ISSN 2249-622X

Asian Journal of Biomedical and Pharmaceutical Sciences 1 (4) 2011, 16-23

RESEARCH ARTICLE

Screening of Methanolic Extract of *Pongamia Pinnata* Leaves for its Antiarthritic and Analgesic Activity

S.R. Arote[1]*, P.G. Yeole[2]

[1] Sanjivani College of Pharmaceutical Education and Research, Kopergaon, India.

[2] Institutes of Pharmaceutical Education and Research, Wardha, India.

ABSTRACT

The aim of present work was to screen traditional claim of methanolic extract of *Pongamia pinnata* (PP) leaves for its antiarthritic and analgesic activity. The antiarthritic activity of different doses of PP extract (100,200 and 400 mg/kg) in rats were evaluated on 7th and 14th day of treatment. The paw volume displacement, radiographic analysis, histopathological investigation and secondary changes were measured, as a mark of activity. The analgesic activity of the different doses of the extract in rats was evaluated using hot plate model. The PP extract at the dose of 200 and 400mg/kg showed significant reduction in paw volume on 7th and 14th day of treatment and also significant effect in radiographic and histopathological analysis but, none of the doses of PP extract showed any significant effect as analgesic.

KEYWORDS: *Pongamia pinnata,* antiarthritic, analgesic

INTRODUCTION

Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which further may be responsible for the deformity and disability especially in a substantial socio-economic impact and hence need to be addressed at all times [1,2].Overall it involves a complicated pathogenesis, with pathological changes in multiple targets [3] Complete Freund's adjuvant (CFA) induced arthritis is an experimental model which considered closest to simulating human rheumatoid arthritis. The appearance of secondary lesions (uninjected paw swelling) are the manifestation of cell mediated immunity (T cell response particularly CD4+ T cells). Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [4] Although these drugs are widely used for relieving pain but are associated with numerous untoward effects like hyperacidity, gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been ideal in all the cases. Therefore, alternate analgesic and anti-inflammatory drugs without serious side effects are being searched all over the world. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine has been paid great attention [5] The interest in drugs of plant origin is due to several reasons namely, limitations of conventional medicine due to various side effects associated with their use. Moreover, a large percentage of the world's population does not have easy access to conventional pharmacological treatment as compared to natural therapies [6, 7]. Folk medicine and ecological awareness suggest that they usually cost less than synthetic drugs and undesirable side effects are less frequent [8] Literature survey of *Pongamia pinnata* has claimed to have anti-inflammatory, anti-plasmodial, anti-nociceptive, and anti-hyperglycemic properties [9]Review of the limited scientific documentation on anti-inflammatory and anti-nociceptive activities was in conformation with the claims mentioned in literature, making it worthwhile to select this plant for validation of the unexplored claims. In light of this, the objective of the present study entitled **"**Screening of methanolic extract of *Pongamia pinnata* leaves for its antiarthritic and analgesic activity" was undertaken.

MATERIALS AND METHODS

PLANT MATERIAL:

Leaves of *Pongamia pinnata* (PP) were purchased from local vendors and were identified and authenticated from National institute of science communication and information sources (NISCAIR). Certification No: NISCAIR/RHMD/Consult/08-09/1052/83/06.

CHEMICALS AND DRUGS:

Complete Freund's adjuvant (CFA), Methotrexate, Pentazocine

* ***Corresponding author:*** S.R. Arote **/** ***Email:*** pharmacology37@yahoo.in

PREPARATION OF EXTRACT:

Pongamia pinnata (PP) leaves were dried and charged to extractor along with methanol. The mass was heated for 5-6 hours in a closed system by re-pumping the extract to herb bed. This procedure was repeated. The extracts were combined, filtered and concentrated under vacuum. This was subjected to spray drying to separate extract in the powder form. This powder was further subjected to multimill to obtain fine mesh size powder. It was sieved by a sifter and mixed in the blender to obtain a uniform particle sized powder [10]

STORAGE OF EXTRACTS:

Methanolic extract of PP was stored in tightly closed glass bottles in refrigerator at 2-8 ^{0}C.

PREPARATION OF EXTRACT SOLUTIONS:

Test solutions (T.S) of Methanolic extract of PP was prepared in distilled water in order to make concentration 100 mg/ml.

ANIMALS:

Wistar albino mice (18- 22gm) and rats (120-150gm) were used. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hour. light 12 hour. dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water ad libitum. Local Institutional Animal Ethical Committee (IAEC) approved the protocol. All experiments were carried out between 12:00- 16:00 h.

PREPARATION OF DRUG SOLUTION:

Accurately weighed quantity of powdered extract of PP was dissolved in the distilled water to prepare the appropriate stock solution of the drug i.e. 10 mg/ml, 20 mg/ml and 40 mg/ml respectively. The doses were administered orally by selecting the appropriate concentration (10ml/kg) of the stock solution.

ROUTE OF ADMINISTRATION:

Methanolic extract of PP was administered by oral route. CFA was administered by sub plantar route and Pentazocin by subcutaneous route.

ACUTE TOXICITY STUDY:

Healthy adult male wistar albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development [11] The mice were administered with the different doses of methanolic extract of PP or distilled water (10ml/kg). The dose progression or reduction was carried out as suggested by the AOT-425 guidelines.

STATISTICAL ANALYSIS:

The comparison was made against the vehicle treated control group and the data was expressed as mean ± SEM. The data was analysed using suitable test with respect to individual models mentioned later.

METHODS:

The different doses (100, 200, 400 mg/kg) of the Methanolic extract of PP were screened for following pharmacological activities.

- Evaluation of antiarthritic activity using using Freund's complete adjuvant model.
- Evaluation of analgesic activity using hot plate analgesia meter.

