Int J Trans Res Ind Med 2022, 4(2): (P) 12 - 17

Research Article

ISSN 2582-0109

International Journal of Translational Research in Indian Medicine
www.ijtrim.com
Volume 4, Issue 2 – 2022

INVESTIGATION OF IMMUNOMODULATORY AND ANTI-ARTHRITIC POTENTIAL OF SIDDHA FORMULATION GURU PARPAM USING COMPLETE FREUND'S ADJUVANT (CFA) INDUCED ARTHRITIS IN WISTAR RATS

K.Pavithra [1], M.G.Anbarasi [2], N.Anbu [3]

[1,2] P.G.Scholar, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai-600106, Tamil Nadu, India.
[3] Professor and Head of the Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai- 600106, Tamil Nadu, India.

ABSTRACT

Polyarthritis (PA) refers to a joint ailment that affects at least five joints. One or more indications of inflammation, including discomfort, mobility limitation, edema, warmth, and redness, are present in the joints affected. In the case when pain is the sole symptom, it is difficult to identify polyarthritis from the causes of polyarticular joint pain (PJP), such as fibromyalgia or osteoarthritis. Conventional medicine for management of PA offers potential life threatening side effects. Hence exploration of drugs from alternate complementary medicine expected to imparts significant outcomes in managing chronic inflammatory disease like PA. The main aim of the present preclinical investigation is to evaluate the anti-arthritic efficacy of the siddha formulation Guru Parpam (GP) on freund's adjuvant induced arthritis in wistar rats. Siddha formulation GP 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. Treatment with GP at both the dose level have shown significant decrease in arthritic scoring to 1.6 which signifies the anti-arthritic potential of the formulation GP in the experimental animals. X-ray radio graphical image of normal control paw of rat's projects integrated join with normal morphology. Sub-chondral erosion on joints was observed in arthritic control paw. Treatment with trial drug GP at the dose of 250 and 500 mg/kg shown significant reduction in joint swelling with reversal in bone and tissue morphology. In conclusion of the siddha drug Guru Parpam could be considered as drug of choice in PA in near future.

KEY WORDS: ***Arthritis, Siddha, Guru Parpam, Freund's adjuvant, Joints, Paw edema, Paw volume***

Corresponding Author: K.Pavithra, P.G.Scholar, Department of General Medicine, Government Siddha Medical College, Arumbakkam, Chennai- 600106, Tamil Nadu, India

1. Introduction

Arthritis is an umbrella term for more than 100 chronic, painful, and debilitating arthritic illnesses. Arthritis, notably osteoarthritis, is a substantial contribution to the worldwide disability burden, and the number of years of disability related to arthritis grew by 75 percent between 1990 and 2013 [1], demonstrating that this illness is a developing concern internationally. Combined with a rising trend in the frequency of arthritis [2].It has a detrimental effect on life and restricts people's movement [3, 4]. In persons with arthritis, joints distort due to increased volume and loss of function, as well as cartilage and bone degradation.

Autoimmunity and chronic inflammation are triggered in polyarthritis (PA) by an imbalance of pro- and anti-inflammatory cytokines [5]. PA is defined by synovial membrane angiogenesis, which contributes to disease progression, as well as the generation of inflammatory cells that infiltrate and damage synovial tissue [6]. Cytokines produced from macrophages and fibroblasts increase the production of both cytokines and chemokines in arthritis [7]. Interleukin 1 beta (IL-1) and tumor necrosis factor alpha (TNF-) are frequently targeted in ploy arthritis therapeutic techniques [8].

Immunology is critical for understanding rheumatic disorders [9]. Monocytes, mast cells, macrophages, polymorphonuclear cells, and fibroblast synoviocytes are all present in joint synovium [10]. Activated macrophages and synoviocytes release significant quantities of proteases, IL-1, and TNF. These cytokines play a significant role in the cascades of information transmission between inflammatory cells in arthritis synovitis [11].

Therapeutic options for arthritis include NSAIDS (Non-Steroidal Anti-Inflammatory Drugs) such as indomethacin, aceclofenac, phenylbutazone, and ibuprofen, as well as DMARDs (Disease Modifying Anti-Rheumatic Drugs) such as cyclosporin A and methotrexate. Anticytokine treatment, such as infliximab, adalimumab, and others, as well as immune suppressive medications, are frequently used to decrease inflammation and discomfort in arthritis. Despite of the clinical efficacy majority of the aforementioned drugs invites potential undesirable side effects upon long term usage [12].

