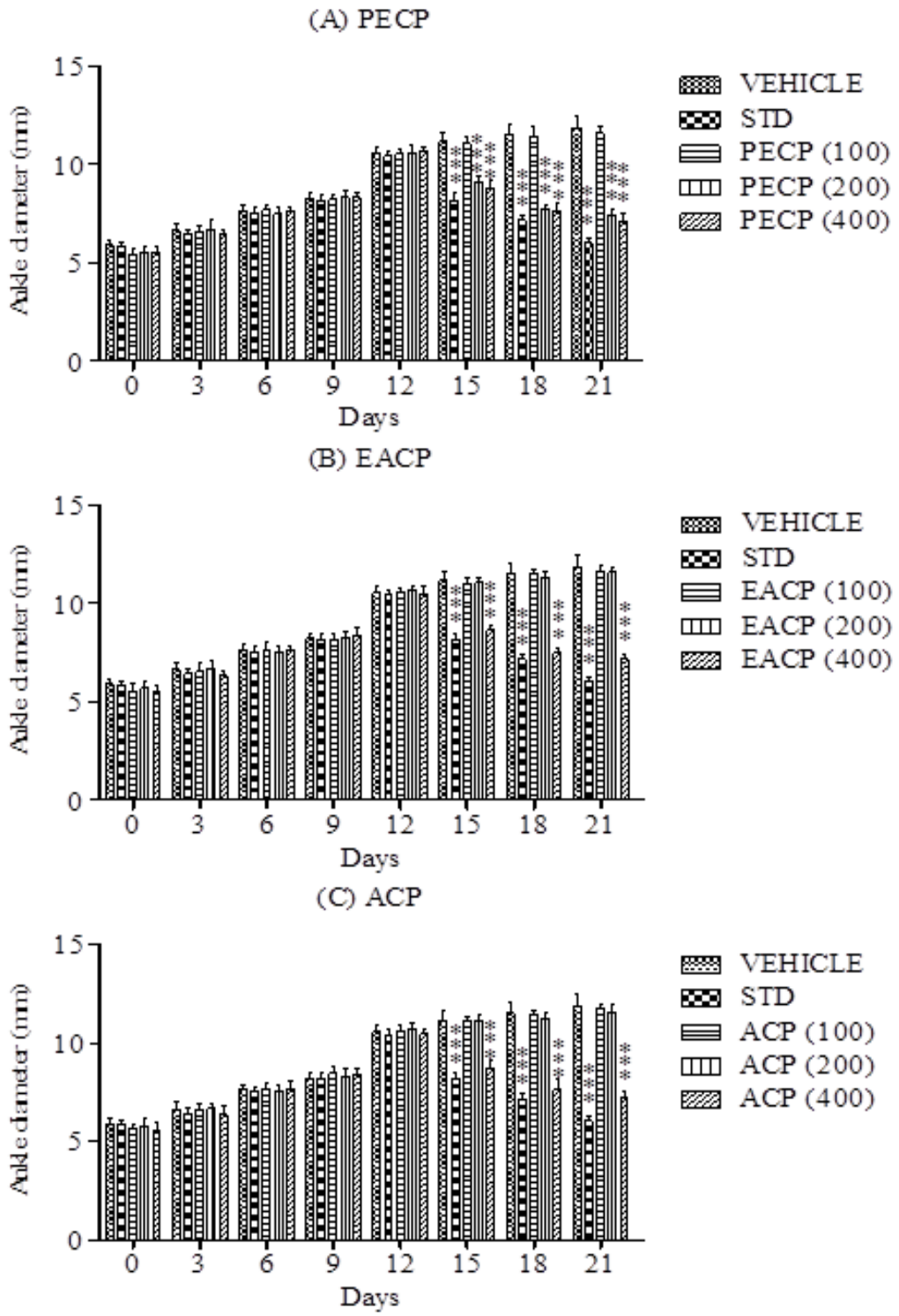


**Figure 4: Effect of (A) Petroleum ether, (B) Ethyl acetate, (C) Alcohol extract of *Clerodendrum phlomidis* on change in ankle diameter in FCA induced arthritis in rats.**

Values are Mean±SE from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

In the present study, the reduction in haemoglobin and RBC count, and increased WBC count are common feature of microbial infectious inflammatory diseases.[34-35] So in arthritic group, there was decrease in haemoglobin and red blood cells count and; increased in total leukocyte number. In the present study, administration of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) significantly improved the haemoglobin and RBC count, and significantly suppressed WBC count as compared to vehicle treated animals in FCA induced arthritis in rats. These findings suggest *Clerodendrum phlomidis* may have beneficial effect for joint preservation.