EVALUATION OF ANTI ARTHRITIC ACTIVITY USING FREUND'S COMPLETE ADJUVANT MODEL:

Thirty pre-selected wistar rats were made arthritic by single intra-dermal injection of 0.1 ml of Complete Freund's adjuvant (CFA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of rats. Rats were randomly divided into five groups, each containing six rats. Wherein group I served as control and received vehicle (10ml/kg) whereas group II, III and IV served as test drug groups and received different doses of methanolic extract of PP (100, 200, 400 mg/kg), Group V served as reference standard and received methotrexate 0.75 mg/kg. On 0th day the left hind paw volume of all the rats as a volume displacement was measured using digital plethysmometer. Immediately after this, respective drug treatment (as mentioned above) was started and continued till next 14 days. 60 minutes after the first dose CFA treatment was given as subplantar injection to induce rheumatoid arthritis in all rats. 60 minutes after dosing, the volume of displacement was measured on 7th day. While on 14th day volume of displacement as well as severity of secondary lesions were noted [12, 13]. Thereafter the supportive parameters that are radiographic analysis and Histological investigations were carried out as mentioned below.

RADIOGRAPHIC ANALYSIS:

On the 14th day immediately after measurement of paw volume displacement, the one rat from each group was anaesthetised by intra peritoneal injection of Ketamine (100 mg/kg) and subjected to the radiological examination using Agfa digital System and Seimens X ray machine. These radiographs were evaluated for soft tissue swelling

and bone erosion, joint space narrowing independent qualified person [12]

HISTPATHOLOGICAL INVESTIGATIONS:

After the radiograph study rats were sacrificed and the knee joint was transected, paws were then transferred into formalin solution and subjected to histopathological investigation [12]

EVALUATION OF ANALGESIC ACTIVITY USING HOT PLATE ANALGESIA METER:

Thirty pre-selected rats were randomly divided into five groups, each containing six rats. Wherein group I served as control and received vehicle (10ml/kg) whereas group II, III and IV served as test drug groups and received methanolic extract of PP (100, 200, 400 mg/kg, p.o.), Group V served as reference standard and received Pentazocine (30 mg/kg, sc.). The rats were treated for a period of 14 days with the different drugs. The analgesic effect was studied using digital hot plate (Columbus- USA) instrument wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded 60 minutes after the administration of respective dose on 7th and 14th day. The temperature of the plate was maintained at 55°C ± 02° C. A cut off reaction time of 30 seconds was chosen in order to avoid injury [14].

RESULTS

EVALUATION OF ANTI ARTHRITIC ACTIVITY USING FREUND'S COMPLETE ADJUVANT MODEL

PAW VOLUME DISPLACEMENT (IN ML):

As shown in figure 1, the paw volume displacement of rats of all groups recorded on the 0th day was found to be similar. The intra group did not show any significant change due to selection of similar weight and age range animals. The paw volume displacements of control group, rats pretreated with PP extract 100, 200 and 400 mg/kg and Methotrexate (0.75mg/kg) rats on the 7th day after CFA injection were found to be 2.102 ± 0.026, 2.078 ± s0.024, 1.992 ± 0.032, 1.859 ± 0.025 and 1.712 ±0.031 respectively. Control rats and PP extract 100 mg/kg treated rats showed significant inflammation whereas the PP extract 200 mg/kg, 400 mg/kg and Methotrexate treated rats showed significant reduction in inflammation. The PP 400 mg/kg and Methotrexate were equipotent (P<0.01) and more significant than PP 200 mg/kg treatment (P<0.05). On 14th day, evaluation showed significant reduction in the inflammation with PP 100, 200 and 400 mg/kg treatment as compared against vehicle treated control rats. Here PP 100mg/kg was less significant (P<0.05) than the other two higher doses and Methotrexate treatment (P<0.01)

