



## Evaluation of antiarthritic activity of *pergularia daemia* in Freund's adjuvant induced arthritic rat model

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### ABSTRACT

*Pergularia daemia* (*P. daemia*) is commonly known as Utaranavel, Uttaravarun, Dudhibel. *Pergularia daemia* plant has been documented for presence of triterpenes, saponins, cardenolides and alkaloids. Considering these biological potentials of *P. daemia* we further investigated the plant for their anti-arthritis activities. Extraction of *P. daemia* was carried out by continuous hot percolation, using Soxhlet apparatus. The observation made on different days of treatment period in Freund's complete adjuvant induced arthritis showed that there was a less increased paw swelling and ankle diameters in Diclofenac sodium (DCS) and *P. daemia* treated animals as compared to control group. The *P. daemia* was found to be effective in controlling secondary lesions. *P. daemia* also inhibited the radiological changes that occurred in CFA induced arthritis. Body weight gain in groups receiving *P. daemia* and DCS was more than control animals. *P. daemia* was found to be effective in reducing arthritis score, flexion pain test score and mobility test score. Stance score in *P. daemia* and DCS group was more than control group which indicates that the *P. daemia* normalized the condition of arthritic rats. *P. daemia* treated groups showed decrease in WBC count and ESR while increases haemoglobin content and RBC count. The concentration of C-reactive protein (CRP) and Rheumatoid factor (RF) was decreased in groups which received *P. daemia* at different doses. Thymus weight was decreased while spleen weight was increased on treatment with *P. daemia* in CFA induced arthritis. Significant activity of *P. daemia* in CFA induced arthritis suggests its usefulness in control of various chronic inflammatory diseases like rheumatoid arthritis.

**Keywords:** *Pergularia daemia* (PD), inflammation, arthritis, complete Freund's adjuvant (CFA).

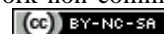
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## INTRODUCTION

Herbal drugs have great growth potential in the global market. The R & D thrust in the pharmaceutical sector is focused on development of new innovative indigenous plant-based drugs through investigation of leads from the traditional system of medicine<sup>[15]</sup>. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant based medicines for their primary healthcare needs<sup>[24]</sup>.

Among the estimated 400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically. This shows a need for investigation of various chemical constituents, its activity and phytopharmacological evaluation of herbal drugs. The efficacy and safety of herbal medicine have turned the major pharmaceutical population towards medicinal plant's research<sup>[5]</sup>.

Arthritis is one of the most common chronic inflammatory conditions, and Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown aetiology. The disease is characterized by articular inflammation and by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints. Arthritis may also develop as the result of increased urate concentration in plasma, resulting in deposition of sodium urate crystals in synovial tissues. The underlying cause being overproduction or impaired excretion of uric acid<sup>[14]</sup>.

Analgesia (painkillers) and anti-inflammatory drugs, including steroids are used to suppress the symptoms, while disease-modifying antirheumatic drugs (DMARDs), newer therapies such as anti-tumour necrosis factor (TNF)- $\alpha$  therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept are often required to inhibit or halt the underlying immune process. In recent days, researchers are directed towards traditional system of medicine for the discovery of drugs that are long acting anti-inflammatory with minimum side effects. Although there is no ideal animal model for RA at this time, rat adjuvant arthritis shares many features of human RA and the sensitivity of this model to antiarthritic agents support the view the adjuvant arthritis is the best available model of rheumatoid arthritis<sup>[3,21,22]</sup>.

Although the plant possesses many potential therapeutic activities in traditional system of medicinal practice and possessing rich phytoconstituents, they are not evaluated for their

pharmacological activities in detail<sup>[26]</sup>. Taking these facts into considerations, the present study deals with the evaluation of antiarthritic activity and its changes in haematological and biochemical parameters of the different extract of *Pergularia daemia* in Freund's adjuvant induced arthritic rats.

## Objectives

The plants *Pergularia daemia* used for the treatment of inflammation, in the folk medicine of different cultures, triterpenes are the most widely diffused and active compounds. *Pergularia daemia* plant has been documented for **presence of** presence of triterpenes, saponins, cardenolides and alkaloids<sup>[19]</sup>. Several studies reported isolation and characterization of various constituents from *Pergularia daemia*. *Pergularia daemia* has anthelmintic, emetic, thermogenic, expectorant, antipyretic and laxative action. Leaves juice is given in catarrhal affections, asthma, and infantile diarrhoea. Saponins, as well as triterpenes, have been investigated for numerous biological activities such as anti-inflammatory, antimalarial, and anti-HIV<sup>[8]</sup>. Triterpene acids viz. betulinic acid, a mixture of ursolic and oleanolic acids is reported to possess significant anti-inflammatory activity<sup>[1]</sup>. The present investigation was undertaken to demonstrate the pharmacological potential of *Pergularia daemia* by using Freund's adjuvant induced arthritic rat model.

## MATERIALS AND METHODS

### A. Phytochemical Screening

The *Pergularia daemia* plant extract showed positive test for Carbohydrates, Glycosides, Alkaloids, Phytosterol, Saponin, Fixed oils and Fats, Flavanoids, and Coumarins as shown in table 1.

### B. Pharmacological study

#### 1. Anti Arthritic study

##### 1.1. Complete Freund's adjuvant induced arthritis in rat

**Procedure:** The albino Wistar rats of either sex (100-150g) were divided into five groups (n = 6). The first group represented control group and receives saline or 5% Tween 80 solution at a dose of 10 mg/kg, p.o. The second group received the standard drug diclofenac sodium at a dose of 10 mg/kg, p.o. The 3rd, 4th, and 5th, groups receive petroleum ether, Chloroform extract and ethanolic extracts (100mg/kg, each), respectively by oral route. After 30 min, 0.1 mL complete Freund's adjuvant (Sigma, U.S.A) was injected into the sub plantar region of left hind paw on day '0'. Saline or extracts were administered orally once daily, from the initial day i.e. from the day of adjuvant

injection (0 day) and continued till 21 consecutive days<sup>[17]</sup>. The anti-arthritic effect of the extracts as well as diclofenac sodium was evaluate by measuring paw volume of inject paw on 4th, 8th, 14th and 21st day of study by using digital vernier caliper (Mitutoyo, Japan). The mean changes in injected paw volume with respect to initial paw volume are calculated on respective days and % inhibition of paw volume with respect to control group was calculated<sup>[12]</sup>.

### Parameters evaluated

**1) Primary lesions:** The paw volume and ankle diameter of injected paw was measured using digital vernier caliper (Mitutoyo, Japan) respectively, on day 0 before administration of adjuvant and at every seven days of the entire treatment period ending on day 21<sup>[20,4]</sup>.

**2) Secondary lesions:** The paw volume and ankle diameter of non-injected contra-lateral paw was measured using digital vernier caliper (Mitutoyo, Japan) respectively, on day 0 before administration of adjuvant and at every seven days of the treatment period ending on day 21<sup>[20,4]</sup>.

**3) Photographs:** Photographs of injected paws of all animals were taken by using digital camera (Sanyo, Japan) on day 7, 14 and 21 after the adjuvant injection<sup>[6]</sup>.

**4) Radiological analysis**<sup>[2,6,27]</sup>: On the day 21, animals were anesthetized with anaesthetic ether. Radiographs of the adjuvant –injected hind paws were taken with X-ray instrument (GE-525 DX, USA) by Fuji computerized radiographic systems (Japan). The film focus distance was 60 inches and machine was operated at 43 kV peak, 2 mA. Radiograph of each rat was evaluated for radiographic changes by a board certified radiologist for following changes and scores were given according to the severity-

- A) Soft tissue swelling around joints of hind paws (0-4).
- B) Periarticular bone resorption (0-4).
- C) Periarticular bony erosions (0-4).
- D) Joint space narrowing (0-4).

**5) Body weight:** Body weights of all the animals were recorded at the interval of 3 days from the day 0 to day 21<sup>[9,13]</sup>.

**6) Scoring systems:** Following scoring systems were used to access the degree of arthritis. The scores were assign according to the severity of disease progression. Scores were evaluated at every 3<sup>rd</sup> day after adjuvant injection for the entire period of drug treatment<sup>[10,11]</sup>.

**A) Arthritis score:** Each rat was observed at every 3<sup>rd</sup> day and scored for severity of swelling and redness of paws & joints. The grading for arthritis was done on 0 to 4 scale where 0 represents least amount of definite swelling and 4 represents maximum swelling. This scoring system involved observations of all four paws of rat & separate score was given for each limb<sup>[23]</sup>.

