Int J Trans Res Ind Med 2019, Sep – Dec; 1(3): (P) 31- 36

Research Article

ISSN 2582-0109

International Journal of Translational Research in Indian Medicine
www.ijtrim.com
Volume 1, Issue 3 – 2019

SCREENING OF ANTI-ARTHRITIC POTENTIAL OF SIDDHA FORMULATION RASA CHENDOORAM ON COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS IN WISTAR RATS

S. Sabarmathi [*1], V.Sudha [2], N.Anbu [3], D.Sivaraman [4]

*[*1&2] P.G Scholar, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.*
[3] Head, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.
[4] Scientist, Centre for Laboratory Animal Technology and Research, Col.Dr.Jeppiaar Research Park, Sathyabama Institute of Science and Technology , Jeppiaar Nagar, Rajiv Gandhi road, Chennai - 600 119,Tamil Nadu, India.

ABSTRACT

Rheumatoid arthritis (RA) is a common multisystem autoimmune disease characterized by chronic inflammation in particular synovial tissue and associated with over production of proinflammatory cytokines. Drugs commonly used for the treatment of inflammation and arthritis include glucocorticoids like cortisone and prednisone etc., NSAIDS like Ibuprofen and naproxen etc., disease-modifying anti-inflammatory and anti-rheumatic drugs like Methotrexate (MTX) and leflunomide etc., It has been reported through several research that chronic usage of conventional medication offers numerous side effects, severe adverse reactions and toxicity, including some risk of infections in clinical level who are being treated with biological response modifiers. Primary factors that influence the people to shift the paradigm towards traditional medicines is Increases rate of treatment failure with conventional medicines. Siddha system of medicine has greater number of potential formulations for special focus of treatment towards chronic arthritis one such drug listed in the literature is rasa chendooram (RC). The main aim of the present study is to evaluate the ant-arthritic potential of the siddha formulation rasa chendooram in complete freund's adjuvant (CFA) induced arthritis model. CFA induced arthritis is a scientifically justified standard experimental procedure for the induction of chronic immune-pathological RA in laboratory animals with similar cellular immunity response and pathological mechanism as in human. Results of the study indicates that the siddha drug RC significantly alleviated arthritic swelling (paw volume and thickness) and also shown reduction in arthritic score in treated group. These results were well justified by the radiological and histopathological outcomes. Hence it was concluded from the data's of the investigation that the formulation RC may be an excellent drug of choice in treating rheumatoid arthritis even at clinical level with proper validation.

KEY WORDS: ***Autoimmune disease, Rheumatoid arthritis, Siddha system, Rasa chendooram, Complete freund's adjuvant, Arthritic score, Radiology, Histopathology***

Corresponding Author: S. Sabarmathi
Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
Email: sabarmathi44@gmail.com

1. Introduction

Immune system of our body plays a crucial role, as an overactive immune system may lead to certain fatal disease because of various hypersensitive or allergic reactions which may cause numerous derangements; loss of normal capacity to differentiate self from non-self-resulting in immune reactions against our own's cells and tissues called autoimmune diseases. Certain common autoimmune diseases like myasthenia gravis, serum sickness, pernicious anemia, reactive arthritis etc., are the severe issues for medical and pharmaceutical community because of unknown etiology [1]. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the disease as compared to males [2]. RA is a chronic, inflammatory, and systemic autoimmune disease [3].

Uncontrolled RA characterized by progressive damages of synovial, cartilage, and bone is associated, probably, with extra-articular signs [4,5]. RA may possibly progress to severe disability with direct negative impacts on life style and increase in mortality rate [6]. The overall prevalence of clinically diagnosed RA was 0.5–2% of the population with higher prevalence in developed countries [7,8].All ages are susceptible to develop RA, but the incidence increased significantly in people aged over 40 years, especially women who are two to three times more susceptible to RA than men [9].