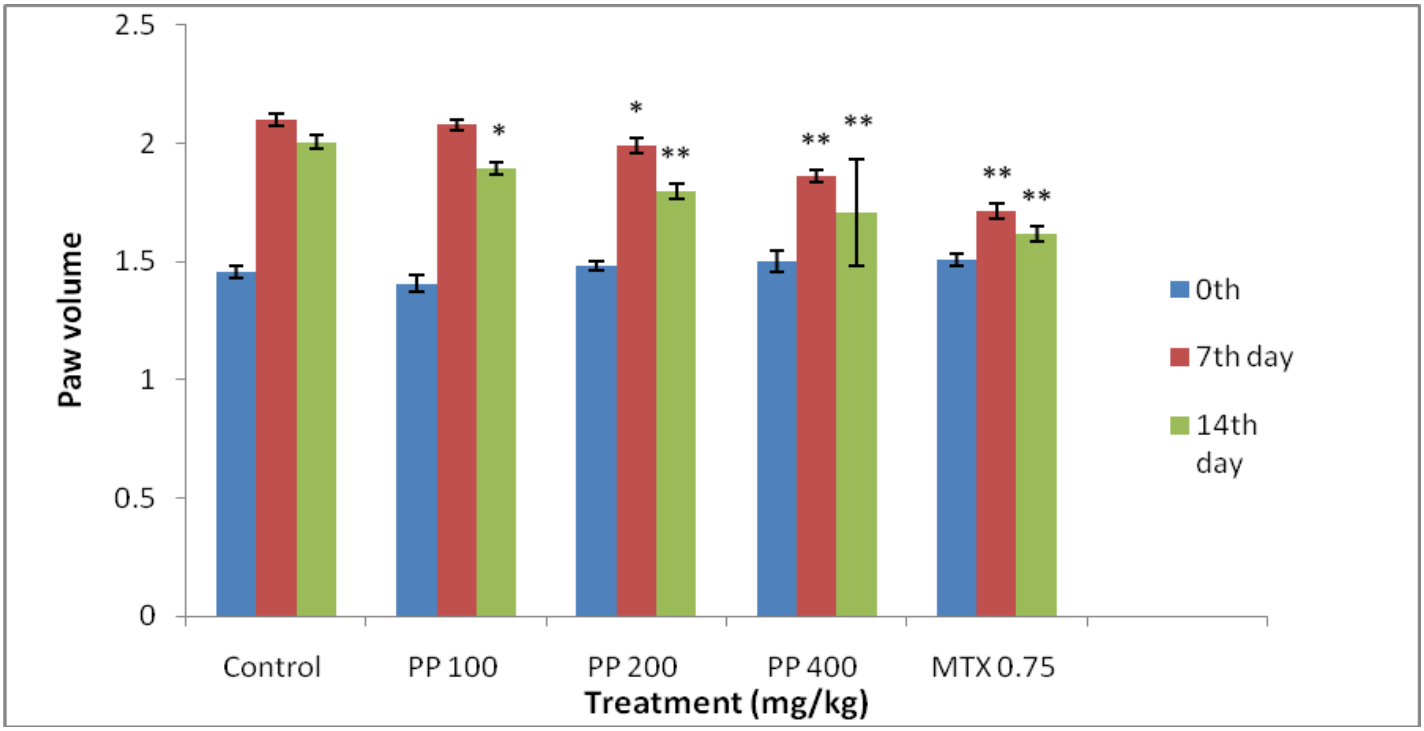


Figure 1: Effect on paw volume and percentage inhibition of paw volume in Complete Freund's adjuvant induced arthritic rats.

Results are expressed as mean ± SEM. (n = 6).Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts 't' test. *P<0.05, **P<0.01.

Sr. No.	Treatment	Secondary lesions
1	Control	+++
2	PP 100 mg/kg	+++
3	PP 200 mg/kg	++
4	PP 400 mg/kg	+
5	Methotrexate	-

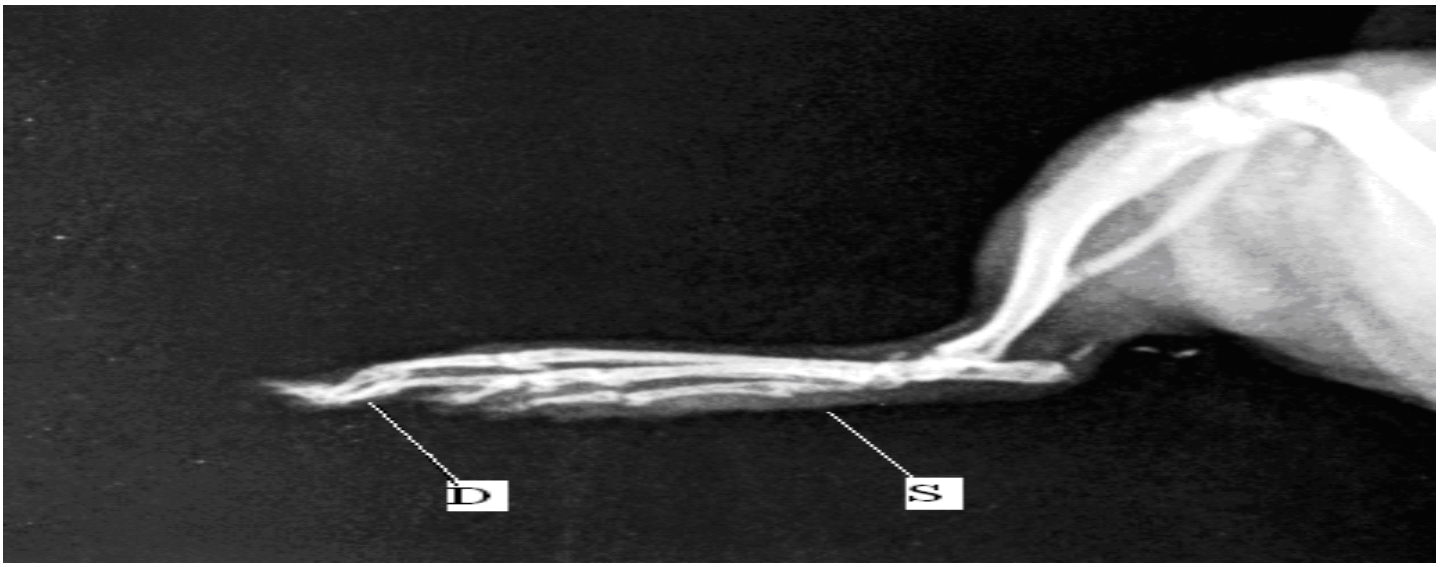
Table 1: Effect of PP extract on secondary lesions on 14th day