**B) Flexion pain test:** The ankle joint was flexed dorsally until the toe touched the anterior part of the leg. The test was performed 5 times with an inter-test interval of 5 sec and the pain was scored 0, if their was no squeaking and no leg withdrawal; 1, if their was either squeaking or leg withdrawal; 2, if both squeaking and leg withdrawal were present.

**C) Mobility score:** The mobility of rats while they moved freely in a large empty cage were also assessed according to the adaptations of following scale-

- 0 – Normal
- 1 – Limping
- 2 – Walks with difficulty
- 3 – Paw not touched

**D) Stance score:** The stance of rats when they placed in large empty cage were assessed according to the following scale –

- 1 = Paw lifted continuously
- 2 = Paw touching but with no weight bearing
- 3 = Some weight bearing on the paw
- 4 = Normal

### 7) Haematological parameters

On the day 21, animals were anesthetized with anaesthetic ether and blood was withdrawn from retro orbital plexus for estimating following haematological parameters.

**A) Total leukocyte (WBC) count:** Increased values suggest inflammation or infection<sup>[13,25]</sup>.

**B) Red blood cell (RBC) count:** Decreased value indicates the severity of disease.

**C) Haemoglobin:** Decreased values are indicative of anemia<sup>[13]</sup>.

**D) Erythrocyte sedimentation rate:** The erythrocyte sedimentation rate (ESR), also called a sedimentation rate, sed rate or Biernacki reaction, is a non-specific measure of inflammation that is commonly used as a medical screening test. ESR was determined by wintrobe method<sup>[6,20]</sup>.

**E) C- reactive protein**<sup>[7,28]</sup>: On the day 21, animals were anesthetized with anaesthetic ether and blood was withdrawn from retro orbital plexus and serum was separated. The semiquantitative determination of c- reactive protien was performed by using commercial CRP kit obtained from Agappe diagnostics Pvt. Ltd., kerala, India,

according to manufacturer's instructions as follows –

1. 50 µL diluted glycine saline buffer was placed on to each of five circles of the slide.
2. Using a 50 µL (0.05 mL) micropipette, 50 µL (0.05 mL) of the serum sample was added to the drop of glycine-saline buffer in the 1<sup>st</sup> circle.
3. Using the same micropipette, the sample was mixed with saline by aspirating back & forth several times.
4. Aspirated 50 µL (0.05 mL) from the 1<sup>st</sup> circle and transferred to 2<sup>nd</sup> circle. Same operation was repeated upto 5<sup>th</sup> circle. 0.05 mL was aspirated from the 5<sup>th</sup> circle and discarded.

Following dilutions were obtained:

Dilution: 1/2, 1/4, 1/8, 1/16, 1/32

5. Then one drop of CRP-Latex antigen was added to each of above diluted circles and the slide was rocked gently to and fro for 2 min. The agglutination was observed under good source of light.

**Calculation:** Concentration of CRP in serum can be calculated as follows,

**CRP Concentration (mg/dL) = Sensitivity × Titre**

Where, CRP sensitivity is 0.6 mg/dL

**F) Rheumatoid factor:** On the day 21, animals were anesthetized with anesthetic ether and blood was withdrawn from retro orbital plexus and serum was separated. The semiquantitative determination of rheumatoid factor was performed by using commercial Rhelax RF kit obtained from Tulip diagnostics Pvt. Ltd., Goa, India. according to manufacturer's instructions as follows –

1. Using isotonic saline solution serial dilutions of the serum sample was prepared as 1:2, 1:4, 1:8, 1:16 and 1:32.
2. Each dilution of the serum sample was pipetted onto separate reaction circle.
3. One drop of Rhelax RF latex reagent was added to each drop of diluted serum sample on the slide. Dropper tip do not touched the liquid on the slide.
4. Using a mixing stick, the sample and latex reagent was mixed uniformly over the entire circle. Rock the slide gently, back and forth and observed for agglutination macroscopically at two minutes.

**Calculations:** Agglutination in the highest serum dilution corresponds to the approximate amount of rheumatoid factors in IU/mL present in the test specimen.

RF is calculated by using following formula –

**RF (IU/mL) = S × D**

Where, S = Sensitivity of the reagent

D = Highest dilution of the serum showing agglutination

**8) Thymus & spleen weights:** On the day 21, animals were sacrificed with overdose of anesthetic

ether. The thymus and spleen of all animals were removed and weighed [9,16].

## RESULTS AND DISCUSSION

**Phytochemical Screening:** The *Pergularia daemia* plant extract showed positive test for Carbohydrates, Glycosides, Alkaloids, Phytosterol, Saponin, Fixed oils and Fats, Flavanoids, and Coumarins as shown in table 1.

### Anti arthritic study

#### Complete freund's adjuvant induced arthritis in rat

##### *Effect on injected & non-injected paw volume:*

The observation made on different days of treatment period in freund's complete adjuvant induced arthritis showed that there was a less increased in paw swelling in diclofenac sodium and *Pergularia daemia* treated animals as compared to control group as shown in Fig. 1. The assessment made on the 21<sup>st</sup> day showed that, treatment with *Pergularia daemia* as well as diclofenac significantly ( $P < 0.0001$ ) reduce the injected paw swelling (primary lesions) as compared with control group as shown in Fig. 1. The *Pergularia daemia* extract of Pet. Ether, Chloroform and Ethanol extract at the doses of 100 mg/kg, p.o. showed percentage rise in paw volume by 113, 109 and 91% respectively. Whereas DCS showed rise in paw volume by 83% on day 21<sup>st</sup>.

*Pergularia daemia* also showed significant inhibition of rise in non-injected paw volume (secondary lesions) on day-21 after adjuvant injection. The Chloroform and Ethanol extract of *Pergularia daemia* at the doses of 100 mg/kg, p.o. showed inhibition of secondary lesion which was significantly ( $P < 0.0001$ ) more than DCS treated standard group as shown in Fig. 2.

##### *Effect on injected & non-injected ankle diameter:*

Ankle diameters of injected and non-injected paws were recorded at predetermined intervals during the entire evaluation period of 21 days. The percentage rise in ankle diameter for *Pergularia daemia* for Pet. Ether, Chloroform and Ethanol extract (100 mg/kg, p.o.) and DCS was 51, 43, 36 & 15% respectively on day-21. *Pergularia daemia* showed significant and dose dependent inhibition of rise in ankle diameter as shown in Fig. 3. The results of effect of *Pergularia daemia* on secondary non-injected paw ankle diameter showed dose dependant inhibition of increased ankle diameter. *Pergularia daemia* for Pet. Ether, Chloroform and Ethanol extract (100 mg/kg, p.o.) showed more significant inhibition ( $P < 0.0001$ ) than that of DCS treated group as shown in Fig. 4.

**Effect on radiographic changes:** The hind paw radiographs of injected paws were evaluated for the following changes like: soft tissue swelling around joints of hind paws, periarticular bone resorption, periarticular, bony erosions and joint space narrowing. The average score for control group was 10, while for Pet. ether, Chloroform & Ethanol (100 mg/kg, p.o.) it was 5, 1 and 1 respectively and for diclofenac treated group the score was 1. Thus *Pergularia daemia* extract and DCS treated group shows less severe radiographic changes as compare to the control group as shown in Fig. 6.

**Effect on body weight changes:** The body weight gain was more for groups which received *Pergularia daemia* for Pet. Ether, Chloroform and Ethanol extract (100 mg/kg, p.o.) and standard diclofenac sodium as shown in Fig. 7. when compared to control group which may be due to the restoration of absorption capacity of intestine. *Pergularia daemia* extract of Chloroform at dose of 100 mg/kg, p.o. showed less weight gain in comparison to groups received *Pergularia daemia* extract of Pet. Ether and Ethanol 100 mg/kg, p.o. doses but was statistically significant as compared with control group.

**Effect of different extracts of *Pergularia daemia* on various scoring systems:** Arthritis score is increased from day-7 to day-21, the maximum score was peaked at 21<sup>st</sup> day. Effect of *Pergularia daemia* on arthritis score showed dose dependent reduction in arthritis score as compared to vehicle treated group. Ethanol extract showed maximum effect at dose of 100 mg/kg, p.o. and comparable to the group treated with standard drug DCS. The inhibition was more significant ( $P < 0.0001$ ) on days 14 and 21 as shown in Fig. 8.

