



Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (3):367-373
(<http://scholarsresearchlibrary.com/archive.html>)



Anti-arthritic activity of chloroform extract of *Barringtonia acutangula* (L) Gaertn. leaves on wister rats

M. Thirumal^{*1}, R. Vijaya Bharathi², B. Kumudhaveni² and G. Kishore¹

¹Department of Pharmacognosy, Jaya College of Pharmacy, Chennai, India

²Department of Pharmacognosy, Madras Medical College, Chennai, India

ABSTRACT

Barringtonia acutangula (family: *Lecythidaceae*), known as Indian Oak in English was used to treat joint pains (Rheumatoid disease). In this study the anti-arthritic effect of Chloroform extract of the leaves was evaluated and compared to untreated control. Complete Freund's Adjuvant (CFA)-induced arthritis model was done by using two techniques includes Prophylactic and Therapeutic model. Arthritis was induced by injecting CFA subcutaneously into the left hind paw. Paw volumes were recorded on the day of injection, 7, 14 and 21st day by using plethysmometer and compared. The results showed that Chloroform extract of the leaves of *B. acutangula*(CEBA)has significant anti- arthritic activity when compared to control group in both studies. This study supports the traditional use of *B. acutangula* for the treatment rheumatoid arthritis and suggests further studies to produce clinically useful herbal drug.

Key words: *Barringtonia acutangula*, Complete Freund's adjuvant, plethysmometer, Prophylactic model, Therapeutic model.

INTRODUCTION

Rheumatoid disease is one of the most common chronic inflammatory condition which develops over months or years in developed countries and is a common cause of disability. One in three patients with rheumatoid arthritis is likely to be disabled. The joint changes, which probably represent an autoimmune reaction, comprise inflammation, proliferation of the synovial and erosion of cartilage and bone. The primary inflammatory cytokines, IL-1, TNF- α have a major role in pathogenesis. Complete Freund's adjuvant (CFA) possess heat killed *Mycobacterium* in a W/O emulsion. After s.c injection, CFA induces adjuvant arthritis that can act as a model to test the anti-arthritic and anti-inflammatory effects of investigational substance and the effect evaluated in this model seems to be parallel to that evaluated in human disease [1, 2, 3, 4, 5].CFA containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis*per milliliter sterile paraffin oil. As *Mycobacteriumtuberculosis*in chronic infectious condition produces arthritis in human hence the model is suitable for the evaluation of arthritic activity. Acute inflammatory response induced by CFA is linked with leukocyte infiltration, mast cell activation and release of cytokines and free radicals [6]. This process is characterized by rapid increased with macrophage activation and secretion of bioactive product that play an important role in tissue destruction, vascular and fibrosis over a period of time [7]. Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain; they are associated with certain undesirable side effects [8]. With these obstacles, the field of arthritis research has put forward exponentially towards alternative therapies i.e., herbal therapies which is used by 80% of population throughout the globe and they have been considered safe and effective in all elevating chronic pain associated with arthritis [9, 10, 11].

Barringtonia acutangula is an evergreen tree belongs to the family *Lecythidaceae*. The literature survey reveals that various parts of *B. acutangula* have been used in traditionally to treat various diseases like cough, hemiplegia, pain

in joints, splenic disorders, stomach disorders, poisoning, anthelmintic, dyspnoea, leprosy, intermittent fever, eye diseases and diarrhoea [12, 13]. There were no reports on systematic and scientific study of Anti-Rheumatoid activity on leaf extracts. In the present study, the Anti-Rheumatoid activity of chloroform extract of the leaves of *B. acutangula* is being evaluated.

MATERIALS AND METHODS

Plant Collection and authentication

Fresh leaves of *Barringtonia acutangula* (L.) Gaertn. (Lecythidaceae) were collected from Madras Medical College premises, Chennai, Tamilnadu. The plant was identified, and authenticated by comparing with an authentic specimen by botanist Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai, T.N, India. Bearing a voucher Number **PARC/2008/197**.

Plant preparation and extraction

The dried, coarsely powdered leaves were extracted with Chloroform using Soxhlet's apparatus by hot percolation method for 24 hrs. The concentrated extract were dried on a water bath and preserved in a vacuum desiccator for further studies.

Phytochemical screening

Preliminary phytochemical analysis tests were carried out using extracts from plant [14, 15].

