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International Journal of Current Research
Vol. 6, Issue, 10, pp.9258--9263, October, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTI-ARTHRITIC ACTIVITY ON INDIGENOUS SIDDHA HERBO-MINERAL FORMULATION "POONEERU DIRAVAGAM" IN EXPERIMENTAL ANIMAL MODELS

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ARTICLE INFO

Article History:
Received 20th July, 2014
Received in revised form
07th August, 2014
Accepted 04th September, 2014
Published online 25th October, 2014

Key words:

Anti-arthritic activity,
Freund's complete adjuvant,
Pooneeru,
Rheumatoid arthritis,
Siddha.

ABSTRACT

Background: Rheumatoid arthritis is an auto immune disorder associated with chronic inflammatory, destructive, and deforming symmetrical Polyarthritis associated with symmetrical involvement of joints.

Aim: The present study evaluates anti-arthritic efficacy of herbo-mineral formulation pooneeru diravagam (PD) in animal models.

Materials and Methods: The anti-arthritic activity of Pooneeru diravagam (in doses of 0.2 ml/kg and 0.4 ml/kg of body wt.) Was evaluated using the Freund's complete adjuvant in to the sub plantar region of the left hind paw. Prednisolone (10mg/kg, p.o) were used as standard drug and administered as CMC suspension by oral route. This study includes examination of the paws thickness measurement, paw volume, paw withdrawal latency and Histopathological analysis. Negative control animal receives saline 1ml on day of immunization with adjuvant. The drug treatments were administered from the day of adjuvant injection (0 day), 30 minutes before adjuvant injection and continued till 14th day. Disease severity was evaluated by the measurement of hind paw volumes on day 0, 1, 4, 7, 10 and 14th day. The joint diameters of left hind paw were measured using a Vernier caliper on the above mentioned testing days after induction of arthritis.

Results: The marked reduction of the arthritis score by Pooneeru Diravagam as observed in our study indicates a possible immuno modulatory effect. Significant ($p<0.05$) decrease in mean paw edema level of treated group compare to control. Significantly inhibit the progression of the arthritis in animal models.

Conclusion: The present study reveals that the Pooneeru diravagam (PD) is a promising drug in the treatment of Rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis is an auto immune disorder associated with chronic inflammatory, destructive, and deforming symmetrical Polyarthritis associated with symmetrical involvement of joints (Alagappan, 2011). 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 is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial

Socio-economic impact (Buch and Emery 2002)

Rheumatoid factor is an autoantibody directed against Immunoglobulin-G (IgG) and is found in the blood of 50-80% of patients with RA. Rheumatoid factor is an important prognostic marker. Those who test positive are more likely to have a worse prognosis with respect to joint destruction, physical/occupational disability, and quality of life in general (Rheumatoid Arthritis and Midlife Women). The symptom logical description of the clinical entity "Uthiravatha Suronitham" is identical to those of the symptoms described under the clinical diagnosis "Rheumatoid Arthritis (RA)" in modern science. About 1% of the world's population is affected by rheumatoid arthritis, women three times more often than men. It is 4 times more common in smokers than non-smokers. The incidence of RA is in the region of 3 cases per 10,000 populations per annum. Onset is uncommon under the age of 15 and from on the incidence rises with age until the age of 80 (Kore *et al.,* 2011). Amongst the various experimental animal models of arthritis, induction of arthritis by Freund's adjuvant is one of the standardized method which mimics the human patho-physiological state including chronic swelling in multiple joints due to accumulation of inflammatory cells, joint cartilage erosion, bone destruction and used to investigate the activity of various potent anti inflammatory and anti-arthritic agents (Singh and Majumdar, 1996).

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Presently many non-steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain; they are associated with certain undesirable side effects. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis (Rao *et al.,* 1999).

Arthritis Method

Over time, the gliding drugs are taken by more than 30 million people worldwide; of these, 40% of consumers are older than 60. Population studies have shown that 10–20% of all people who are 65 years or older either are currently receiving or have recently received a prescription for nonsteroidal anti-rheumatic drugs. During the next 20 years the number of people over 65 is expected to increase from 380 million to 600 million surface wears out every day anti-arthritic (URL: http://web.squ.edu.om/med-Lib/MED_CD/E_CDs/Surgery/ CHAPTERS/CH72.PDF). This revival of herbal and other complementary therapies in the management of chronic diseases (RA and other inflammatory disorders) is well documented (Eisenberg *et al.,*1998). The present study was carried out evaluating the safety of Pooneeru diravagam by determining toxicity after acute and sub-acute per oral administration in swiss albino rats.