Flexion pain test score was employed to assess effect of *Pergularia daemia* on inhibition of arthritic pain. This test score increases as disease progress takes place. *Pergularia daemia* and DCS decreases flexion pain test score significantly on day-7 and day-21 in a dose dependant manner. The effect of *Pergularia daemia* at 14<sup>th</sup> day was not significant and do not show dose dependent correlation as shown in Fig. 9.

Results of mobility score are shown in Fig. 10. *Pergularia daemia* showed significant reduction in mobility score at a dose of 100 mg/kg, p.o. of ethanol only on 21<sup>st</sup> day. Reduction in mobility score on 14<sup>th</sup> day is not significant but in a dose dependent manner.

In case of stance scoring system the maximum score referred to the normal condition of animals while reduction in score indicates severity of

disease progression. Effect of *Pergularia daemia* on stance score was shown in Fig. 11. *Pergularia daemia* extract of Pet. Ether, Chloroform and Ethanol at doses of 100 mg/kg, p.o. showed significant as well as dose dependant increase in stance score which was comparable with the effect of DCS.

**Effect of *Pergularia daemia* on haematological parameters in CFA induced arthritis in rat**

**1. WBC count:** Total leukocyte count was found to be decreased in *pergularia daemia* and DCS treated group as compared to negative control group. The WBC count in *pergularia daemia* of Ethanol treated group at dose of 100 mg/kg, p.o. has count of  $5.6 \times 10^3 \text{ mm}^3$ , while DCS treated group showed WBC count of  $5.5 \times 10^3 \text{ mm}^3$ . The effect of *pergularia daemia* was comparable to that of standard group was shown in Fig. 12.

**2. RBC count:** The effect of *Pergularia daemia* on RBC count is shown in Fig. 13. The increase in RBC count in *Pergularia daemia* extract (100 mg/kg, p.o.) was in a dose dependent manner. The RBC count in Chloroform and ethanol 100 mg/kg, p.o. treated group was significant and for ethanol 100 mg/kg, p.o. the count was more than that of the DCS treated group.

**3. Haemoglobin content:** The results indicated that haemoglobin count was normalized in *Pergularia daemia* and DCS treated group as compared to control group is shown in Fig. 14. This indicates protective effect of *Pergularia daemia* against anemia that occurs due to arthritic condition. *Pergularia daemia* containing Chloroform and Ethanol extract at doses of 100 mg/kg, p.o. treated group showed significant levels of haemoglobin as compared to the negative control group.

**4. Erythrocyte sedimentation rate (ESR):** The result indicated that, ESR was lowered in *Pergularia daemia* and diclofenac sodium treated group as compared to control group is shown in Fig. 15. *Pergularia daemia* containing Pet. Ether and Ethanol at dose of 100 mg/kg, p.o. treated groups showed significantly lower values for ESR as compared to the control group.

**5. C-reactive protien (CRP):** The rate of synthesis and secretion of CRP is increases within hours of an acute injury or the onset of inflammation. So, if CRP concentration is greater than 0.6 mg/dL a visible agglutination is observed in the presence of CRP latex reagent. The levels of CRP in serum were measured by semi-quantitative method. *Pergularia daemia* (100 mg/kg, p.o.) significantly lowered the level of CRP in a dose dependent

manner is shown in Fig. 16. CRP level in *Pergularia daemia* containing Ethanol extract (100 mg/kg, p.o.) group (1.4 mg/dL) was lower than that of the DCS treated group (1.8 mg/dL).

**6. Rheumatoid factor:** Any serum sample containing 10 IU/mL or more of rheumatoid factor will show a clear agglutination. The levels of RF in serum were measured semi-quantitatively. In this study, serum sample of control showed 73 IU/mL of RF. *Pergularia daemia* at the dose 100 mg/kg, p.o. showed significantly lowered concentration of RF at concentration of 37, 33, 23 IU/mL respectively. The concentrations of RF in ethanol extract (100 mg/kg, p.o.) treated group was similar to that of the positive control group treated with DCS is shown in Fig. 17.

**1.7 Effect of *Pergularia daemia* on lymphoid organ weights:** The decrease in weight of thymus indicates the suppression of immune response. *Pergularia daemia* containing Chloroform and ethanol extract at doses of 100 mg/kg, p.o. lowered the weight of spleen. The effect of *Pergularia daemia* was dose dependent. The weight of spleen in Chloroform and ethanol extract (100 mg/kg,

p.o.) treated group was less than weight of spleen in DCS treated group is shown in Fig. 18.

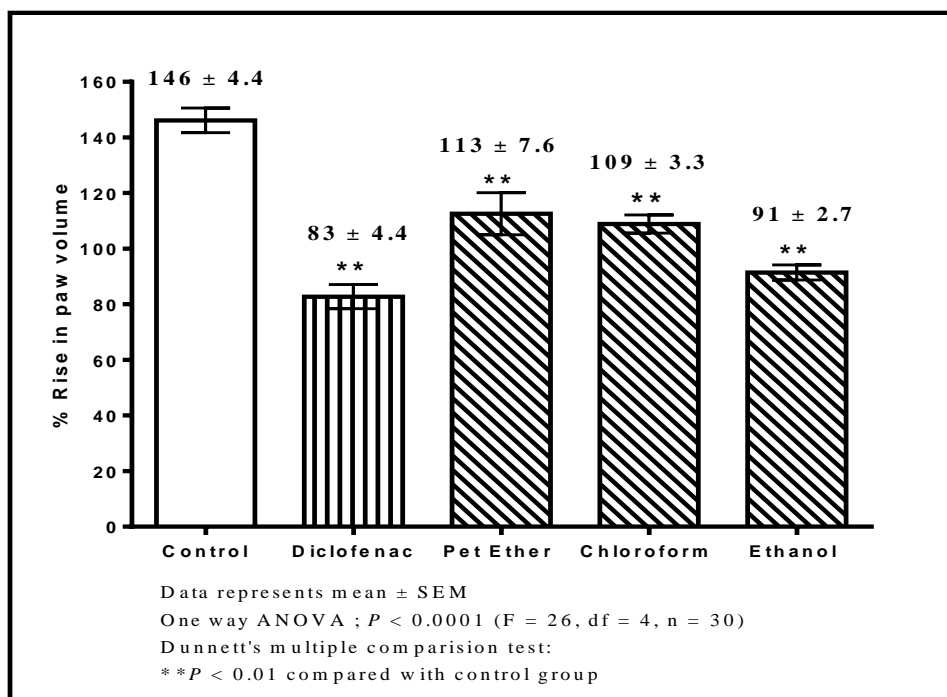
*Pergularia daemia* also lowered the weight of thymus gland at all the dose level tested, in a dose dependent manner. The ethanol extract at the dose of 100 mg/kg, p.o. decrease the thymus weight more than that of the standard DCS treated group is shown in Fig. 19.

**CONCLUSION**

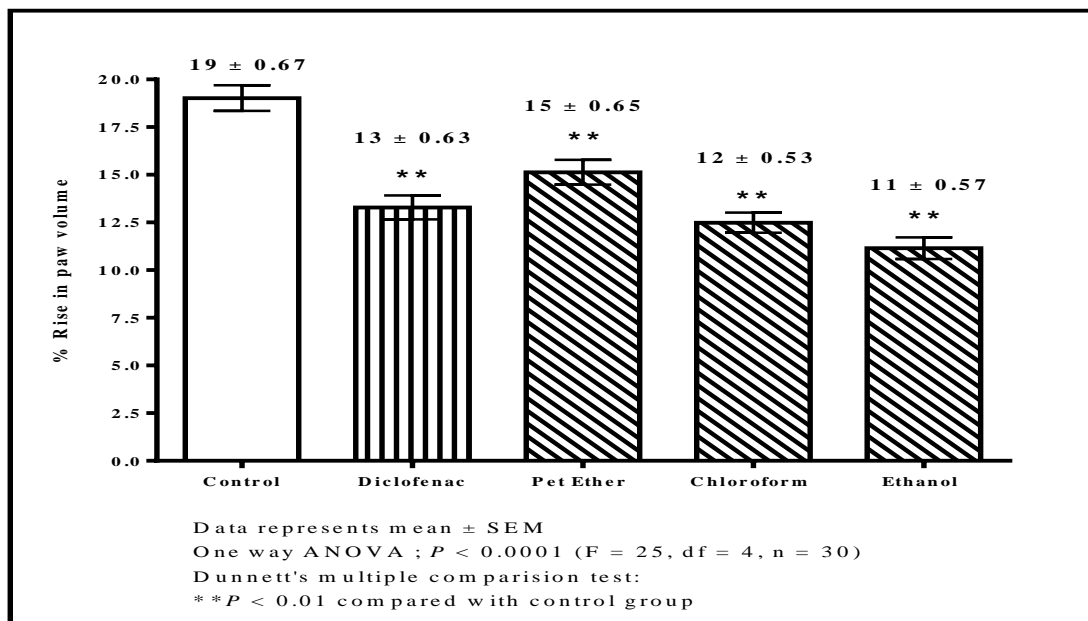
From the results & discussion we can conclude that *Pergularia daemia* extracts shows significant anti arthritic effect against FCA induced arthritis in rat. *PD* showed the reduction in arthritis score, which is the characteristic of immunosuppressant drug.