Currently, RA treatment primarily includes a combination of patient education, rest and exercise, joint protection, medication and occasionally surgery [10]. Medication for RA includes nonsteroidal anti-inflammatory and disease modifying antirheumatic drugs, as well as T-cell activation inhibitors, B-cell depleters, tumor necrosis factor (TNF)-α inhibitors, interleukin (IL)-6 inhibitors and Janus kinase (JAK) inhibitors.

Disease modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunomide, hydroxychloroquine, and corticosteroids like prednisolone, methylprednisolone have all been associated with adverse effects. Because of this reason, patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief and are amongst the highest users of complementary and alternative medicine [11]. Rasa chendooram is a potential siddha formulation which consist of active ingredients that includes Rasam (Hydragyram), Gandhagam (Sulphur) and Lime juice. This preparation is advocated for treating various disorders. But still now not explored for its anti-rheumatic property. Hence The main objective of the present investigation is to evaluate the anti- arthritic potential of the formulation RC using CFA induced arthritic rat model.

2.Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 220-240 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬+ 2o C and relative humidity 50–65%. They were provided with food and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/X/091/2018.

2.2. Experimental Design and Induction of arthritis [12]

The animals were grouped into three groups of 6 animals each. Group I (Right paw normal control and left paw serves as arthritic control) -received normal saline, Group II (low dose treatment group) animal received 0.1 ml of freund's adjuvant into the left hind and treated with 100 mg/kg of RC from day 1 to 21. Group III (High dose treatment group) animal received 0.1 ml of freund's adjuvant into the left hind and treated with 200 mg/kg of RC from day 1 to 21

2.3. Measurement of Paw volume [13]

Paw volume and Paw thickness will be measured on 0, 7th, 14th, and 21st, days by using Plethysmometer and verneir caliper respectively. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days.

2.4. Measurement of Paw Edema [14]

Paw thickness was used as a measurement of inflammation-induced edema. Briefly, the dorsoventral thickness of each hind paw was measured

using a caliper placed at the border of the phalanges and metatarsals. The measurement was taken when each edge of the caliper was just touching the dorsal and ventral surface of the hind paw

2.5. Assessment of Arthritic Score [15]

0 = no edema or swelling, 1 = slight edema and limited erythema, 2 = slight edema and erythema from the ankle to the tarsal bone, 3 = moderate edema and erythema from the ankle to the tarsal bone, and 4 = edema and erythema from the ankle to the entire leg.

2.6. Histopathological Analysis [16]

At the end of the study period animals were euthanized with high dose of anesthetic agents and the hind paws of control and experimental rats was dissected out and fixed in 10% buffered neutral formal saline and processed. Bone samples were immersed in PLP fixative (2% paraformaldehyde containing 0.075 M lysine and 0.01 M sodium periodate solution, pH 7.4) at 4ºC. These were then subsequently demineralized with 10 per cent EDTA solution and dehydrated with increasing concentration of ethanol before being embedded in paraffin. The paraffin blocks were then placed in microtome and 5 µm transverse sections were obtained

2.4. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3.Results

3.1. Effect of RC on Paw volume of arthritis and treatment group rats

It was observed that there was a significant increase in the paw volume of CFA injected paws when compare to the normal control paw, which denotes the induction of arthritis characterized by swelling and edema. Treatment with RC at both the dose level have shown significant decrease in the paw volume and paw edema in the peak threshold time of 14th to 21st day. As shown in Table 1.

Table 1: Effect of RC on Paw volume of arthritis and treatment group rats

Group	Paw Volume in ml			
	0th Day	7th Day	14th Day	21st Day
Control Paw	0.93 ± 0.06	0.9 ± 0.04	0.88 ± 0.05	0.91 ± 0.04
CFA-Arthritic Control Paw	0.96 ± 0.06	2.033 ± 0.08*	2.45 ± 0.10*	2.75 ± 0.05*
CFA+ 100 mg/kg RC	1 ± 0.07	1.45 ± 0.06*	1.967 ± 0.04*	1.8 ± 0.07*
CFA+ 200 mg/kg RC	0.96 ± 0.09	1.25 ± 0.04*	1.65 ± 0.05*	1.48 ± 0.03*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.2. Effect of RC on Paw edema of Freund's adjuvant induced Arthritis

It was observed that there was a significant increase in the paw thickness of CFA injected paws when compare to the normal control paw, which denotes the induction of edema and swelling. Treatment with RC at both the dose level have shown significant decrease in the paw edema level. As shown in Table 2.