The Siddha medicine pioneered the treatment of inflammation and other degenerative conditions; the majority of Siddha prperation are made up of minerals, metals and botanical substances with new therapeutic properties. Siddha supplements are well-known for their high safety index, as well as the fact that vibrant ingredients of the formulation expected to enhance the healing system by appropriately boosting the cellular biochemical route. The main aim of the present preclinical investigation is to evaluate the anti-arthritic efficacy of the siddha formulation Guru Parpam (GP) on freund's adjuvant induced arthritis in wistar rats

2. Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats weighing between 200-230 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 (Sai feeds, Bangalore, India) 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. SU/CLATR/IAEC/XVII/182/2021

2.2. Experimental Design and Induction of arthritis [13]

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 250 mg/kg of GP 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 500 mg/kg of GP from day 1 to 21.

2.3. Measurement of Paw volume [14]

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 [15]

Cross 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 [16]

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 [17]

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.

2.7. 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 GP on Paw volume of arthritis and treatment group rats

It was observed 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 GP 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 GP on Paw volume of arthritis and treatment group rats

Group	Paw Volume in ml			
	0th Day	7th Day	14th Day	21st Day
Control Paw	0.8667 ± 0.03	0.8267 ± 0.04	0.8733 ± 0.07	0.81 ± 0.05
CFA-Arthritic Control Paw	0.8933 ± 0.03	2.11 ± 0.05*	2.56 ± 0.06**	2.905 ± 0.05*
CFA+ 250 mg/kg GP	0.9517 ± 0.05	1.853 ± 0.08*	2.05 ± 0.09*	1.737 ± 0.07*
CFA+ 500 mg/kg GP	0.7533 ± 0.04	1.69 ± 0.12*	1.918 ± 0.09*	1.488 ± 0.07*

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

3.2. Effect of GP on Arthritic score

Results of arthritic assessment scoring reveals the induction severity of arthritis in CFA injected paws with the maximum scoring of 3.6. Treatment with GP at both the dose level have shown significant decrease in arthritic scoring to 1.6 which signifies the anti-arthritic potential of the formulation GP in the experimental animals. As shown in table 2.

Table 2: Effect of GP on Arthritic score of experimental rats

Group	Paw Thickness in mm		
	7th Day	14th Day	21st Day
CFA- Arthritic Control Paw	2.833 ± 0.16	3.333 ± 0.42	3.667 ± 0.33
CFA+ 250 mg/kg GP	2.667 ± 0.55	3 ± 0.25*	2.167 ± 0.40*
CFA+ 500 mg/kg GP	2.333 ± 0.49*	2.833 ± 0.40*	1.667 ± 0.21*

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

3.3. Effect of GP on Paw edema of Freund's adjuvant induced Arthritis

It was observed 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 GP at both the dose level have shown significant decrease in the paw volume and paw edema. As shown in table 3.

Table 3: Effect of GP on Paw edema of Freund's adjuvant induced Arthritis

Group	Arthritic Assessment score			
	0th Day	7th Day	14th Day	21st Day
CFA-Arthritic Control Paw	3.033 ± 0.18	4.7 ± 0.12	6.25 ± 0.17	6.883 ± 0.14
CFA+ 250 mg/kg GP	3.733 ± 0.33	4.317 ± 0.24*	5.967 ± 0.13*	5.1 ± 0.08*
CFA+ 500 mg/kg GP	3.433 ± 0.23	3.967 ± 0.11*	5.683 ± 0.31*	4.85 ± 0.19*

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

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

X-ray radio graphical image of normal control paw of rats projects integrated join with normal morphology. Sub-chondral erosion on joints was observed in arthritic control paw. Treatment with trial drug GP at the dose of 250 and 500 mg/kg shown significant reduction in joint swelling with reversal in bone and tissue morphology. As shown in figure 1.