Animals

Healthy male Wistar albino rats weighing 120–180 g and from the Central Animal House of Darshan Institute of Pharmacological Studies, Puliur, Karur TK, Tamilnadu (**20/243/2008**) were used throughout the study. They were kept under standard environmental conditions at 25 °C with 12:12 h light–dark cycle in ventilated plastic cages. The rats were fed with a standard rat feed and water ad libitum. The experiment was performed in accordance with the guidelines was approved by IAEC.

Acute oral toxicity study

Acute oral toxicity [16] study was performed as per OECD-423 guidelines (acute toxic class method). Wister male rats (n=6) were used for the study. The animals were kept fasting for overnight providing only water, after which the ethanolic extracts were administered orally at the dose level of 5mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 100 and 2000 mg/kg body weight.

Complete Freund's adjuvant induced Arthritis[5, 17]

Adjuvant arthritis in rats has been described by Pearson and Wood (1959) exhibiting many similarities to human rheumatoid arthritis. Adjuvant induced antiarthritic activity was done by using two models:

1. Prophylactic model
2. Therapeutic model

Two protocols, termed “preventative” (or “prophylactic”) and “therapeutic” (or “established”) adjuvant arthritis, have gained wide usage for assessing a drug's potential anti-arthritic activity. Wister male rats with an initial body weight of 130 to 200g are used. On day 1, they were injected into the sub plantar region of the left hind paw with 0.1ml of complete Freund's adjuvant. It consists of 6 mg *Mycobacterium tuberculosis* being suspended in heavy paraffin oil by thoroughly grinding with mortar and pestle to give a concentration of 6mg /ml. Dosing with the test compounds or the standard was started on the same day and continued for 21 days. Paw volumes of all animals were recorded on the day of injection, whereby paw volume was measured plethysmographically on day 7, 14 and 21. The volume of the injected paw was measured again, indicating the primary lesion and the influence of therapeutic agents on this phase. The severity of the induced adjuvant disease on injected paw was measured by using a plethysmometer. The procedure has been modified based upon Javier *et al.*, (2003). To evaluate the therapeutic activity, the animals were dosed with the test compound or the standard from day 14 to 21. On day 21, Paw volume was determined again.

Grouping of Animals

Prophylactic model

Group I (Arthritic control) – Received daily dose of 1ml of 0.1 % CMC per orally/day from day 1-21.

Groups II (standard – Indomethacin 1 mg/kg b.w) – Received daily dose of 1ml of Indomethacin orally per /day from day 1-21.

Groups III & IV- Received daily with Chloroform extract of *B. acutangula* (200 and 400 mg/kg body wt. respectively), per orally/day from day 1-21.

Therapeutic model

Group I (Arthritic control) – Received daily dose of 1ml of 0.1 % CMC per orally/day from day 15-21.

Groups II (standard – Dexamethasone 2 mg/kg b.w) – Received daily dose of 1ml Dexamethasone per orally/day from day 15-21.

Groups III & IV- Received daily with Chloroform extract of *B. acutangula* (200 and 400 mg/kg body wt. respectively), per orally/day from 15-21.

Parameter Measured

Paw Volume was measured on day 0 and then weekly for 21 days. Paw volume was measured by using volume displacement plethysmometer.

Histopathological Analysis

The hind limbs were removed just distal to the knee and placed in 10% buffered formalin. The fixed tissues were then decalcified and slides of sagittal slices through the hind paw stained with hematoxylin and eosin. Slides were reviewed for the evaluation of soft tissue swelling, bone demineralization, pannus formation, cartilage erosion and joint space narrowing.

Statistical analysis

The data were expressed as Mean \pm SEM, statistical analysis was performed by one way ANOVA followed by Bornferroni's t-test, p values <0.05 were considered as significant. Highest significant difference test performed with Statistical package for Social Studies (SPSS).

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary Phytochemical screening of powdered drug & chloroform extract of *B. acutangula* leaves was performed by standard methods and the results indicated the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins & phenolic compounds, flavonoids, saponins and gums.

Acute toxicity studies

Acute toxicity studies showed no mortality up to the doses of 2000 mg/kg body weight. So, the extracts safe for long term administration.