1. In CFA induced arthritis model *PD* was effective in all parameters evaluated.
2. *PD* has potent anti-inflammatory, analgesic and anti-arthritic activity in various animal models studied. Probable mechanisms behind these activities might be either inhibition of synthesis and/or release of pro-inflammatory mediators like histamine, 5-HT, bradykinin etc. or inhibition of cyclo-oxygenase or lipo-oxygenase which are responsible for inflammation.

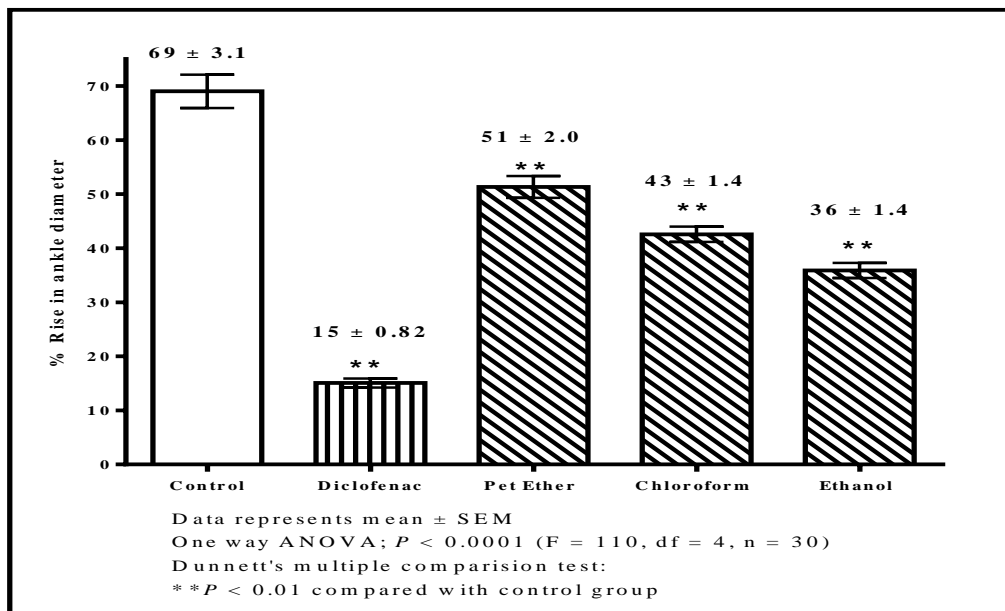
**Figure 1:** Effect of *Pergularia daemia* on Injected Paw Volume (Primary Lesions) in CFA Induced Arthritis in Rat



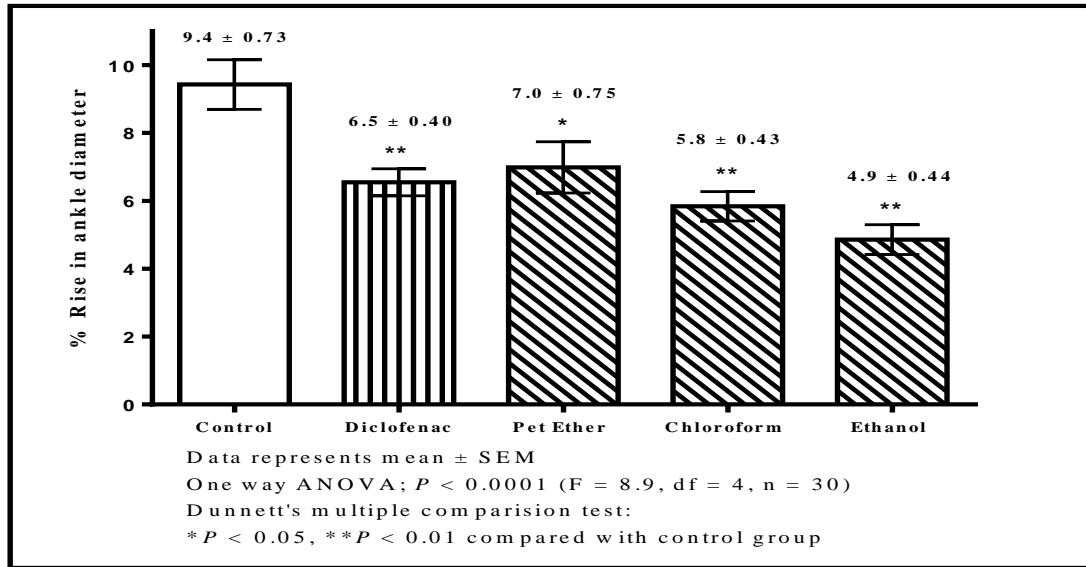
**Figure 2:** Effect of *Pergularia daemia* on Non-injected Paw Volume (Secondary Lesions) in CFA Induced Arthritis in Rat



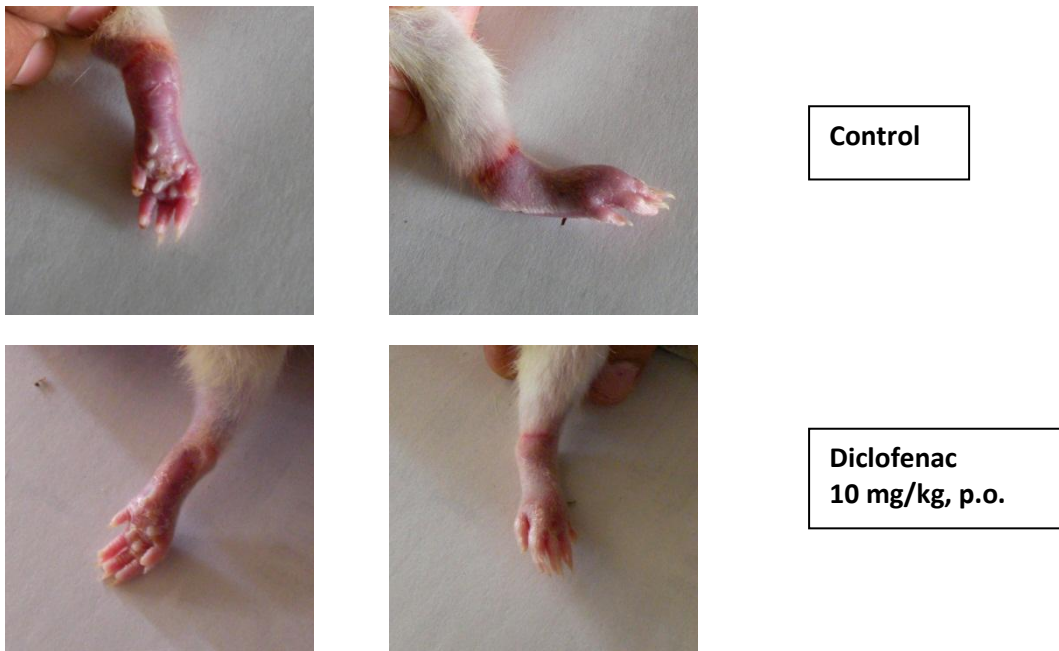
**Figure 3:** Effect of *Pergularia daemia* on Injected Paw Ankle Diameter (Primary Lesions) in CFA Induced Arthritis in Rat



**Figure 4:** Effect of Pergularia daemia on Non-injected Paw Ankle Diameter (Secondary Lesions) in CFA Induced Arthritis in Rat