Table 2: Effect of RC on Paw edema of Freund's adjuvant induced Arthritis

Group	Paw Thickness in mm			
	0th Day	7th Day	14th Day	21st Day
CFA-Arthritic Control Paw	1.8 ± 0.05	3.5 ± 0.11	5.1 ± 0.08	5.4 ± 0.08
CFA+ 100 mg/kg RC	1.517 ± 0.07	2.833 ± 0.07*	3.2 ± 0.10*	2.55 ± 0.10*
CFA+ 200 mg/kg RC	1.583 ± 0.08	2 ± 0.10*	2.783 ± 0.11*	2.317 ± 0.13*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.3. Effect of RC on Arthritic score

It was observed that there was a significant increase in the arthritic score of CFA injected paws when compare to the normal control paw. Treatment with RC at both the dose level have shown significant decrease in the arthritic score. As shown in Table 3.

Table 3: Effect of RC on Arthritic score

Group	Arthritic Assessment score			
	0th Day	7th Day	14th Day	21st Day
CFA-Arthritic Control Paw	3.217 ± 0.09	4.55 ± 0.20	6.533 ± 0.20	7.817 ± 0.22
CFA+ 100 mg/kg RC	3.25 ± 0.13	4.217 ± 0.09*	6.05 ± 0.14*	5.067 ± 0.11*
CFA+ 200 mg/kg RC	3.467 ± 0.08	3.883 ± 0.10*	5.717 ± 0.17*	4.683 ± 0.23*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.4. Effect of RC on Radiological changes in arthritis and treatment group rats

X-ray radiographic image of control group rats reveal integrated join with normal morphology. Sub-chondral erosion on joints was observed in arthritic control group. Treatment with trial drug RC at the dose of 100 and 200 mg/kg shown significant reduction in joint swelling with reversal in bone and tissue morphology. As shown in figure 1.

Figure 2: Effect of RC on Radiological changes in arthritis and treatment group rats

3.5. Effect of RC on Histologic pathological analysis of arthritic control, arthritis and treatment group rats

Histological results have revealed that Prominent histology of Synovial membrane with regular arrangement of cartilage and bone architecture were observed in normal control rat paw. Sample belongs to arthritis control paw has shown induction of arthritis with well-characterized synovial hyperplasia bone destruction in the joint. Mild cartilages destruction with restored histology of synovium were observed in sample belongs to group III. Microscopic observation of sample belongs to group IV exhibits synovial membrane and regular cartilage; the surface of the cartilage in the tibia and femur. As shown in figure 2.

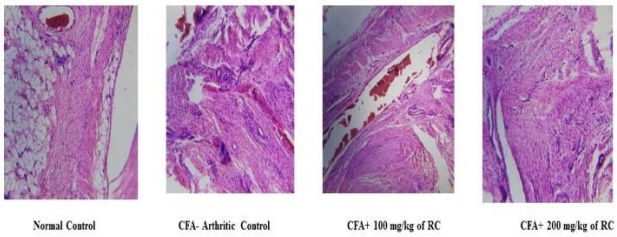

Figure 2: Effect of RC on Histopathological changes of rat paw of control arthritis and treatment group rats

4.Discussion

Numerous biological and non-biological disease-modifying anti-rheumatic drugs have been used in the treatment of RA [17]. In some patients, the best therapeutic efforts do not produce the expected results and are often associated with numerous complications and serious side effects [18]. Therefore, the search for new treatment options continues today. Although a number of drugs have been developed and used for the treatment of RA, such as non-steroidal anti-inflammatory drugs (NSAIDs, e.g., Nimesulide), disease-modifying anti-rheumatic drugs (DMARDs, e.g., Leflunomide) and biological drugs (e.g., Abatacept), they are too expensive, and have selective efficacy and potential unknown threat [19,20]. Traditional medicine has greater advantage of treating chronic illness like RA as its not only efficacious but also available at low cost and with less or no side effects