Anti-Arthritic Activity

Prophylactic model

The results are given in Table 1, Fig 1. On 7th day, the inflammation in standard group was 50% whereas the inflammations in other groups were more than 60%. On 14th Day, the inflammation in standard group was 33%. The inflammation in the Chloroform extract of *B. acutangula* at 400mg/kg b.w had 43% inflammation. The inflammation in 200 mg/kg b.w was 55%. The arthritic control group had maximum inflammation of 79%. On 21st Day, the inflammation in standard group was 5.5%. The inflammation in the Chloroform extract of *B. acutangula* at 400mg/kg b.w had 21% inflammation. The inflammation in 200 mg/kg b.w was 32%. The arthritic control group had maximum inflammation of 88%.

Table 1. CFA induced antiarthritic activity (Prophylactic model)

| Treatment | Paw edema (ml) | | | |
|-------------------------|-----------------|--------------------------|---------------------------|--------------------------|
| | 0 day | 7 th day | 14 th day | 21 day |
| Arthritic control | 1.70 \pm 0.08 | 3 \pm 0.22 (62.5) | 3.28 \pm 0.37(79.16) | 3.45 \pm 0.16 (88.17)* |
| Standard (Indomethacin) | 1.78 \pm 0.07 | 2.7 \pm 0.08 (49.5)* | 2.4 \pm 0.12 (32.83)* | 1.92 \pm 0.08 (5.5)* |
| Dose 200 mg/kg b.w | 1.67 \pm 0.18 | 2.9 \pm 0.07 (72.67)** | 2.62 \pm 0.16 (55.00)** | 2.23 \pm 0.10 (32.5)* |
| Dose 400 mg/kg b.w | 1.73 \pm 0.18 | 2.7 \pm 0.08 (60.5)** | 2.42 \pm 0.15 (43.17)* | 2.03 \pm 0.11 (20.67)* |

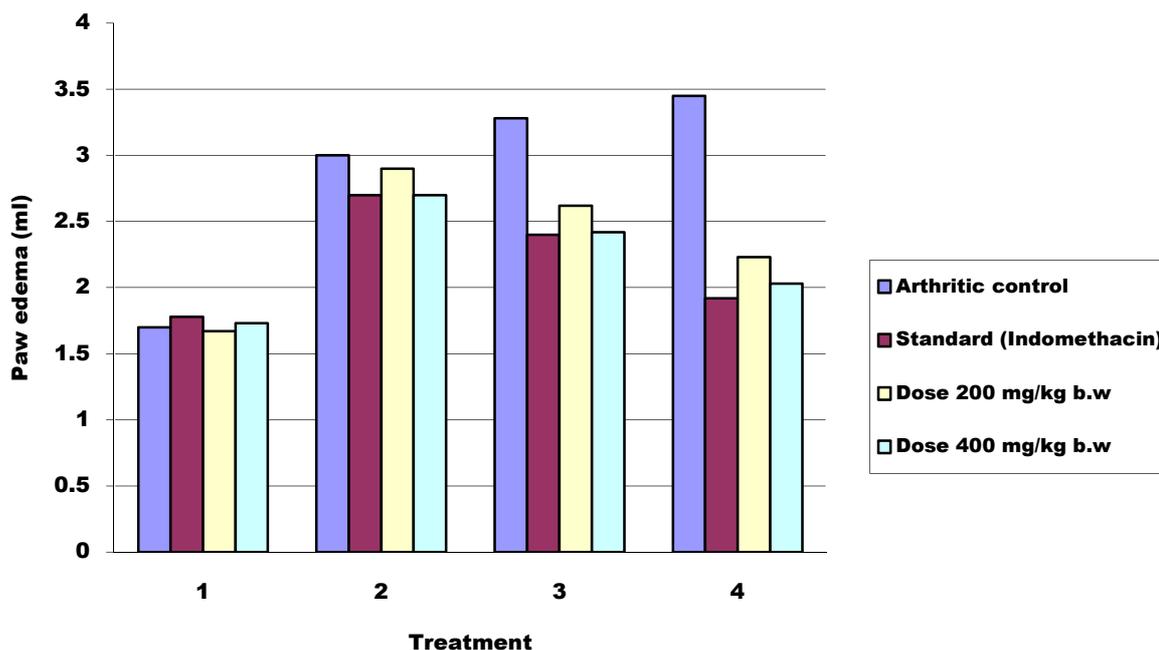
All the values are expressed as mean \pm SEM, n=6