Nil; + Mild; ++ Moderate; +++ Severe

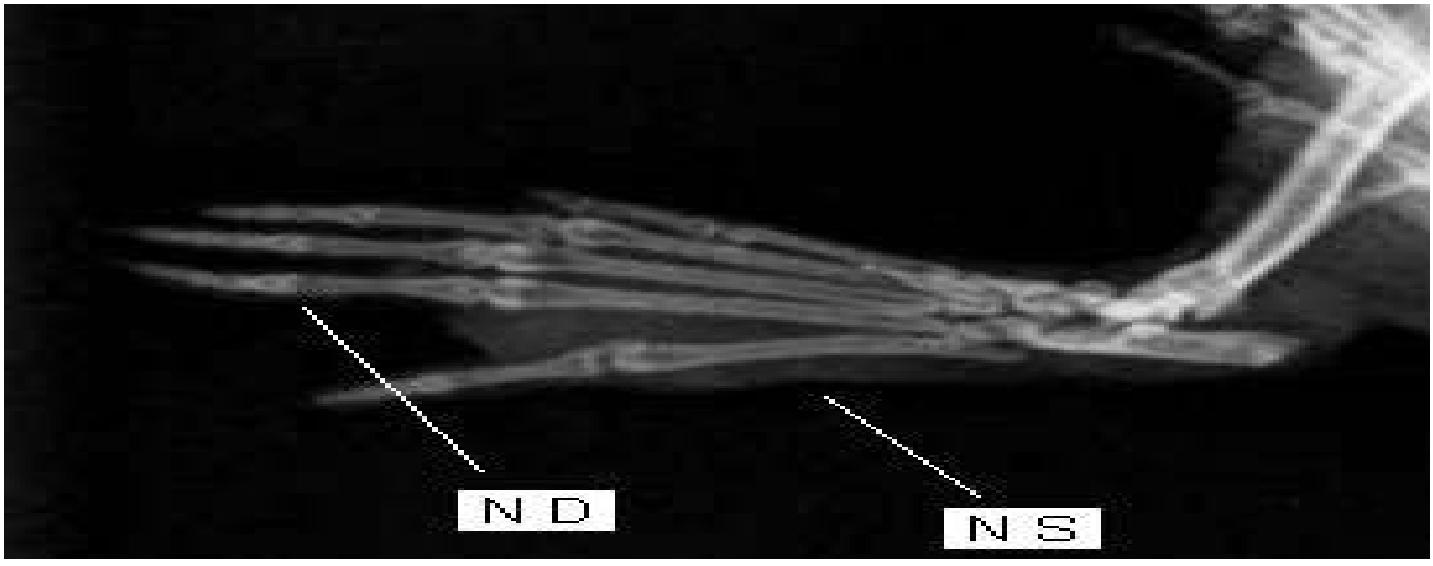
Note: *Secondary lesions on the 14th day were collectively observed in the ear, fore-paws, hind-paws and tail of rats [13]*

RADIOGRAPHIC ANALYSIS:

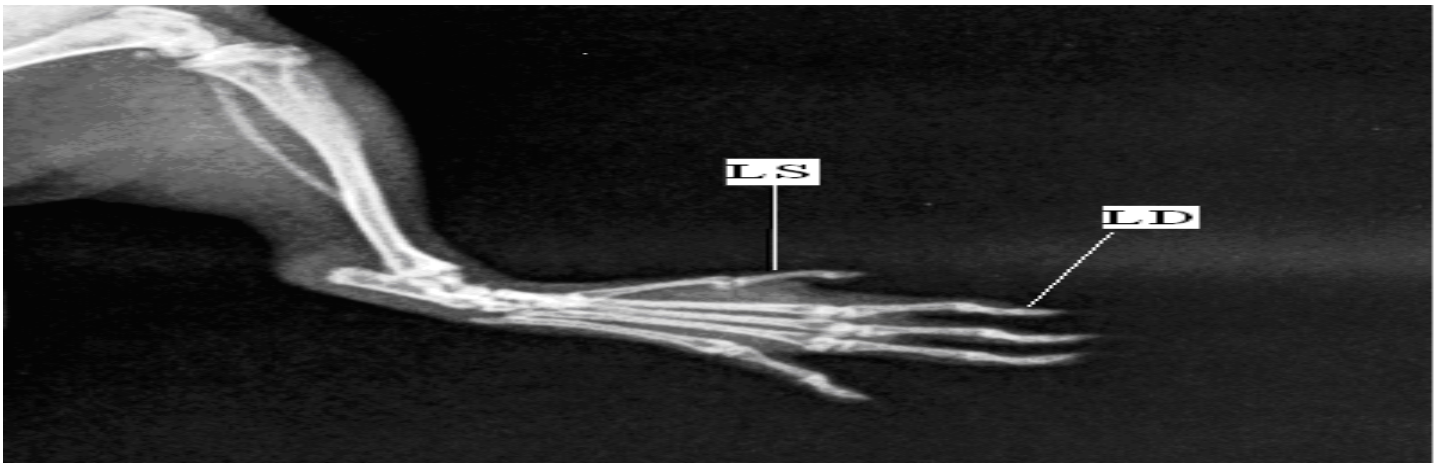
As shown in figure 2, radiographic examination of CFA injected hind paws of control rat exhibited uneven narrowing of the joint spaces, and subsequent bone cartilage destruction in the knee joint and significant soft tissue swelling indicating full blown arthritis. Whereas, PP extracts 200 mg/kg and 400 mg/kg treated rat showed remarkable reduction in soft tissue swelling as well as destruction of the knee joints. Moreover, the joint space is more even as compared against the vehicle treated control rats. Similar but more potent results were seen in rats treated with Methotrexate.