Freund's complete adjuvant induced arthritis model are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics and this model is characterized by a very rapid erosive disease. The bacterial peptidoglycan and muramyl dipeptide present in the CFA are responsible for the induction of adjuvant arthritis [21,22]. It was observed from the present study that there was a significant increase in the paw volume and thickness of CFA injected paws when compare to the normal control paw, which denotes the induction of arthritis characterized by swelling and edema. Treatment with RC at both the dose level have shown significant decrease in the paw volume and paw edema in the peak threshold time of 14th to 21st day.

Results of arthritic assessment scoring reveals the induction severity of arthritis in CFA injected paws with the maximum scoring of 3.8. Treatment with RC

at both the dose level have shown significant decrease in arthritic scoring to 1.6 which signifies the anti-arthritic potential of the formulation RC in the experimental animals

Reduced bone formation and increased bone resorption are a major cause of bone destruction in adjuvant-induced arthritis in rats. In this regard, the radiographic images may give a clear understanding of disease status and its remission. X-ray radiographic image of control group rats reveal integrated join with normal morphology. Sub-chondral erosion on joints was observed in arthritic control group. Treatment with trial drug RC at the dose of 100 and 200 mg/kg shown significant reduction in joint swelling with reversal in bone and tissue morphology.

In RA release of free radicals may induce the production of interleukins (IL) and tumor necrosis factor (TNF-α) from T-cells which ultimately influence the production of growth factors, cytokines and adhesive molecules on immune cells as such factors may cause tissue destruction and inflammation. Pathological changes in RA are hyperplasia of synovial membrane, infiltration of inflammatory cells and neovascularization, which results into cartilage erosion and articular destruction [23]. In the present investigation prominent histology of Synovial membrane with regular arrangement of cartilage and bone architecture were observed in normal control rat paw. Sample belongs to arthritis control paw has shown induction of arthritis with well-characterized synovial hyperplasia bone destruction in the joint. Mild cartilages destruction with restored histology of synovium were observed in sample belongs to group III. Microscopic observation of sample belongs to group IV exhibits synovial membrane and regular cartilage; the surface of the cartilage in the tibia and femur.

5.Conclusion

Rheumatoid arthritis RA is a chronic, inflammatory condition of unknown etiology, affecting approximately 1% of the general population. It is a progressive, disabling, chronic multisystem disease that is characterized by pain, swelling and stiffness of the synovial joints. In certain cases, due to consistent failure and huge expense involved in conventional therapy routed people towards alternative medicines. Based on the results of the present study it was observed that the siddha drug rasa chendooram shown significant improvement in alleviating arthritic swelling and also reduction in arthritic score in treated group. These results were well justified by the radiological and histopathological results. Hence it was concluded from the outcome of the study that the formulation like rasa chendooram may be good drug of choice in treating chronic inflammatory disease like rheumatoid arthritis at clinical level.

Acknowledgement

I wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

6. References

1. Chitme RH, Patel PN. Antiarthritis activity of Aristolochia bracteata extract in experimental animals. Open Natl Pro J. 2009;2:6–15.
2. Tripathy S, Pradhan D, Anjana M. Anti-inflammatory and antiarthritic potential of Ammania baccifera Linn. Int J Pharm Bio Sci. 2010;1:1–7.
3. Chunxia C, Peng Z, Huifang P, Hanli R, Zehua H, Jizhou W. Extracts of Arisaema rhizomatum C.E.C. Fischer attenuate inflammatory response on collagen-induced arthritis in BALB/c mice. J Ethnopharmacol. 2011;133:573–82.
4. Shi F., Zhou D., Ji Z., Xu Z., Yang H. Anti-arthritic activity of luteolin in Freund's complete adjuvant-induced arthritis in rats by suppressing P2X4 pathway. Chem Biol Interact. 2015;226:82–87.
5. Bhalekar M.R., Upadhaya P.G., Nalawade S.D., Madgulkar A.R., Kshirsagar S.J. Anti-rheumatic activity of chloroquine-SLN gel on wistar rats using complete freund's adjuvant (CFA) model. Indian J Rheumatol. 2015;10:58–64.
6. Gomes R.P., Bressan E., Silva T.M., Gevaerd S.M., Tonussi C.R., Domenech S.C. Standardization of an experimental model suitable for studies on the effect of exercise on arthritis. Einstein (São Paulo) 2013;11:76–82.
7. Kapetanovic M., Lindqvist E., Geborek P., Saxne T., Eberhard K. Long-term mortality rate in rheumatoid arthritis patients with disease onset in the 1980s. Scand J Rheumatol. 2011;40:433–438.