**Figure 5:** Photograph of rat paw on Day-21 after adjuvant injection



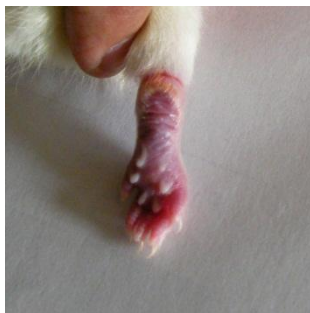




**Pet. Ether  
100 mg/kg, p.o.**

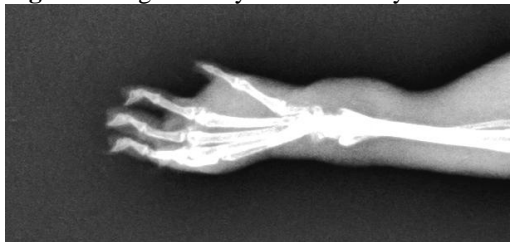


**Chloroform  
100 mg/kg, p.o.**

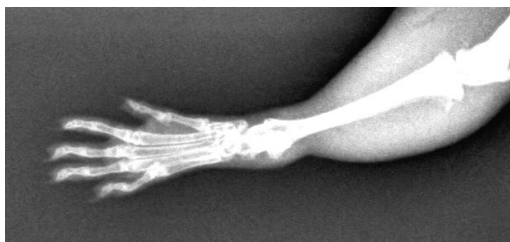


**Ethanol  
100 mg/kg, p.o.**

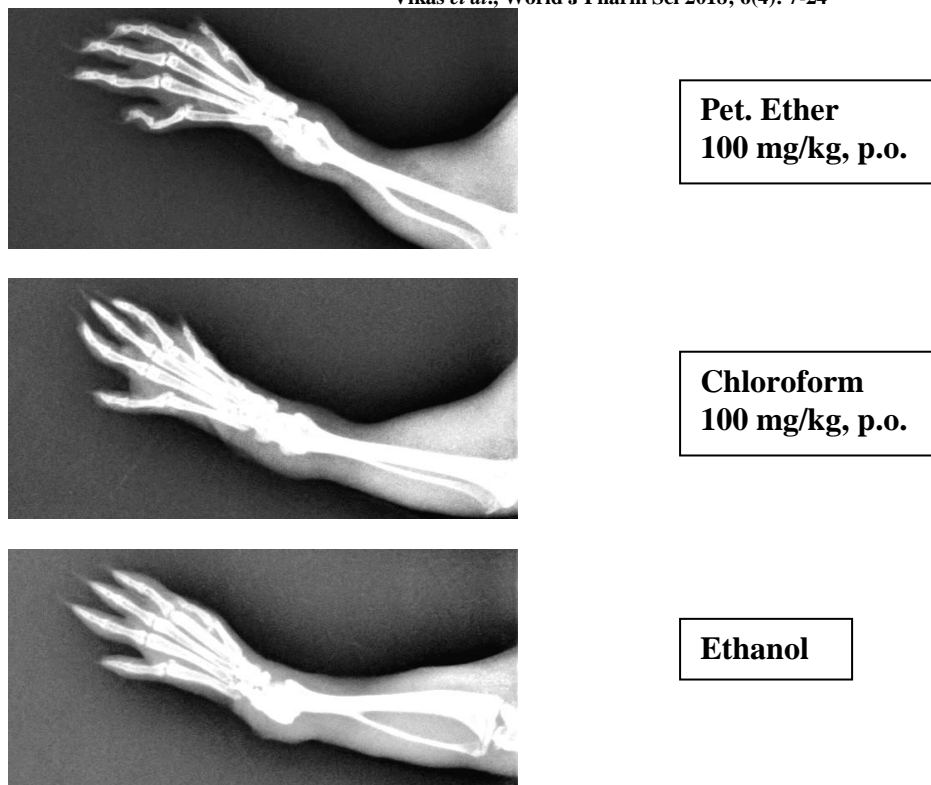
**Figure 6:** Fig 6: X-ray of rats on Day-21 after adjuvant injection



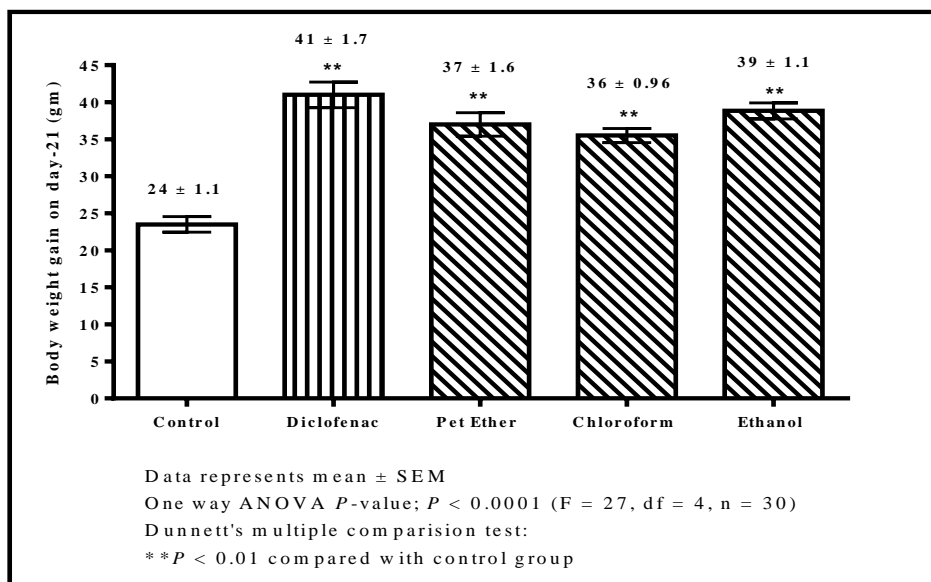
**Control**



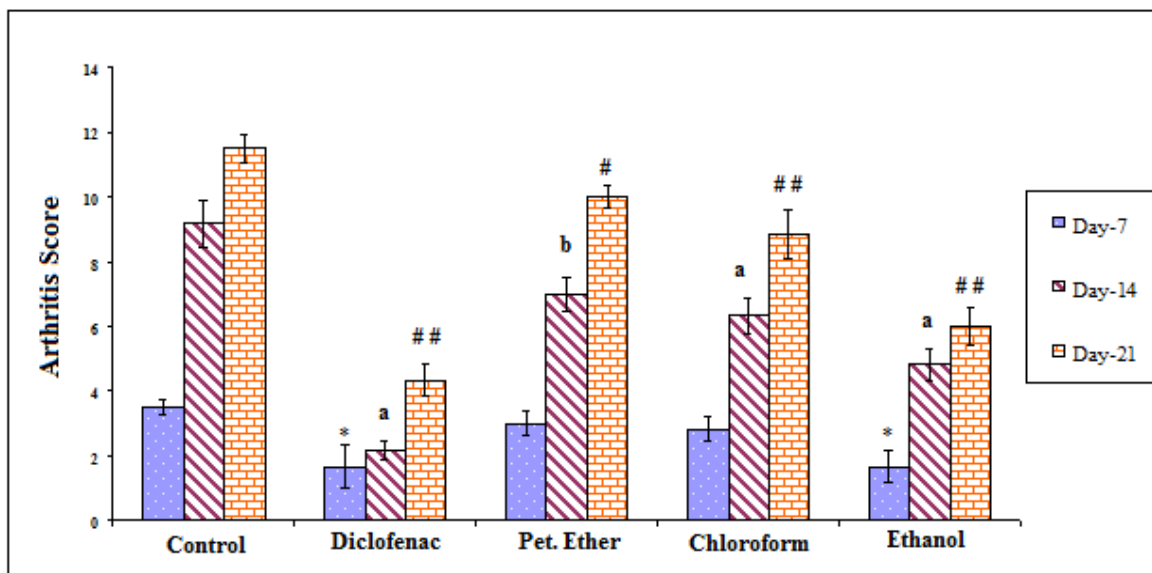
**Diclofenac  
10 mg/kg, p.o.**



**Figure 7:** Effect of *Pergularia daemia* on Body Weight Changes in CFA Induced Arthritis in Rat



**Figure 8:** Effect of *Pergularia daemia* on Arthritis Score in CFA Induced Arthritis in Rat



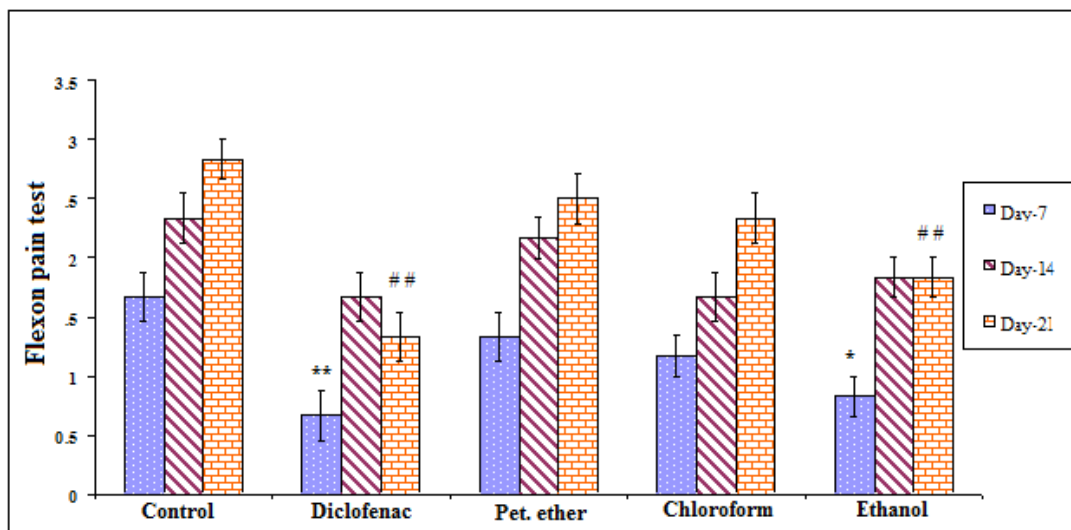
The differences in means of readings at 7, 14 and 21 days were statistically compared for significance by ANOVA followed by dunnett's t-test.