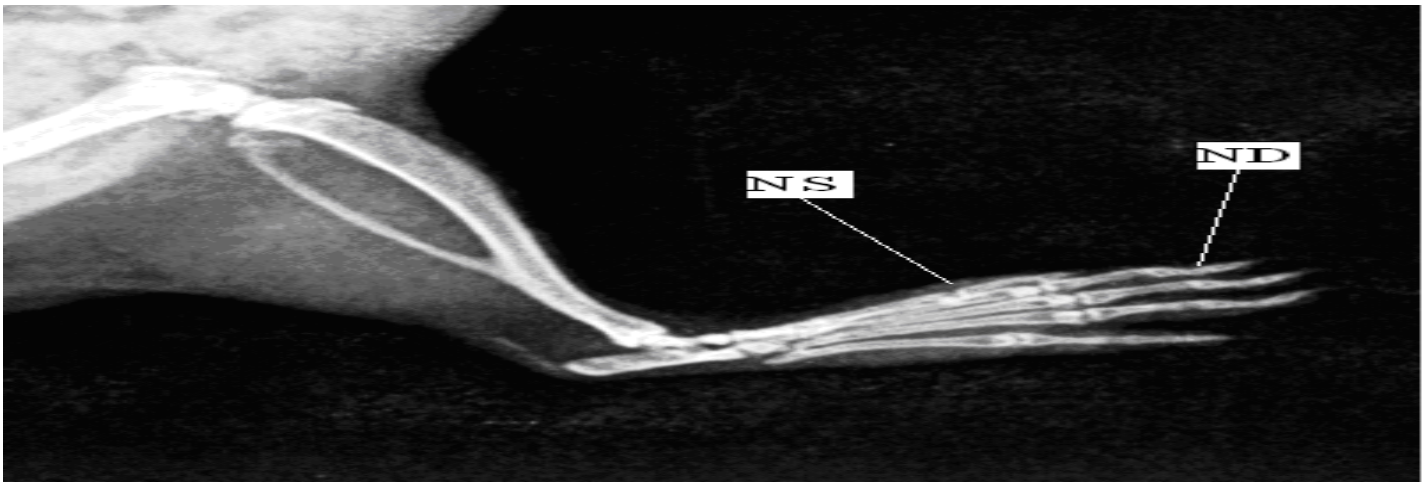
Control



Methotrexate



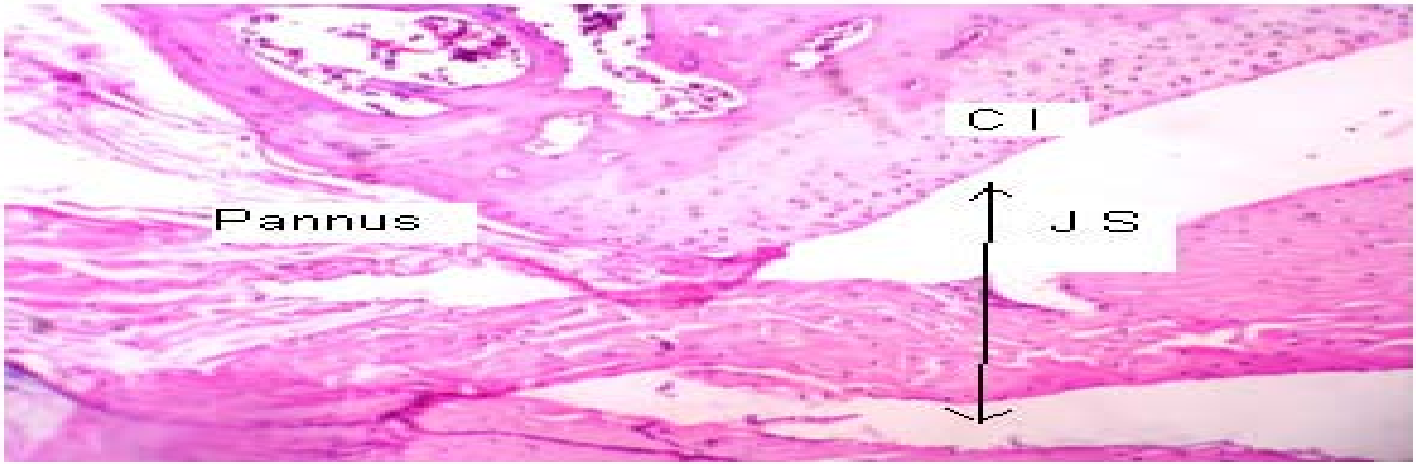
(PP 200mg/kg)



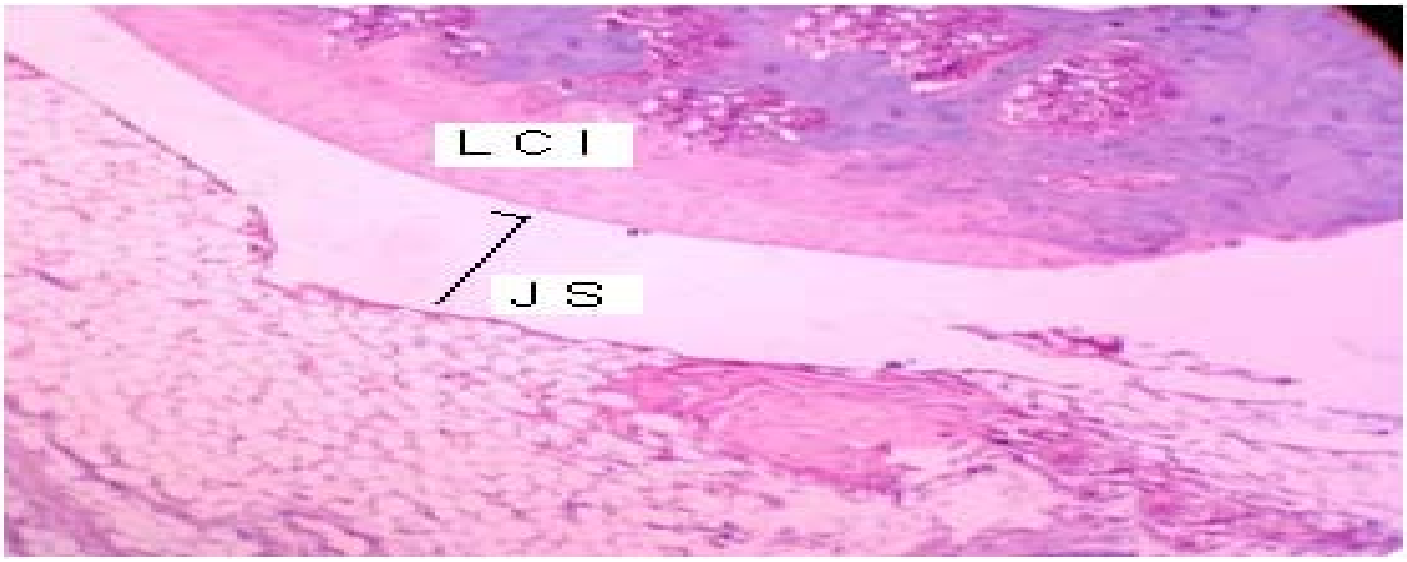
(PP 400mg/kg)
D- Deformity, S- Swelling, LD- Low deformity, LS- low swelling, ND- No deformity, NS- No swelling.