*P \leq 0.05, compared with Arthritic control

**P \leq 0.05, compared with standard

Parentheses –Percentage change in inflammation

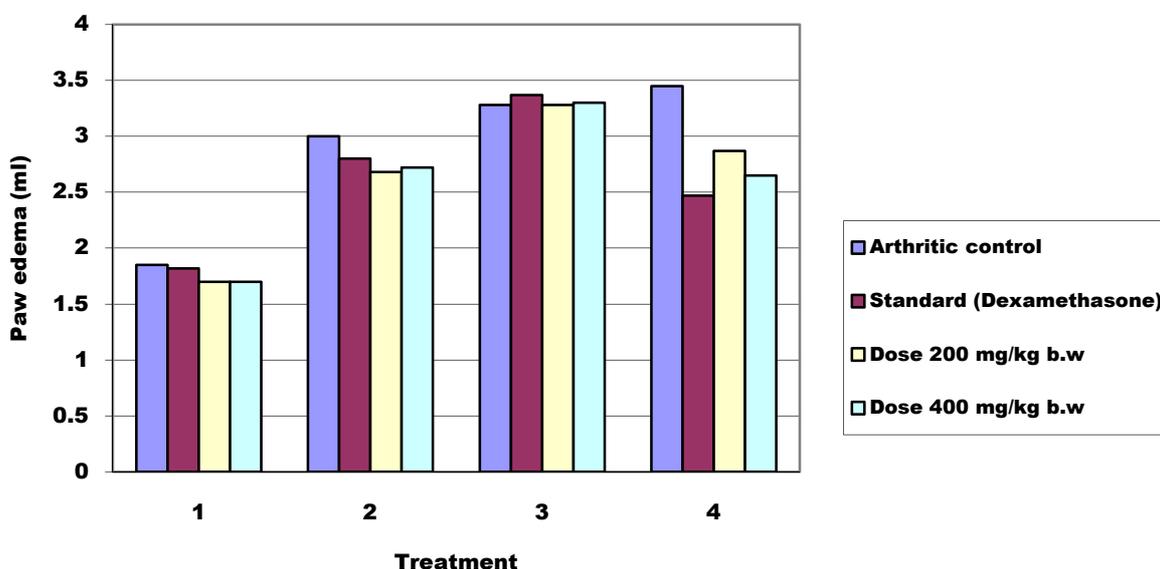
Fig 1. CFA Induced Anti Arthritic Activity (Prophylactic Model)



Therapeutic model

The results are given in Table 2, Fig 2. Up to 14th day, the inflammation in all the groups rose gradually. The inflammation was between 79-95%. On 21st Day, the inflammation in standard group was 36%. The inflammation in the Chloroform extract of *B. acutangula* 200mg/kg b.w was 70%. The inflammation in 400 mg/kg b.w was 56%. The arthritic control group had maximum inflammation of 88%.

Fig 2. CFA Induced Anti Arthritic Activity (Therapeutic Model)



Histopathology of Joints

Soft tissue swelling, massive influx of inflammatory cells and accumulation of abundant mononuclear cells, bone demineralization, cartilage erosions, and joints space narrowing were observed in the arthritic control group. In prophylactic study (Fig 3), it was minimal in Low dose level of Chloroform extract treated group and perfect healing at high dose level and standard drug treated group. In therapeutic study (Fig 4), local collection of lymphocytes and bony trabeculae are seen at the low dose level of Chloroform extract where as mature lamellar bone was found in

high dose level and with the standard drug. There is no inflammation at high dose level of Chloroform extract in both the studies.

Table 2. CFA induced anti arthritic activity (Therapeutic Model)

| Treatment | Paw edema (ml) | | | |
|--------------------------|----------------|---------------------|----------------------|-----------------------|
| | 0 day | 7 th day | 14 th day | 21 day |
| Arthritic control | 1.85 ± 0.08 | 3.0 ± 0.22 (37.16) | 3.28 ± 0.15 (79.17) | 3.45 ± 0.16 (88.17) |
| Standard (Dexamethasone) | 1.82 ± 0.07 | 2.8 ± 0.07 (35.17) | 3.37 ± 0.07 (86.33) | 2.47 ± 0.07 (36.00)* |
| Dose 200 mg/kg b.w | 1.70 ± 0.07 | 2.68 ± 0.05 (36.67) | 3.28 ± 0.06 (94.33) | 2.87 ± 0.10 (69.83)** |
| Dose 400 mg/kg b.w | 1.70 ± 0.07 | 2.72 ± 0.06 (37.33) | 3.30 ± 0.06 (95.67) | 2.65 ± 0.09 (56.33)* |