**For 7<sup>th</sup> Day readings,** ANOVA:  $P < 0.05$ , Dunnett's test: \* $P < 0.05$

**For 14<sup>th</sup> Day readings,** ANOVA:  $P < 0.001$ , Dunnett's test: <sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$

**For 21<sup>st</sup> Day readings,** ANOVA:  $P < 0.001$ , Dunnett's test: <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$

**Figure 9:** Effect of *Pergularia daemia* on Flexion Pain Test Score in CFA Induced Arthritis in Rat.



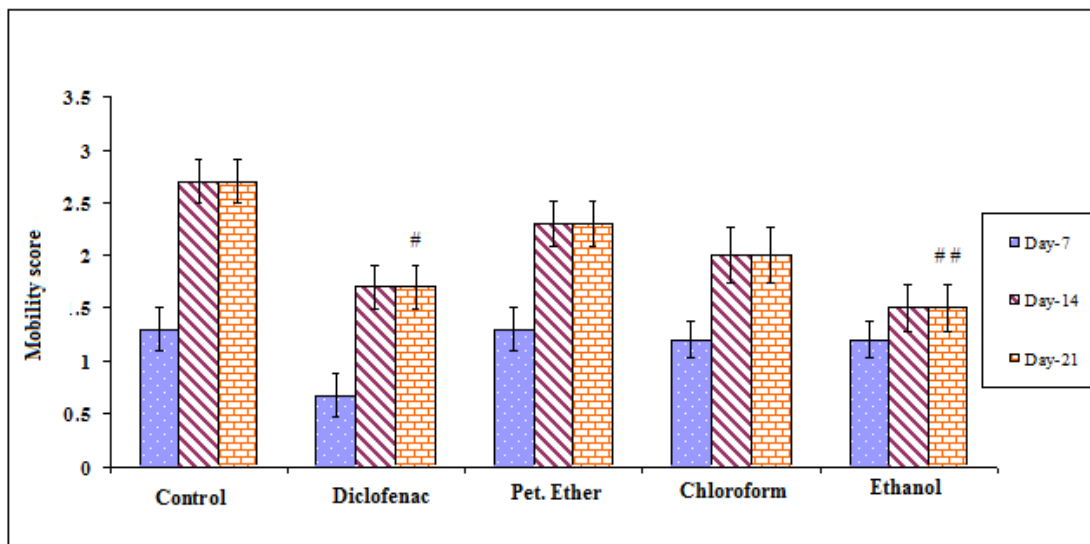
The differences in means of readings at 7, 14 and 21 days were statistically compared for significance by ANOVA followed by dunnett's t-test.

**For 7<sup>th</sup> Day readings,** ANOVA:  $P < 0.001$ , Dunnett's test: \* $P < 0.05$ , \*\* $P < 0.01$

**For 14<sup>th</sup> Day readings,** ANOVA:  $P > 0.05$

**For 21<sup>st</sup> Day readings,** ANOVA:  $P < 0.001$ , Dunnett's test: <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$

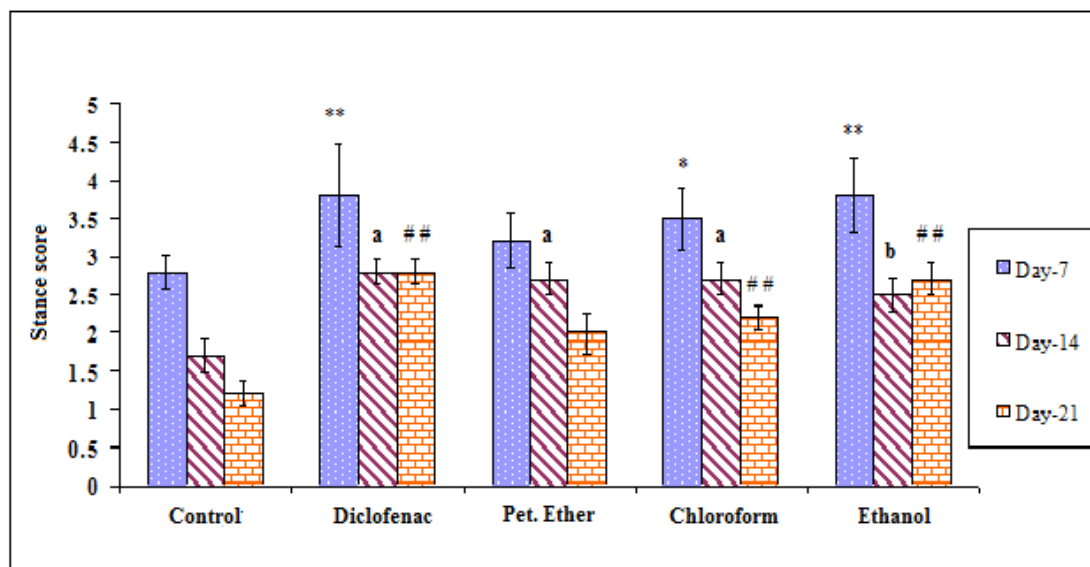
**Figure 10:** Effect of *Pergularia daemia* on Mobility Score in CFA Induced Arthritis in Rat



The differences in means of readings at 7, 14 and 21 days were statistically compared for significance by ANOVA followed by dunnett's t-test.

For 21<sup>st</sup> Day readings, ANOVA:  $P < 0.01$ , Dunnett's test: # $P < 0.05$ , ## $P < 0.01$

**Figure 11:** Effect of *Pergularia daemia* on Stance Score in CFA Induced Arthritis in Rat



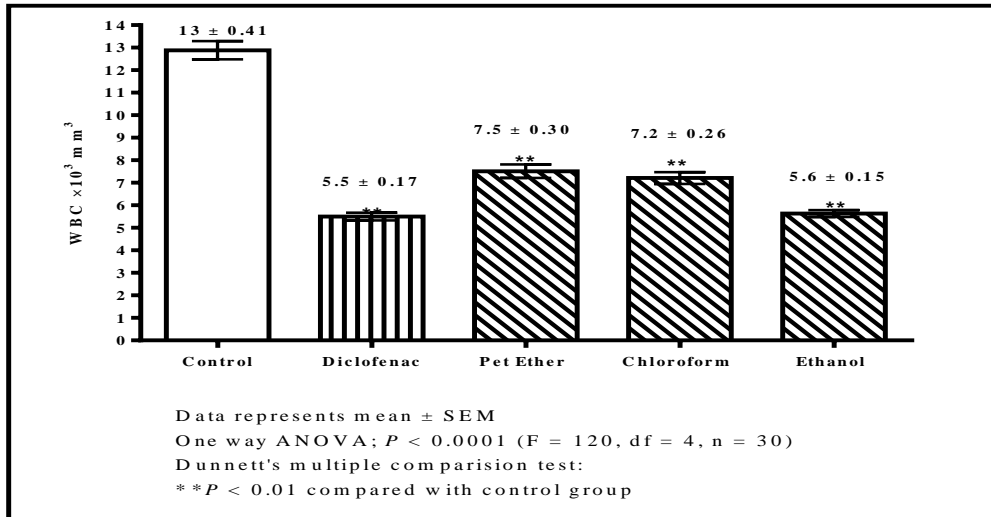
The differences in means of readings at 7, 14 and 21 days were statistically compared for significance by ANOVA followed by dunnett's t test.