Now it is a growing concern allover for the development of new safe, potent, less toxic antiarthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded (Kore *et al.,* ?). The present study was carried out to see the efficiency of Indian herbal source against a chronic inflammatory disease i.e. arthritis. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid diseases (Harris *et al*., 1990).

MATERIALS AND METHODS

AIM

Aim of the study is to evaluate the safety and efficacy of the Siddha drug 'Pooneeru Diravagam'

Drugs, Chemicals and Preparation of stock Solution

Prednisolone was obtained from Sigma Aldrich, USA. Incomplete Freund's adjuvant and Mycobacterium tuberculosis H37RA was obtained from DIFFCO Laboratories. CFA emulsified solution was prepared by triturating 1 mg of non-viable, desiccated Mycobacterium tuberculosis in 1 ml of Freund's adjuvant. Test drug were administered as solution form 0.2 and 0.4 ml to swiss albino rats. Prednisolone (10mg/kg, p.o) were used as standard drug and administered as CMC suspension by oral route. Negative control animal receives saline 1ml on day of immunization with adjuvant.

Animals

Wistar rats of either sex (100–150 g weight) were used in this study. The animals were housed in the poly vinyl chloride cages (PVC) cages at 22 ± 2°C with free access to pellet food and water. This study protocol was approved by the Institutional Animal Ethical committee (IAEC) No. CLBMCP /0731/01/1415, C.L.Baid metha foundation for pharmaceutical education and research, Jyothi nagar, old mahabalipuram road, Thorapakkam, Chennai-96, Tamil Nadu, India

CFA-Induced Arthritis in Rats

Each treatment group contained six wistar rats. The rats were randomly divided into four groups: CFA control, Pooneeru diravagam (0.2 ml kg−1 day−1, p.o.), Pooneeru diravagam (0.4 ml kg−1 day−1, p.o.), and Prednisolone (10 mg kg−1 day−1, p.o.). On day 0, arthritis was induced by injection of 100 μL CFA, which contains heat-killed and dried Mycobacterium tuberculosis into the paw of the left hind limb of each rat. The Severity of Arthritis was measured with the consideration of the primary and secondary lesions, by paw volumes of injected and non-injected paws, were evaluated using a plethysmometer, after adjuvant administration (KalpeshRamdasPatil *et al.,* 2011). After the induction of arthritis (day 0), the increase in paw volume was calculated. The lesions were measured again on the 7th, 14th, and 21st days after injection of the adjuvant (Singh *et al.,* 2003; Bani *et al.,* 2007). The severity of arthritis was recorded by a blinded observer using the visual arthritis scoring systems (Kumar *et al.,* 2006; Laird *et al.,* 2001).

Statistical Analysis

The results are expressed as the mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnet's multiple comparisons test. Data were considered statistically significant if $P<0.05$.

RESULTS

Observations such as the paw volumes, Paw thickness were recorded on the 1st, 4th, 7th, 10th, and 14th days after adjuvant injection. Finally Histopathological analysis of efficacy study was carried out on 15th day. The CFA-induced arthritis control group showed signs of arthritis development, as seen by the increase in the paw volumes in both CFA-injected and CFA-non-injected paws, which indicates primary and secondary arthritic lesions (barto). Other indications, such as a paw thickness and alterations in the arthritis scores, also showed induction of arthritis in the CFA-treated control group rats. The assessment made on the 14th day showed that the standard drug Prednisolone and Pooneeru diravagam treatments had significantly reduced the adjuvant-induced primary and secondary lesions in the respective treatment groups as compared with the CFA control group. It is noteworthy that the reduction in the secondary lesions was comparable in the Prednisolone treated and Pooneeru diravagam 0.4 ml/kg treated groups. Pooneeru diravagam treatment favorably affected the pain scores, indicating a marked decrease in the pain associated with the adjuvant-induced arthritis. All the estimated pain

scores, including the flexion pain test score, mobility score and stance score were significantly altered in Prednisolone treated and Pooneeru diravagam (0.2 ml/kg) treated rats. The reduction in the mobility score was greater in the Pooneeru diravagam (0.4 ml/kg) treated group as compared with the standard drug treated group.