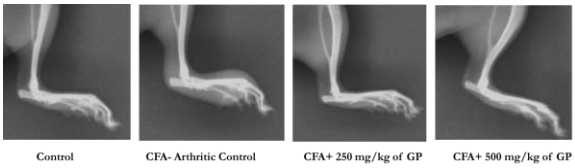

Figure 1: Effect of GP on Radiological changes in arthritis and treatment group rats

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

Sample belongs to normal control paw reveals prominent histology of synovial membrane with regular arrangement of cartilage, bone and muscle architecture on rat paw. Sample belongs to arthritis control paw has shown induction of arthritis with well-characterized synovial hyperplasia associated bone destruction in the joint. Sample belongs to low dose drug treatment reveals moderate cartilages destruction with restoring histology of synovium were observed in sample belongs to group III. Microscopic observation of sample belongs to high dose drug treatment rats exhibits histomorphology of synovial membrane almost to that of the normal and prominent arrangement cartilage. As shown in figure 2.

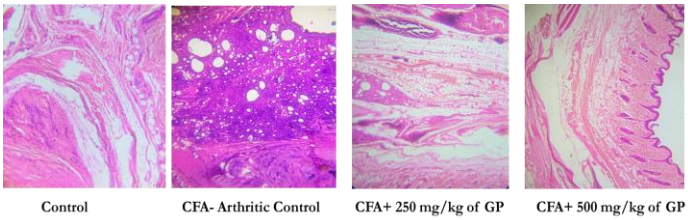

Figure 2: Effect of GP on Histopathological changes of rat paw of control arthritis and treatment group rats

4. Discussion

Polyarthritis is a systemic inflammatory disease that manifests as proliferative synovitis in the joints, resulting in the loss of articular cartilage and underlying bone. Around 0.5 percent to 1% of individuals worldwide are affected with arthritis, with women being affected two to three times more commonly than males; RA is also linked with a high death rate [18]. Treatment for polyrthritis is a combination of steroidal, non-steroidal, and immunosuppressive medications intended to manage inflammatory symptoms and pain, these medications are linked with a variety of unwanted side effects.

CFA is a commonly utilized arthritis model in which susceptible strains of rats are injected with heat-killed Mycobacterium tuberculosis [19]. A quick, reliable, robust, and easily quantifiable polyarthritis occurs upon CFA injection [20]. Notably, the joint pathology reported in the rat model is similar to that observed in human arthritis, particularly polyarthritis [21]. CFA has lately gained popularity as a method for determining the efficiency of herbal medications used to treat arthritis.

Inflammation is a nonspecific immune defence process that is activated in response to mechanical injuries, microbial infections, burns, allergies, and other unpleasant stimuli. Harmful stimuli activate nociceptors by releasing a variety of chemical mediators, including excitatory amino acids, vasoactive amines (histamine, serotonin), proteins, peptides, nitric oxide (NO), arachidonic acids (prostaglandins E2, leukotrienes), and cytokines [TNF- and interleukin-1], all of which act on specific receptors and ion channels [22].It was observed 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 GP at both the dose level have shown

significant decrease in the paw volume and paw edema.

Polyarthritis is a chronic, debilitating, and progressive autoimmune disease characterized by persistent proliferative synovitis and synovial inflammation, as well as severe bone and cartilage degeneration, culminating in considerable joint damage and decreased functioning [23]. This pathology can progress rapidly in an individual and impact several areas of the body, becoming inflammatory or severely painful. It is most prevalent in the elderly, but also affects individuals with degenerative bone disease or immune system malfunction. This condition, which may also be caused by the immune system attacking the synovial membrane, is characterized by swelling, stiffness, discomfort, and a decrease or loss of joint function [24]. X-ray radio graphical image of normal control paw of rats projects integrated join with normal morphology. Sub-chondral erosion on joints was observed in arthritic control paw. Treatment with trial drug GP at the dose of 250 and 500 mg/kg shown significant reduction in joint swelling with reversal in bone and tissue morphology.

5.Conclusion

Bronchial asthma relapse higher prevalence across the globe, as the conventional medicines fails to offer adequate relief imparts the positive impression on availing traditional siddha medicines. It was concluded from the data's of current cross sectional study is that siddha physicians utilised versatile formulations which includes Chooranam, Parpam, Chendooram, Chunnam, Legiyam, Nei, Mathirai and Kudineer for managing symptoms associated with bronchial asthma. Further it was also observed that siddha therapy found clinically effective with lower incidence of adverse event in economical mean and at lesser time. Hence availing siddha medicines found to ideal in limiting the symptoms associated with bronchial asthma.

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.