For 7<sup>th</sup> Day readings, ANOVA:  $P < 0.01$ , Dunnett's test: \* $P < 0.05$ , \*\* $P < 0.01$

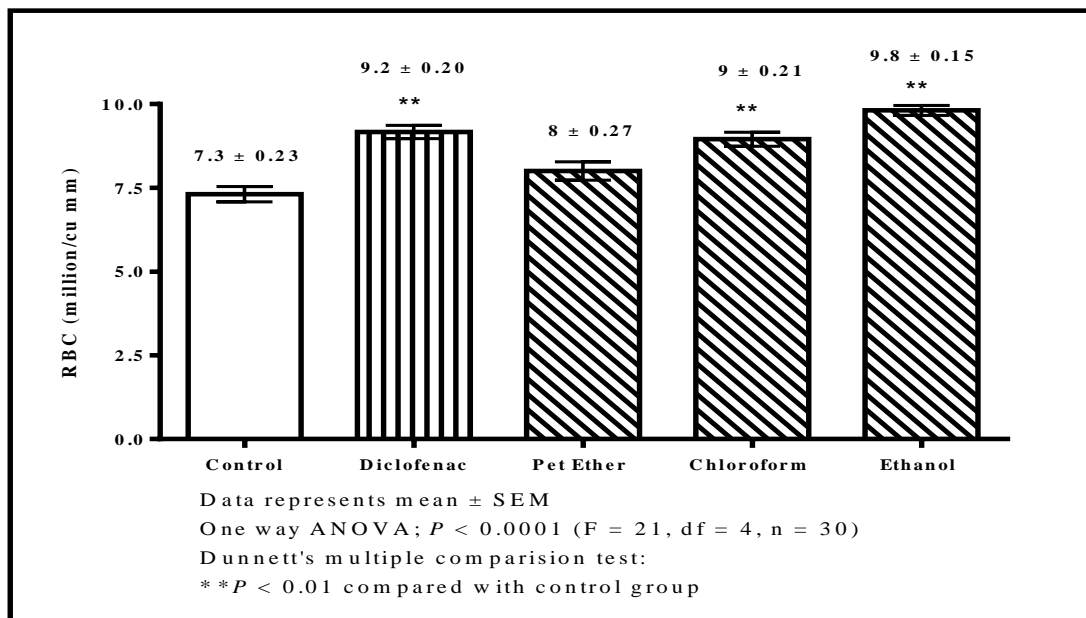
For 14<sup>th</sup> Day readings, ANOVA:  $P = 0.01$ , Dunnett's test: <sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$

For 21<sup>st</sup> Day readings, ANOVA:  $P = 0.001$ , Dunnett's test: # $P < 0.05$ , ## $P < 0.01$

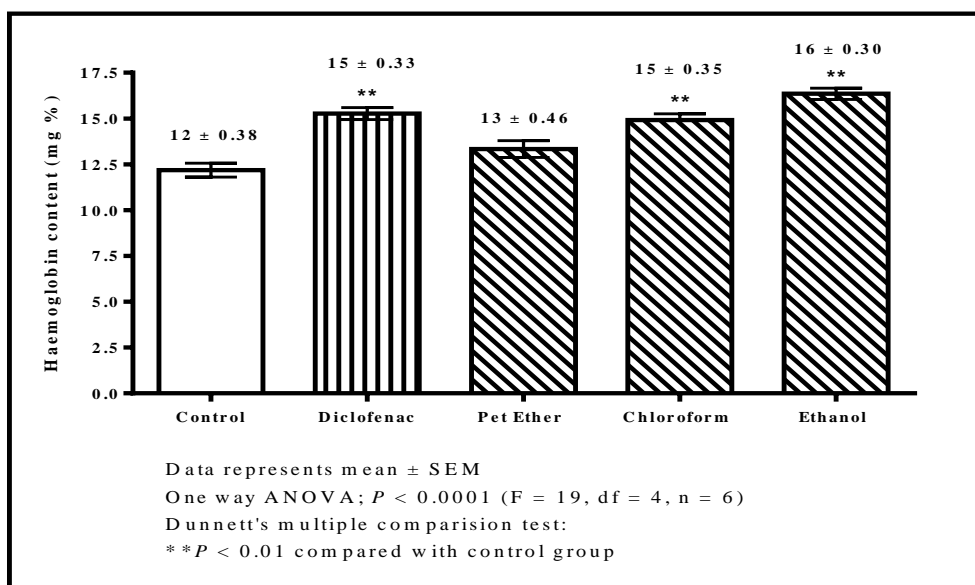
**Figure 12:** Effect of *Pergularia daemia* on WBC Count in CFA Induced Arthritis in Rat



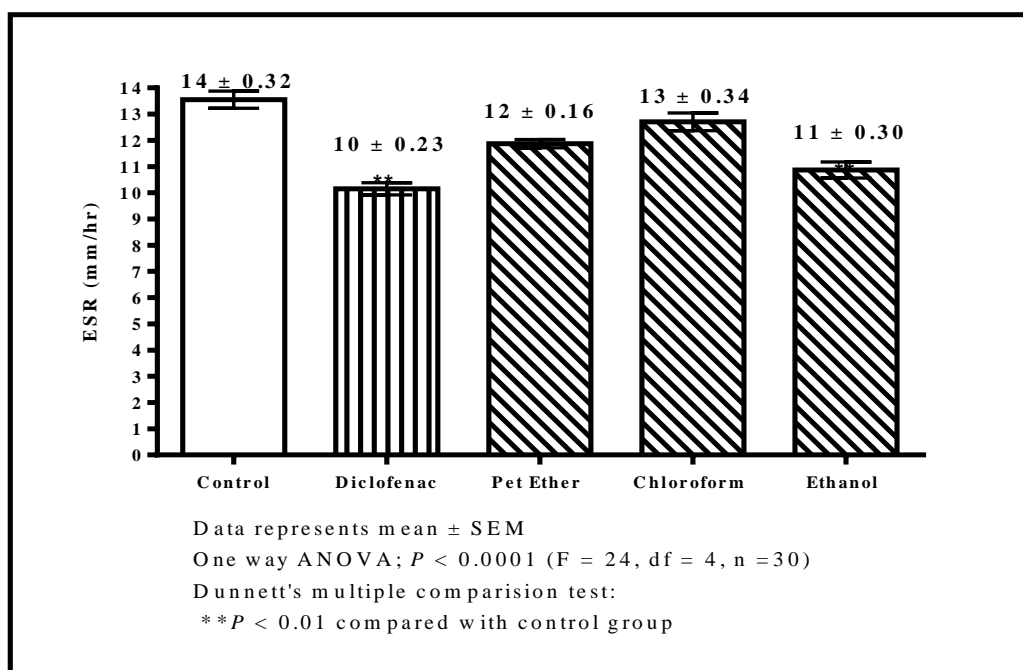
**Figure 13:** Effect of *Pergularia daemia* on RBC Count in CFA Induced Arthritis in Rat



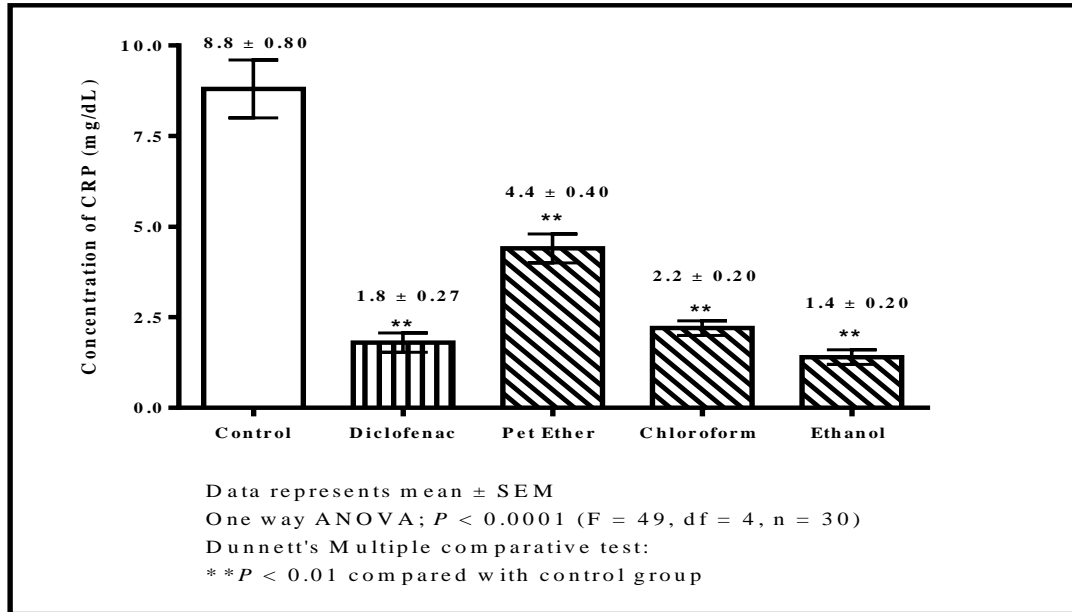
**Figure 14 :** Effect of *Pergularia daemia* on Haemoglobin Content in CFA Induced Arthritis in Rat