HISTOLOGICAL INVESTIGATION:

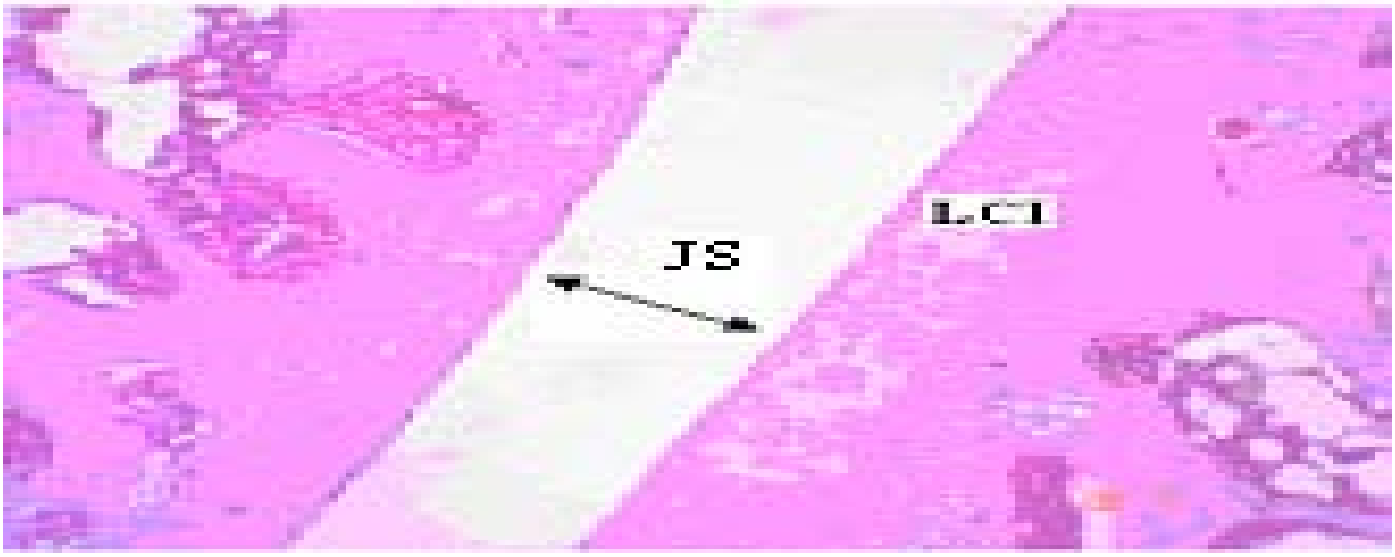
As shown in figure 3, histological study reported appearance of peculiar features like cellular infiltration, synovial hyperplasia and pannus formation, narrowing of joint space, and bone erosion in the knee joints sections in the vehicle treated control rats. These changes were significantly reversed with the treatment of PP 200 and 400 mg /kg treatment in the dose dependent manner whereas 100 mg/kg dose was found to be ineffective in this regard.