It is clearly observed in the Paw volume, thickness and histopathology of joint that the soft tissue swelling around the joints, periarticular bone resorption, periarticular bony erosions and joint space narrowing in the rats treated with Pooneeru diravagam have been protected from the CFA-induced arthritis-related joint changes. The left hind paw volumes of all animals were measured just before CFA injection on day 0 and thereafter at different time intervals till day 14 using a mercury plethysmograph. The change in paw volume was measured as the difference between the final and initial paw volume.

pathogeneses of inflammation but also explore the anti-inflammatory mechanisms as well as to identify the suitability of drugs for specific inflammatory disease. Inflammatory response protects the body by triggering innate and acquired immunity under conditions such as tissue damage and infections, but chronic inflammatory responses can result in diseases such as cardio-vascular disease, diabetes, pulmonary disease and Rheumatoid Arthritis (D'Arcy *et al.,* 1960). The Freund's complete adjuvant (FCA) induced arthritis model in rats is the most common model. This preclinical model predicted the activities of a number of compounds that are currently used in the treatment of rheumatoid arthritis are being tested in clinical trials. There are 4 phases of arthritis on the basis of biochemical markers of arthritis (1) Day 1-4 with acute local inflammation and systemic effects (liver), (2) Days 7-12 with remission of acute inflammation and periarthritis, (3) Days 12-28 with chronic inflammation, periarthritis and osteogenic activity,(4) Day 35 onwards (indefinitely) with

Paw volume (ml) was measured on days /Mean Displacement Value(ml)						
	0 day	1 day	4 day	7 day	10 day	14 day
Negative control treated)						
Meannnn	0.2483	0.9633	1.032	1.055	1.06	1.083
Std. Deviation	0.03061	0.02066	0.01169	0.005477	0.01095	0.005164
Std. Error	0.01249	0.008433	0.004773	0.002236	0.004472	0.002108
Prednisolone (10mg/kg) p.o						
Mean	0.2367	0.57	0.53	0.4533	0.3217	0.28
Std. Deviation	0.01862	0.008944	0.008944	0.02422	0.01941	0.01095
Std. Error	0.007601	0.003651	0.003651	0.009888	0.007923	0.004472
low dose 0.2 ml						
Mean	0.275	0.8533	0.7467	0.6467	0.5833	0.565
Std. Deviation	0.01049	0.02944	0.02582	0.0216	0.01506	0.01761
Std. Error	0.004282	0.01202	0.01054	0.008819	0.006146	0.007188
High dose 0.4 ml						
Mean	0.28	0.7567	0.57	0.46	0.375	0.345
Std. Deviation	0.006325	0.02658	0.01549	0.008944	0.005477	0.01871
Std. Error	0.002582	0.01085	0.006325	0.003651	0.002236	0.007638

Mean changes in paw volume using mercury Plethysmometer in adjuvant-induced arthritis in rats n=6, values are expressed as mean ± SEM

Paw thickness measurement

The joint diameters of left hind paw were measured using a Vernier caliper on the above mentioned testing days after induction of arthritis.

	PAW THICKNESS BY VERNIER CALIPER					
TREATMENT	0 day	1 day	4 day	7 day	10 day	14 day
control (un treated)						
Mean	0.4533	0.9633	1.06	1.143	1.162	1.175
Std. Deviation	0.01751	0.02658	0.01897	0.01751	0.02787	0.01643
Std. Error	0.007149	0.01085	0.007746	0.007149	0.01138	0.006708
standard Prednisolone 10mg/kg						
Mean	0.4567	0.87	0.785	0.7267	0.6667	0.515
Std. Deviation	0.01966	0.02366	0.01517	0.01966	0.01633	0.01643
Std. Error	0.008028	0.009661	0.006191	0.008028	0.006667	0.006708
LOW DOSE (0.2 ml)						
Mean	0.4433	0.98	1.037	0.9483	0.85	0.7483
Std. Deviation	0.01506	0.008944	0.02338	0.02401	0.02	0.01169
Std. Error	0.006146	0.003651	0.009545	0.009804	0.008165	0.004773
HIGH DOSE (0.2 ml)						
Mean	0.4517	0.925	0.9117	0.855	0.7433	0.62
Std. Deviation	0.01722	0.01643	0.01169	0.01049	0.01751	0.008944
Std. Error	0.007032	0.006708	0.004773	0.004282	0.007149	0.003651