8. References

1. Global Burden of Disease Collaborators Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800.
2. Vos T, Flaxman AD, Naghavi M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the global burden of disease study. Lancet. 2012;380:2163–2196. doi: 10.1016/S0140-6736(12)61729-2
3. NCCCC (National Collaborating Centre for Chronic Conditions) Rheumatoid arthritis: National clinical guideline for management and treatment in adults. London: Royal College of Physicians (UK); 2009. p. 275. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care. 2012;18(13 Suppl):S295–302.
4. McInnes I. B., Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nature Reviews Immunology. 2007;7(6):429–442.
5. Firestein G. S. Evolving concepts of rheumatoid arthritis. Nature. 2003;423(6937):356–361.
6. Furst D. E., Emery P. Rheumatoid arthritis pathophysiology: update on emerging cytokine and cytokine-associated cell targets. Rheumatology. 2014;53(9):1560–1569.
7. Bresnihan B., Alvaro-Gracia J. M., Cobby M., et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis and Rheumatism. 1998;41(12):2196–2204.
8. Zhuang Y., Lyn S., Lv Y., et al. Pharmacokinetics and safety of golimumab in healthy Chinese subjects following a single subcutaneous administration in a randomized phase I trial. Clinical Drug Investigation. 2013;33(11):795–800.
9. Choi W., Choi C., Kim Y. R., Kim S., Na C., Lee H. HerDing: herb recommendation system to treat diseases using genes and chemicals. Database. 2016;2016

10. Jung H. W., Mahesh R., Park J. H., Boo Y. C., Park K. M., Park Y.-K. Bisabolangelone isolated from Ostericum koreanum inhibits the production of inflammatory mediators by down-regulation of NF-κB and ERK MAP kinase activity in LPS-stimulated RAW264.7 cells. International Immunopharmacology. 2010;10(2):155–162.
11. Kore K.J. Anti-Arthritic activity of Hydroalcoholic extract of Lawsonia Innermis. International Journal of Drug Development & Research. 2011, 3 (4): 217-224.
12. Li Y, Kakkar R, Wang J. In vivo and in vitro Approach to Anti-arthritic and Anti-inflammatory Effect of Crocetin by Alteration of Nuclear Factor-E2-Related Factor 2/hem Oxygenase (HO)-1 and NF-κB Expression. Front Pharmacol. 2018;9:1341.
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. Shah A. S., Alagawadi K. R. Anti-inflammatory, analgesic and antipyretic properties of Thespesia populnea Soland ex. Correa seed extracts and its fractions in animal models. Journal of Ethnopharmacology. 2011;137(3):1504–1509.
18. Tomczyk M., Latté K. P. Potentilla-a review of its phytochemical and pharmacological profile. Journal of Ethnopharmacology. 2009;122(2):184–204.
19. Durai M., Kim H. R., Moudgil K. D. The regulatory C-terminal determinants within mycobacterial heat shock protein 65 are cryptic and cross-reactive with the dominant self homologs: implications for the pathogenesis of autoimmune arthritis. The Journal of Immunology. 2004;173(1):181–188.
20. Patel S. S., Shah P. V. Evaluation of anti-inflammatory potential of the multidrug herbomineral formulation in male Wistar rats against rheumatoid arthritis. Journal of Ayurveda and Integrative Medicine. 2013;4(2):86–93.
21. Andersen M. L., Santos E. H. R., Seabra M. D. L. V., da Silva A. A. B., Tufik S. Evaluation of acute and chronic treatments with Harpagophytum procumbens on Freund's adjuvant-induced arthritis in rats. Journal of Ethnopharmacology. 2004;91(2-3):325–330.
22. Kim W, Park S, Choi C, et al. Evaluation of Anti-Inflammatory Potential of the New Ganghwaljetongyeum on Adjuvant-Induced Inflammatory Arthritis in Rats. Evid Based Complement Alternat Med. 2016;2016:1230294.
23. Jeon H., Yoon W. J., Ham Y. M., Yoon S. A., Kang S. C. Anti-arthritis effect through the anti-inflammatory effect of Sargassum muticum extract in collagen-induced arthritic (CIA) mice. Molecules. 2019;24(2):p. 276.
24. Murugananthan G., Sudheer K. G., Sathya C. P., Mohan S. Anti-arthritic and anti-inflammatory constituents from medicinal plants. Journal of Applied Pharmaceutical Science. 2013;3(4):161–164.