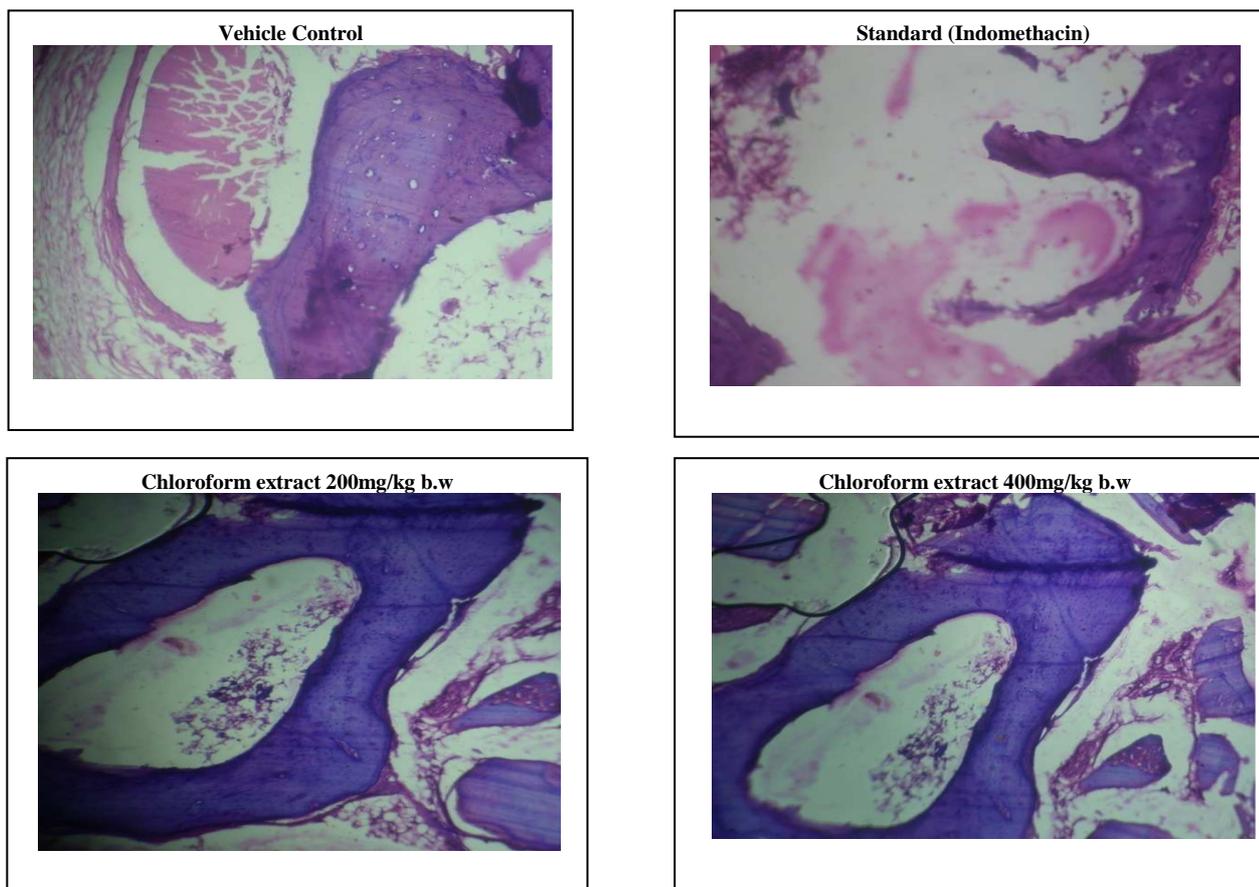
All the values are expressed as mean ± SEM, n=6