DISCUSSION

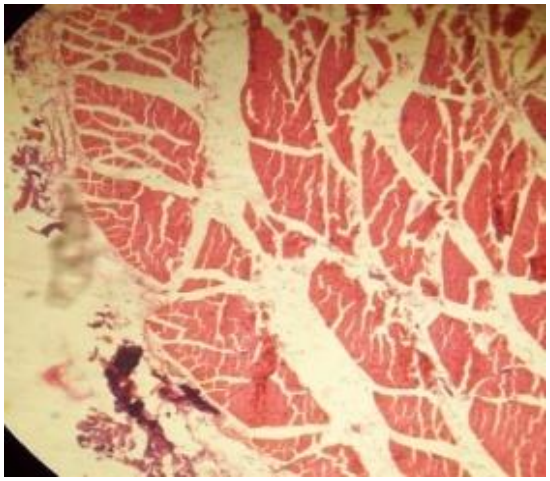
Numerous experimental methods for evaluation of anti-inflammatory drugs have been developed over the last few years. These methods help not only understanding the

permanent articular deformity and minimal (burnout) inflammation. A general increase in 5- HT synthesis within the whole central nervous system during the acute phase of the disease (2-3 weeks post inoculation) with a specific, further

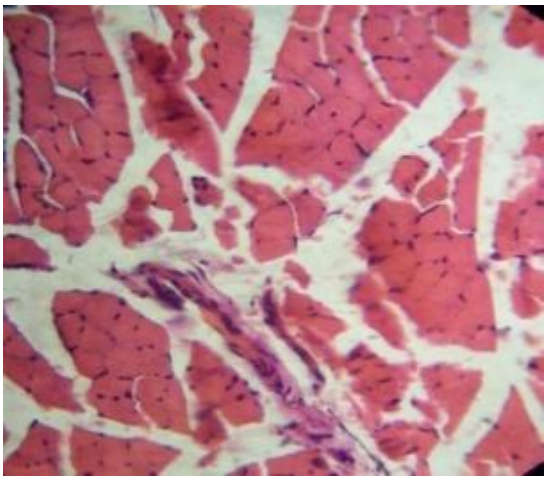
enhancement restricted to the spinal cord during the post acute phase (4-6 weeks post inoculation) (Chitme *et al*., 2009).

was more potent than that of prednisolone. This reveals potent suppression by PD of cell-mediated immunity in arthritic rats. Similarly, it reduced the arthritic score and secondary paw

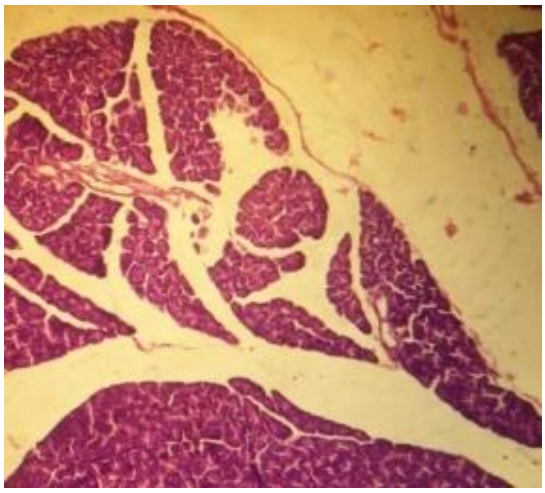
Histopathological Analysis of Efficacy Study



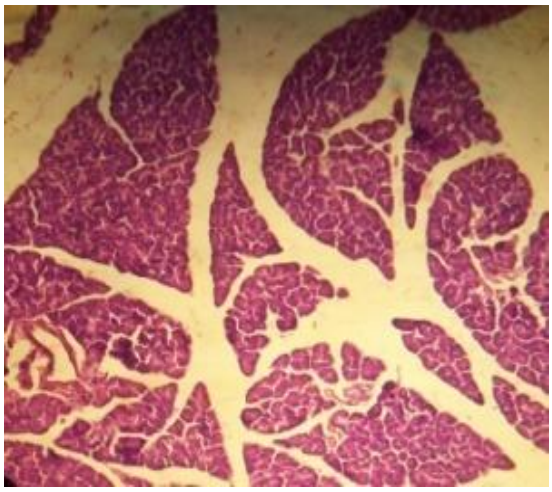
Negative control



Low dose



High dose



Standard

The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The Freund's adjuvant model is chosen as it develops chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-alpha), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability (Lam *et al*., 2004). However standard drug and Pooneeru diravagam significantly suppressed the swelling of the paws and also decreases the paw volume in both acute and chronic phase which may be due to the suppression of inflammatory mediator released due to induction of Freund's adjuvant.