8. Goulielmos G.N., Zervou M.I., Myrthianou E., Burska A., Niewold T.B., Ponchel F. Genetic data: the new challenge of personalized medicine, insights for rheumatoid arthritis patients. Gene. 2016;583:90–101.
9. Crowson C.S., Matteson E.L., Myasoedova E., Michet C.J., Ernste F.C., Warrington K.J. The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheum. 2011;63:633–639.
10. Xie QC and Yang YP: Anti-proliferative of physcion 8-O-β-glucopyranoside isolated from Rumex japonicus Houtt. on A549 cell lines via inducing apoptosis and cell cycle arrest. BMC Complement Alter Med. 14:3772014.
11. Cronan TA, Kaplan RM, Posner L, Blumberg E, Kozin F. Prevalence of the use of unconventional remedies for arthritis in a metropolitan community. Arthritis Rheum. 1989;32:1604–7.
12. Kore K.J. Anti-Arthritic activity of Hydroalcoholic extract of Lawsonia Innermis. International Journal of Drug Development & Research. 2011, 3 (4): 217-224
13. Nair V., Singh S., Gupta Y. Evaluation of disease modifying activity of Coriandrum sativum in experimental models. Indian J Med Res. 2012;135:240–248.
14. Vijayalaxmi A., Bakshi V., Begum N. Anti-arthritic and anti inflammatory activity of beta caryophyllene against Freund's complete adjuvant induced arthritis in wistar rats. Bone Rep Recomm. 2015;1:1–10.
15. Abdin AA, Abd El-Halim MS, Hedeya SE, El-Saadany AA. Effect of atorvastatin with or without prednisolone on Freund's adjuvant induced-arthritis in rats. Eur J Pharmacol. 2012 ;676(1-3):34-40.
16. Suvarna, S.K., C.Layton and J.D. Bancroft. Bancroft’s theory and practice of histological techniques. 7th edn, Churchill Livingstone, London.2013.
17. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res . 2006;37:425–435.
18. Firestein GS. Kelley’s Textbook of Rheumatology. Philadelphia: Saunders; 2005. Etiology and pathogenesis of rheumatoid arthritis.2005:996–1042.
19. Payne R. Limitations of NSAIDs for pain management: toxicity or lack of efficacy? J Pain. 2000;1:14–8.
20. Shanahan JC, Moreland LW, Carter RH. Upcoming biologic agents for the treatment of rheumatic diseases. Curr Opin Rheumatol. 2003;15:226–36.
21. Petchi RR, Vijaya C, Parasuraman S. Anti-arthritic activity of ethanolic extract of Tridax procumbens (Linn.) in Sprague Dawley rats. Pharmacognosy Res. 2013;5:113–7.
22. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J Pharmacol Chemother. 1963;21:127–36.
23. Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauses SL, Jameson JL. 16th ed. II. United States of America: Mc-Graw Hill Companies; Harrison's Principle of Internal Medicine. 2005.

How to cite this Article

S. Sabarmathi , V.Sudha , N.Anbu , D.Sivaraman. Screening of Anti-Arthritic potential of Siddha formulation Rasa Chendooram on Complete Freund's adjuvant induced Arthritis in wistar rats.Int J Trans Res Ind Med .2019; 1(3): 30- 35.