*P ≤ 0.05, compared with Arthritic control

**P ≤ 0.05, compared with standard

Parentheses – Percentage change in inflammation

Fig 3. Histopathology Observations - Prophylactic study

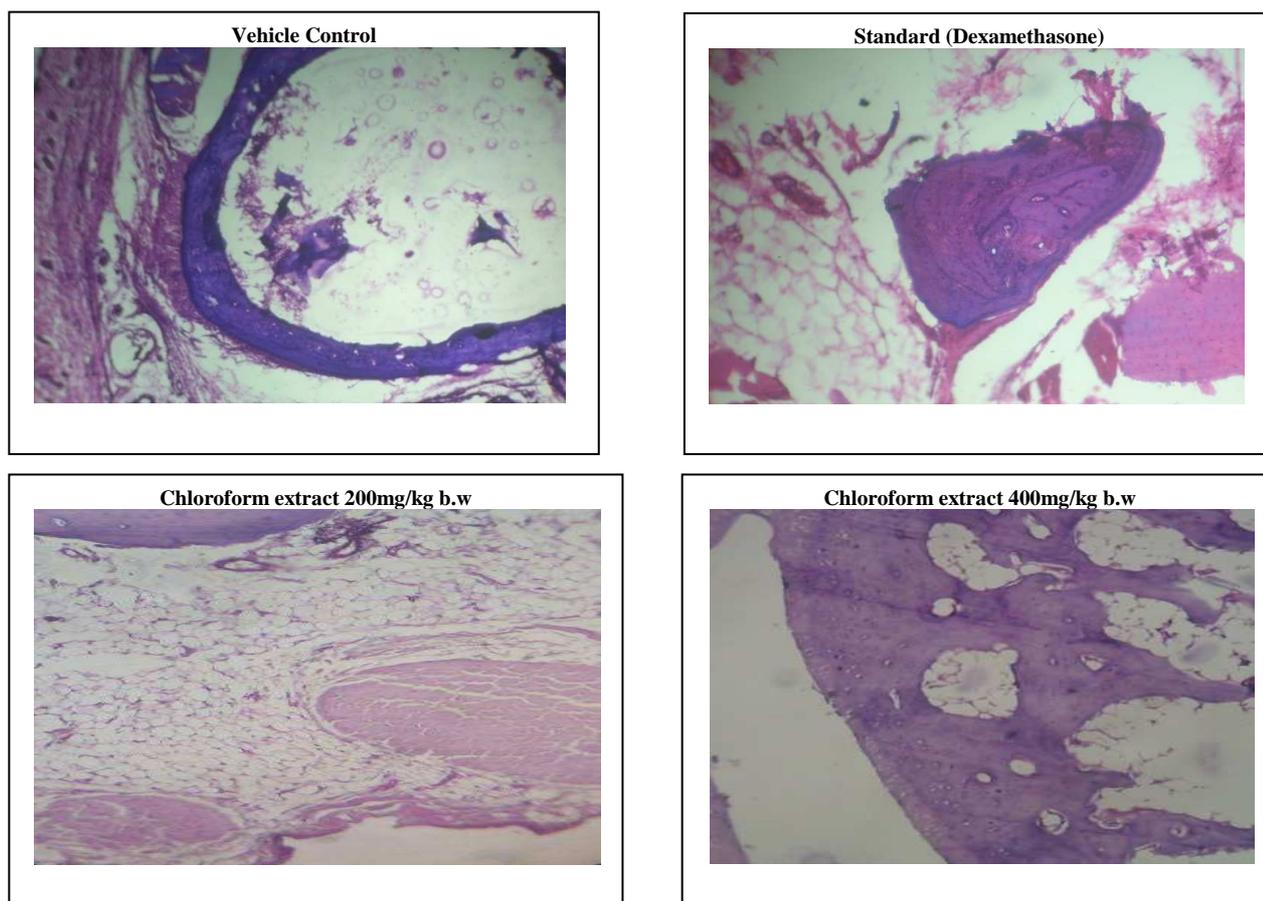


The Chloroform extract of the leaves of *Barringtonia acutangula* has significant anti-arthritic activity when compared to control group in both studies.

In the current study, complete Freund's adjuvant induced arthritis in Wistar rats were selected to induce arthritis, because it is the best and most widely employed empirical model for arthritis with clinical and laboratory features such as chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction and it has close similarities to human rheumatoid diseases [18]. Oxygen derived free radicals and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders. The importance of oxygen free radicals and related activated oxygen free intermediates in the pathogenesis of Rheumatoid arthritis has been identified with increasing incidence [19].

Paw swelling is one of the primary factors in evaluating the degree of inflammation and therapeutic efficacy of the drugs [20]. The initial inflammatory response will be produced within hours, but more vital clinical signs will be observed from the 10th post-inoculation day and thereafter and the changes remain detectable for many weeks [21].

Fig 4. Histopathology Observations – Therapeutic model



The present study demonstrated that chloroform extract of *B. acutangula* is able to suppress the swelling of the paws in both models. This may be due to the suppression of the inflammatory mediator released due to the induction of CFA [22].