Pooneeru diravagam effectively reduced the secondary lesions in arthritic rats. Moreover, this effect of Pooneeru diravagam

swelling. A selective reduction in the arthritis score distinguishes the immunosuppressive effects of a drug from its anti-inflammatory effects. The reduction of the arthritis score by Pooneeru diravagam was observed in our study indicates a possible immune suppressant effect. CFA-induced arthritis in rats is associated with an increase in the plasma levels of RF and CRP.

The treatment with Pooneeru diravagam shows marked reduction in the levels of these biomarkers of inflammation and autoimmune stimulation in the treated rats. This study includes examination of the paws, paw thickness measurement, paw withdrawal latency and Histopathological analysis with the standard drug prednisolone. The visual observations of the rats show that the treatment with Pooneeru diravagam and Prednisolone inhibited the arthritis-associated joint changes. In the PD and Prednisolone treated groups there was restoration of the body weights in rats. The evidence of restoration of the body weight in rats with PD and

prednisolone treated groups may improve the absorption of nutrients from the intestine and reduce the distress caused by the arthritis. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions (Tripathy *et al*., 2010). Effect of Pooneeru diravagam in complete Freund's adjuvant induced arthritic rats. Challenge with CFA (0.1ml) shows development of paw edema which reached peak edema on 21st day of injection. Prednisolone treated group shows significant inhibition of paw edema on day 4^{th} ($P<0.05$), 7^{th} ($P<0.01$), 10th ($P<0.001$) and day 14^{th} ($P<0.001$). PD (0.2ml/kg) shows significant inhibition of paw edema on day 14th and day 21st with $P<0.01$. Also rats treated with PD (0.4ml/kg) shows significant inhibition of paw edema on day 4^{th} ($P<0.05$), 7^{th} ($P<0.05$), 10^{th} ($P<0.01$) and day 14^{th} ($P<0.01$).

Paw thickness was increased up to 7th day of adjuvant induction and after that it slightly decreased. Prednisolone treated group shows significant inhibition of paw diameter on day 7^{th} ($P<0.01$), 10^{th} ($P<0.001$) and day 14^{th} ($P<0.001$). Pooneeru diravagam (0.2ml/kg) shows significant inhibition of paw diameter on day 10^{th} and day 14^{th} with $P<0.01$. Also rats treated with Pooneeru diravagam (0.4ml/kg) shows significant inhibition of paw diameter on day 10^{th} and day 14^{th} with $P<0.01$.

Conclusion

On the basis of the results obtained in this study we conclude and propose that possibly, the potent anti-arthritic effect of *Pooneeru diravagam* may maintain the synovial membrane and inhibiting cytokines and leukotriene infiltration inhibition as evidenced through paw edema volume and paw thickness measurement. This in turn, protecting synovial membrane and thereby improving the health status through its anti-arthritic property. In the present study, based on the above result it can be concluded that the Pooneeru diravagam treatment at a dose of 0.2 and 0.4 ml/kg body wt. significantly inhibit the progression of the arthritis in experimental animal models. So, this potentially a promising drug and can be used clinically with the doses employed in this study.

Acknowledgement

1. I express my sincere heartfelt thanks to my Guide. Dr. M.MOHAMED MUSTHAFA, M.D(S), Head of the Department, Post graduate Sirappu Maruthuvam, Government Siddha medical college and hospital, Chennai-106, for his worthwhile eminent guidance, marvellous masterpiece the development and inching me towards excellence with every endeavour.
2. I express my sincere thanks to Dr.MURALIDHARAN and Dr.SIVARAMAN Department of pharmacology, Institutional Animal Ethical Committee (IAEC), C.L.Baidmetha college of pharmacy, Thorappakkam, Chennai, for their excellent help in toxicological and pharmacological activities in this study.
3. My heartfelt thanks to D.P.S.Thiruvarul kamala Arumugam M.D(S) for his great and valuable support throught this study.
4. My special thanks to my lovable Parents, Brother and sister-in-law for giving me full Co-Operation and Moral support from the very beginning of my career.

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