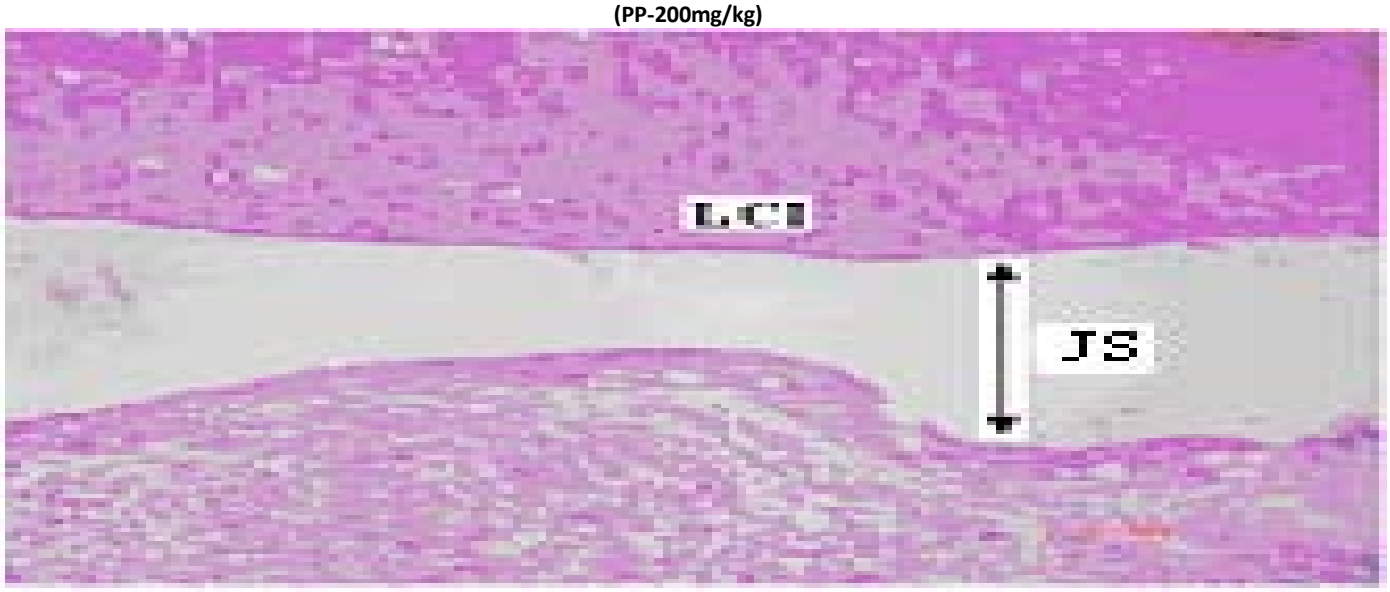
Control



Methotrexate (0.75mg/kg)



(PP-200mg/kg)



(PP-400mg/kg)

EVALUATION OF ANALGESIC ACTIVITY USING HOT PLATE ANALGESIA METER:

As shown in figure 4, the mean reaction times shown by the vehicle treated control, PP extract (100, 200, 400 mg/kg) and Pentazocin treated rats on the 7th day were found to be 11.12 ± 0.296, 10.55 ± 0.610, 10.50 ± 0.284, 10.39 ± 0.361 and 14.47 ± 0.255 respectively. All the doses of PP extract did not show any change in the reaction time as compared against control group rats. Pentazocine significantly increased (P<0.01) the mean reaction time. Similar results were recorded on 14th day.

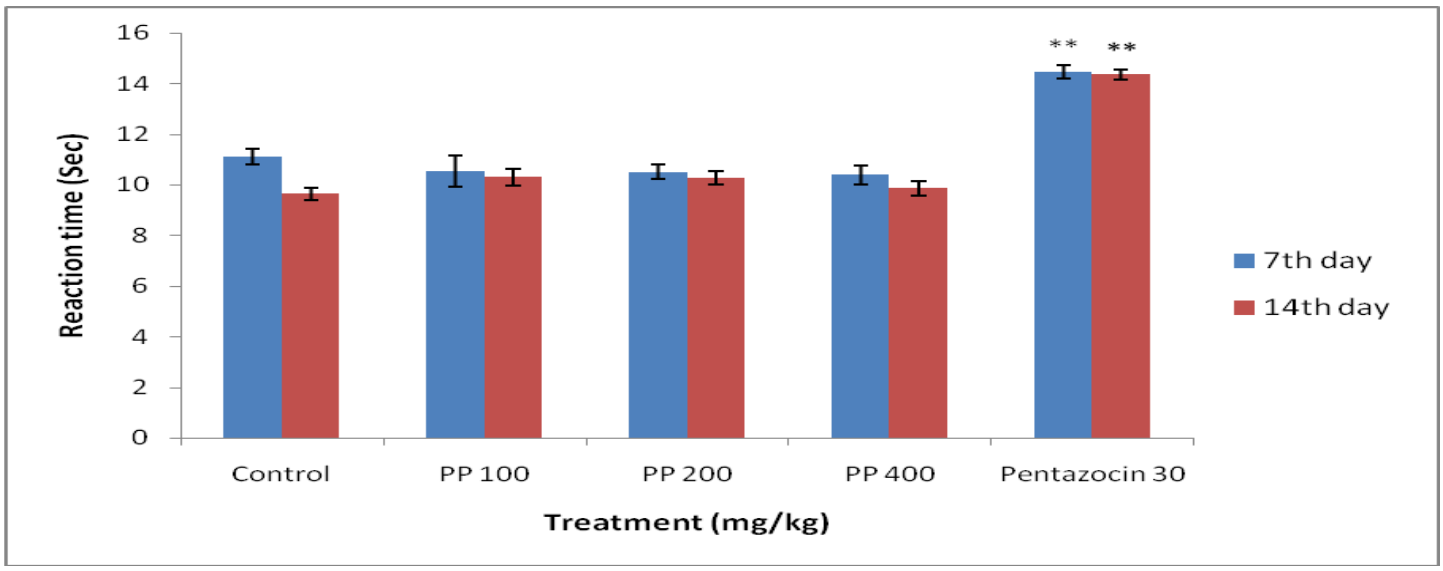


Figure 4: Effect of PP extract and Pentazocin on analgesia induced by hot plate in Rats

Results are expressed as mean ± SEM. (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnett's't' test. *P<0.05, **P<0.01.

DISCUSSION

Pharmacotherapy using plant-derived substances can be currently regarded as a very promising future alternative to current synthetic drug therapy. The advanced techniques and technologies available today enable to investigate chemically well-defined bioactive plant components as sources of novel drugs [15] to evaluate the toxicity profile so as to confirm safety of methanolic extract of *Pongamia pinnata* prior subjected to any preclinical pharmacological screening the acute toxicity study was carried out. Our findings indicated that the extract was found to be devoid of any toxic symptoms and no mortality was found up to 2000 mg/kg [11] from this report three different doses i.e 100, 200 and 400 mg/kg of extract were selected for further study. *Pongamia pinnata* has been recognised in different systems of traditional medicines for the treatment of various different diseases and ailments of human beings. We have scientifically explored some of its important claims and possible uses. It has been traditionally claimed to be useful in joint pain. The plant has already been reported for its significant analgesic and antipyretic effect which suggests possible use in various joint pain management approaches. The current literature survey also revealed that no systematic approach has been made towards documentation of this claim. On the contrary, Rheumatoid arthritis (RA), one of the commonest autoimmune diseases prominently manifested by the joint pain and inflammation has reported a large number of mortality and morbidity and thereby left substantial socioeconomic impact [2] The currently available large number of synthetic drugs, especially steroidal drugs are mainly used for symptomatic relief and also associated with numerous side effects. These limitations in turn demand for alternative value addition therapy. With these difficulties, the field of arthritis research has become a prominent thrust area [3] Modern research in the field of anti-arthritic therapy is directed towards developing potent compounds with wide acceptability, non-toxicity and the ability to suppress the immune response to an antigen. In light of this, the anti-inflammatory activity of *Pongamia pinnata* using Complete Freund's Adjuvant induced arthritis model was performed. The present investigation reported that the higher two doses of the extract showed improvement in arthritis condition by reducing hind paw inflammation and secondary lesions. The improvement in secondary lesions is the hallmark of anti-rheumatoid activity of the extract. These results postulated possible dual role of extract as a symptomatic therapy and preventive remedy which can be considered as a value added outcome as compared to modern therapy. An ideal