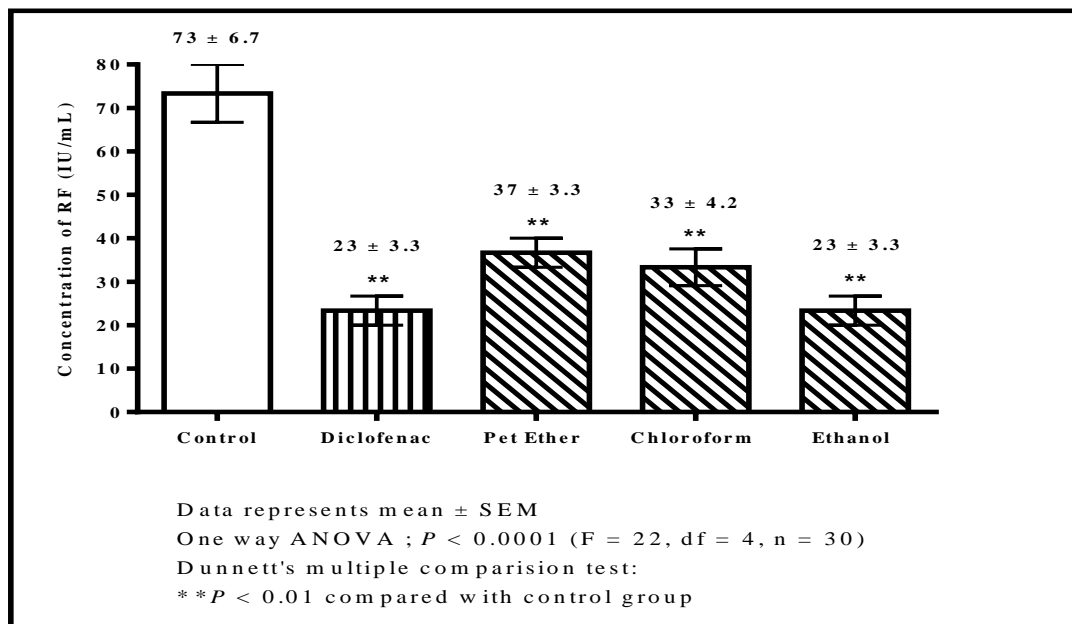
**Figure 15:** Effect of *Pergularia daemia* on ESR in CFA Induced Arthritis in Rat



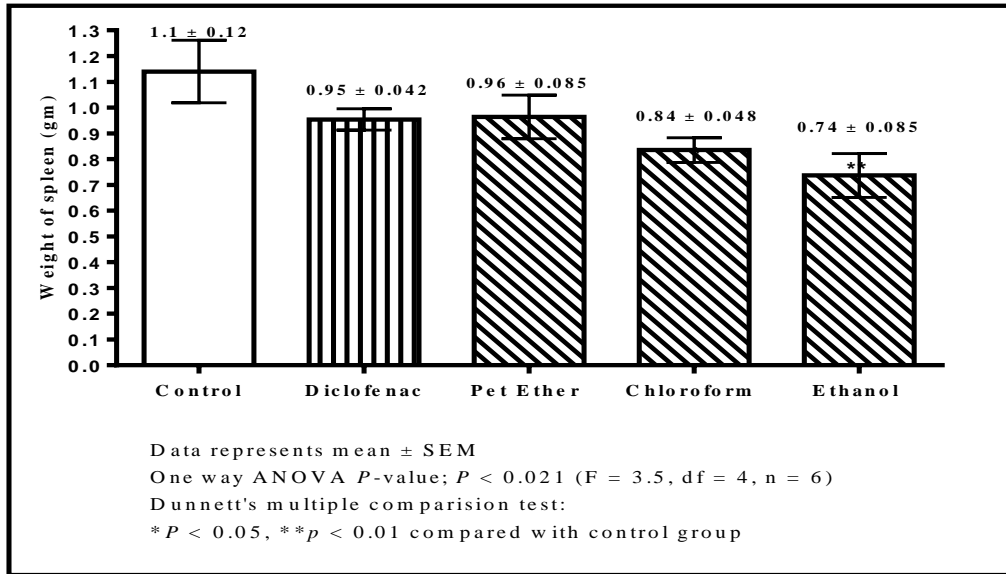
**Figure 16:** Effect of *Pergularia daemia* on Concentration of C - Reactive Protein in CFA Induced Arthritis in Rat



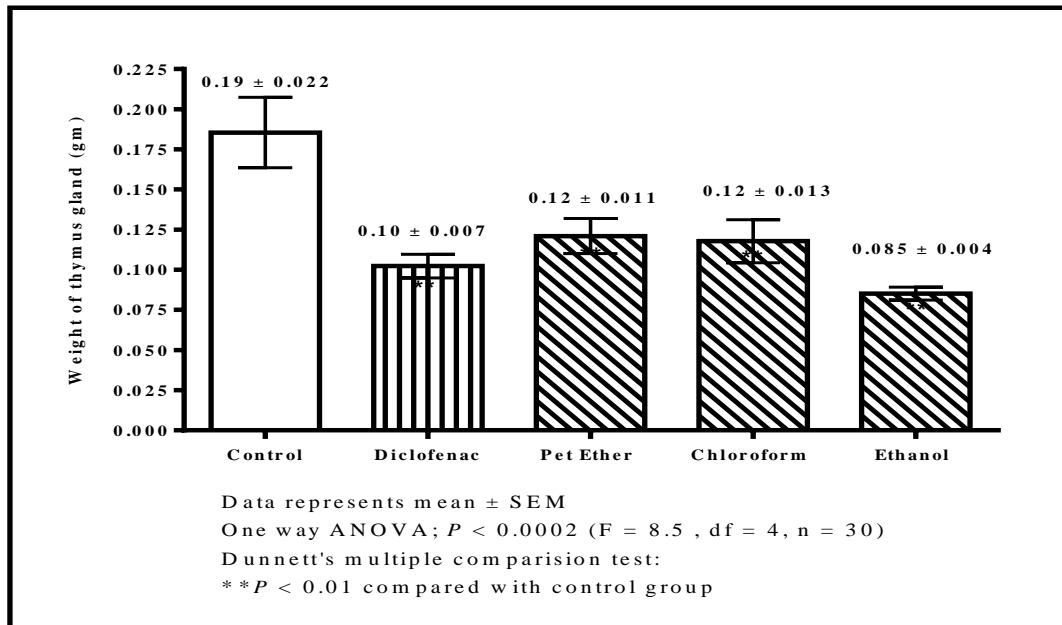
**Figure 17:** Effect of *Pergularia daemia* on Concentration of Rheumatoid Factor in CFA Induced Arthritis in Rat



**Figure 18:** Effect of *Pergularia daemia* on Spleen Weight in CFA Induced Arthritis in Rat



**Fig 19:** Effect of *Pergularia daemia* on Thymus Weight in CFA Induced Arthritis in Rat





**Table1: Data Showing The Preliminary Phytochemical Screening of The Various Leaf Extracts of *Pergularia Daemia***

Sr. No	PHYTOCHEMICAL SCREENING OF THE VARIOUS EXTRACTS OF <i>PERGULARIA DAEMIA</i> CONSTITUENTS	PET. ETHER EXTRACT	CHLOROFORM EXTRACT	ETHANOL EXTRACT
1	Carbohydrates	-	-	-
2	Glycosides	+	+	+
3	Alkaloids	+	+	+
4	Phytosterols	+	+	+
5	Saponins	+	+	+
6	Fixed oils and fats	+	+	+
7	Tannins	-	-	-
8	Proteins & amino acids	-	-	-
9	Flavanoids	+	+	+
10	Coumarins	+	+	+

+ Indicates positive test result

- Indicates negative test result

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