Erythrocyte sedimentation rate (ESR) in the FCA treated animals showed high value compared to drug treated animals. ESR is strongly related with the ability of red cells to aggregate into olderly stacts or rouleaux. Proteins are believed to affect the repellent surface charges on red cells and cause them to aggregate into rouleaux and hence the sedimentation rate increases.[36] Chronic administration with PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) significantly ($p<0.001$) decrease the ESR level in FCA induced arthritis in rats.

C-reactive protein (CRP) is a prototype hepatically derived inflammatory biomarkers. During inflammatory process, CRP level increases due to increased concentration of IL-6 in plasma which is produced by macrophages[37] as well as adipocytes.[38-39] Increased level of CRP in FCA treated group was significantly ($p<0.001$) decreased with the treatment of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.).

Chronic inflammation involves the release of number of mediators like IL-1B, TNF alpha, interferon and prostaglandins. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability.[40] Chronic oral administration of PECP (200 and 400 mg/kg); EACP and ACP (400 mg/kg, p.o.) significantly suppressed the levels of TNF alpha as compared to vehicle treated animals in FCA induced arthritis in rats (Table 1).

**Table 1: Effect of (A) Petroleum ether, (B) Ethyl acetate, (C) Alcohol extract of *Clerodendrum phlomidis* on change in biochemical parameter in FCA induced arthritis in rats.**

| ↓ Treatment Parameters → | Change in Biochemical parameters in FCA induced arthritic animals | | | | | |
|---|---|---|---|---|---|---|
| | **Hb** | **RBC** | **WBC** | **ESR** | **CRP** | **TNF α** |
| VEHICLE | 8.78±0.62 | 3.30±0.31 | 19.52±0.41 | 13.42±0.56 | 440.09±0.24 | 412.68±0.29 |
| STD | 11.68±0.40 *** | 4.49±0.52 *** | 12.68±0.45 *** | 9.22±0.52 *** | 232.47±0.23 *** | 232.19±0.40 *** |
| PECP (100 mg/kg) | 9.28±0.31 | 3.42±0.47 | 18.52±0.56 | 12.39±0.62 | 432.52±0.38 | 398.19±0.35 |
| PECP (200 mg/kg) | 10.58±0.61 *** | 4.19±0.62 *** | 13.98±0.21 *** | 9.98±0.24 *** | 270.32±0.35 *** | 298.52±0.35 *** |
| PECP (400 mg/kg) | 11.20±0.11 *** | 4.32±0.70 *** | 13.09±0.48 *** | 9.50±0.43 *** | 267.12±0.19 *** | 265.22±0.62 *** |
| EACP (100 mg/kg) | 9.37±0.12 | 3.40±0.14 | 18.52±0.29 | 12.58±0.47 | 429.12±0.20 | 329.52±0.29 |
| EACP (200 mg/kg) | 9.22±0.09 | 3.42±0.32 | 17.19±0.42 | 12.12±0.56 | 401.29±0.75 | 307.42±0.25 |
| EACP (400 mg/kg) | 11.32±0.41 *** | 4.32±0.29 *** | 12.98±0.31 *** | 9.37±0.24 *** | 269.20±0.21 *** | 262.59±0.24 *** |
| ACP (100 mg/kg) | 9.29±0.27 | 3.42±0.19 | 18.57±0.24 | 12.52±0.24 | 412.27±0.45 | 322.47±0.50 |
| ACP (200 mg/kg) | 9.15±0.48 | 3.49±0.21 | 17.27±0.23 | 12.17±0.30 | 412.29±0.17 | 305.12±0.27 |
| ACP (400 mg/kg) | 11.09±0.32 *** | 4.37±0.30 *** | 13.72±0.12 *** | 9.52±0.23 *** | 262.32±0.38 *** | 272.49±0.40 *** |

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

From the present investigation, it can be concluded that *Clerodendrum phlomidis* possesses dose dependent significant antiarthritic activity. This activity may attributed due to the phytochemical constituents present in *Clerodendrum phlomidis*. In view of the present work, the potential activity of various ingredients in *Clerodendrum phlomidis* acting synergistically and working in concert for overall antiarthritic activity. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

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