therapy in rheumatoid arthritis is expected in halting of the disease pathology rather than pure symptomatic relief. Radiographic analysis is considered to be the best tool to screen any drug in this regard. In this study, radiographic analysis of the joint showed significant prevention in the progress of joint pathology which is perhaps the most desired effect in Rheumatoid arthritis. In this model of arthritis, complex composition of bacterial adjuvant leads to initiation of a multistage process of immune response. Hence, the test drug effective i.e PP extract indicates immunomosuppresant potential [16, 17]. In synthetic medication, rapid reduction in inflammation in Rheumatoid arthritis is observed with corticosteroids however they are effective for a short term. Corticosteroid become less effective over time and rheumatoid arthritis is usually active for year's together (Beers *et al.,* 2008). The above observed optimal immunosuppressant action coupled with significant anti-inflammatory activity of the extract suggests that it can be a good substitute to the current corticosteroid therapy. In rheumatoid arthritis pain, inflammation and immune response are the key parameters that ultimately govern the disease pathology. PP extract has already been documented to be antinociceptive using various models of peripheral analgesia. However its narcotic analgesic potential has not been documented. The present investigation studied this effect using hot plate analgesiameter and found to be ineffective. This suggests that PP extract can exhibit only peripheral analgesia and not central one [18] This report suggests possible use of this extract as a long term therapy, since drugs with narcotic analgesic property usually results in drug dependency, especially up on long term administration [19] Moreover, peripheral analgesics e.g. NSAID's are sufficient to alleviate pain in rheumatoid arthritis. In this context, the eligibility of the extract as a long term therapy in rheumatoid arthritis is the most important outcome of this study.

CONCLUSION

To conclude methanolic extract of *Pongamia pinnata* showed beneficial activity as a long term therapy in rheumatoid arthritis (RA).

REFERENCES

1. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov 2003; 2: 473–88.

2. Buch M, Emery P. The aetiology and pathogenesis of rheumatoid arthritis. Hospital Pharmacist 2002; 9: 5–10.

3. Gabriel SE. The epidemiology of rheumatoid arthritis. Rheumatic Disease Clinics of North America 2001; 27: 269–282.

4. Janssen SA. Negative effect and Sensitization to pain. Scandinavian Journal of psychology 2002; 43: 131-137.

5. Kumara, NKVMR. Identification of strategies to improve research on medicinal plants used in Sri Lanka. In: WHO Symposium. University of Ruhuna, Galle, Sri Lanka, 2001.

6. Pandey AK, Rai MK. Plant-derived antimycotics: Potential of Asteraceous Plants. In. Plant-derived antimicotics. (Eds. MK Rai, Mares D) 2003; 343-344.

7. Singh G. Studies on the biocidal activities of certain essential oils. JMAPS 1999; 21: 1119-1130.

8. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000; 33(2): 179-189

9. Chopde VV, Tankar AN, Pande VV, Tekade AR, Gowekar NM, Bhandari SR, Khandake, *Pongamia pinnata*: Phytochemical constituents, Traditional uses and Pharmacological properties; A review. International journal of green pharmacy 2008; 72-75.

10. Rangari VD. Pharmacognosy and Phytochemistry. Vol I, 2nd edi, Career publication, Nashik, 2009.

11. OECD Guideline For The Testing of Chemicals: Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment 2000.

12. Wilder RL, Calandra GB, Garvin AJ, Wright KD, Hansen CT. Strain and sex variation in the susceptibility to streptococcal cell wall-induced polyarthritis in the rat. Arthritis and Rheumatism 1982; 25 (9):1064–1072.

13. Newbould BB. Chemotherapy of Arthritis induced in rats by Mycobacterial adjuvant. Brit J Pharmacol 1936; 21: 127-136.

14. Gupta M, Mazumder UK, Chakraborty S. CNS activities of methanolic extract of Moringa oleifera root in mice. Fitoterapia 1999; 70: 244 – 250.

15. Philipson JD. Phytochemistry and pharamacognosy. Phytochemistry 2007; 68:2960–72.

16. Crofford LJ, Wilder RL. Arthritis and autoimmunity in animals. In: Mccarty, D.J., Koopman, W.J. (Eds.), Arthritis and Allied Conditions. Lea and Febiger, London, 1993; 525–539.

17. Walz DT, Di Martimo MJ, Misher A. Adjuvant-induced arthritis in rats. II: drug effects of physiologic, biochemical and immunologic parameters. Journal of Pharmacology 1971; 178: 223–231.

18. Kulkarni SK. Hand book of Experimental Pharmacology. 3rd edi, Vallabh Prakashan, New Delhi, 2004; 92-95.

19. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th edi, Longman group UK Limited, 2003.