From the results obtained, it can be said that herbal *B. acutangula* possess significant antiarthritic property which is comparable to synthetic anti-inflammatory agents.

CONCLUSION

CEBA was subjected to Complete Freund's Adjuvant induced arthritis and anti arthritic property of the extract studied by two methods (Prophylactic and Therapeutic). The effect of chloroform extract were determined after administration at two dose level (200 and 400 mg/kg b.w.) in arthritis induced rats and assessed by histopathological studies.

From the results, it may be concluded that herbal *B. acutangula* possess significant antiarthritic effect may be due to the synergistic effect of antioxidants like Flavonoids, Poly Phenols and Saponins present in the plant. This synergistic effect is comparable to synthetic anti-inflammatory agents. All these biological activities may be said to be a promising findings brought out by the present study. These contributions can be used as parameters for the authentication of plant as well as for developing newer drugs based on their activity. It can be optimistic that the present work suggests an herbal drug of multiple therapeutic advantages and likely to be a powerful antiarthritic drug. Further clinical studies are needed to establish its safety and its usefulness in arthritic patients.

REFERENCES

- [1] H.P.Rang, M.M. Dale, J.M. Ritter and P.K.Moore. Pharmacology, 5th edition, Anti-inflammatory and immunosuppressant drugs, Elsevier, New Delhi, India, 2003) 255.
- [2] V.K Alluri, C.B.M. Rao, D. Sundararaju, K. Sengupta and G. Trimurtulu, *Am. J. Infect. Dis.*, 2009, 5, 68-73.
- [3] W. Jia, W. Gao, N. Cui and P. Xiao, *J. Ethnopharmacol.*, 2003, 89, 139-141.

- [4] B. Newbould, *Br. J. Pharmacol. Chemother.*, **1963**, 21, 127-136.
- [5] C. Pearson and F. Wood, *Arthritis Rheumatol.*, **1959**, 2, 440-459.
- [6] P.A. Nigrovi, D.M. Lee, *Arthritis research and Therapy.*, **2005**, 7, 1-11.
- [7] S.M. Wahl, *Ann NY Acad Sci.*, **1985**, 460, 224-231.
- [8] LS Goodman, A. Gilman; *The Pharmacological Basis of Therapeutics*. Macmillan, New York, **1980**, 1, 682-709.
- [9] J.K. Rao, K. Mihaliak, K. Kroenke, J. Bradely, W.M. Tierney, M. Weinberger, *Ann Internal Med.*, **1999**, 131, 409-416.
- [10] K.L. Soeken, S.A. Miller, E. Ernst, *Rheumatology.*, **2003**, 42, 652-659.
- [11] V. Sheetal, S.P. Singh. *Veterinary World.*, **2008**, 1, 347-350.
- [12] Yoganarashimhan; *Medicinal Plants of India-Tamil Nadu*, Vol. 2, Cyber Media Ltd, Bangalore, India, **2000**, 71.
- [13] S.K. Jain; *Dictionary of Indian Folk Medicine and Ethnobotany*, Deep Publications, New Delhi, India, **1991**, 33.
- [14] C.K. Kokate; *Practical Pharmacognosy*, 4th edn, Vallabh Prakashan, New Delhi, **1994**.
- [15] J.B. Harborne; *Phytochemical methods: a guide to modern techniques of plant analysis*, 2nd edn, Chapman and Hall, London New York, **1983**.
- [16] D.J. Ecobichon; *The basis of toxicology testing*, CRC press, New York, **1997**, 43-86.
- [17] Javier Rojas, Miguel Paya, Jose N. Domínguez, M. Luisa Ferrández, *European Journal of Pharmacology.*, **2003**, 465, 183-189.
- [18] S. Singh, D.K. Majumdar, *Int. J. Pharmacol.*, **1996**, 34(3), 218-222.
- [19] P. Renuka Devi, S. Krishna Kumari and C. Kilavani, *Indian Journal of clinical biochemistry.*, **2007**, 22(1), 143-147.
- [20] V.H. Begum, J. Sadique, *Indian J Exp. Biol.*, **1988**, 26, 877-882.
- [21] F.C. Colpaert, T. Meert, P. Witte, P. Schmitt, *Life Sci.*, **1982**, 31, 67-75.
- [22] S. Tripathy, S.P. Sahoo, D. Pradhan, S. Sahoo, D.K. Satapathy, *Afr J Pharm Pharmacol.*, **2009**, 3, 614-617.