ORIGINAL ARTICLE

Evalution of Anti-Arthritic Potential of Siddha Formulation ChanthirothayaMathirai on Freund's Adjuvant-Induced arthritis in Wistar rats

P. V. Sudhaa Devi, S. M. Chitra, N. Anbu

Department of PG Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India

Abstract

Introduction: Rheumatoid arthritis (RA) is an autoimmune, chronic multisystem disease with various systemic manifestations resulting in joint damage and physical disability. The Siddha system of medicine is one of the traditional system of Indian medicine, which comprises ennumber of siddha formulations for RA. The tablet ChanthirothayaMathirai (CHTM), a herbomineral siddha formulation is found to be efficacious for RA. The present study focuses on theevaluation of anti-arthritic potential of Siddha formulation CHTM on complete Freund's adjuvant (CFA)-induced arthritis model. **Materials and Methods:** The animals were grouped into three. Group I received normal saline, Group II received 0.1 ml of CFA in the left hind and treated with 250 mg/kg of CHTM from day 1 to 21, and Group III received 0.1 ml of CFA into the left hind and treated with 500 mg/kg of CHTM from day 1 to 21. The biochemical parameters were carried out on all groups after 21 days. The presence of any adverse effects was also recorded throughout the study. **Results:** A significant increase in the paw volume and thickness of CFA-injected paws compared to the normal control paws was observed. Arthritic assessment scoring revealed that CFA-injected paws were with the maximum scoring of 3.8. The treatment with CHTM at both doses has significantly decreased arthritic scoring to 2.3. Results showed marginal signs of rejuvenation with mild cartilages destruction with restoring histology of synovium and bone morphology were observed in low-dose drug-treated rats. High-dose drug-treated rats showed normal histomorphology of synovium. **Conclusion:** The study concluded that the trial drug CHTM at the dose of 250 and 500 mg/kg showed a significant reduction in joint swelling with a reversal in bone and tissue morphology. Thus, CHTM formulation offers a strong protective effect against Freund's complete adjuvant-induced paw edema in rats. Hence, the drug CHTM has been scientifically validated for its anti-arthritic potential in management of RA.

Keywords: Anti-arthritic, Chanthirothaya Mathirai, Freund's adjuvant, Siddha formulation

INTRODUCTION

Arthritis is a term that refers to a variety of joint ailments such as rheumatoid arthritis (RA), osteoarthritis (OA), and others that affect one or more joints.[1] RA is a chronic autoimmune illness, in which the host's immune system attacks its cells.[2] RA has been posing a worldwide hazard, over 15% of India's population, or 180 million individuals, are impacted by RA.[3] RA is more prevalent than other illnesses such as cancer and diabetes. According to a study, RA has been the second leading cause of disability and substantially contributes to the worldwide disability load.[4] A person with severe RA is prone to difficulties such as unable to walk or move and not able to do everyday activities.[5] It might result in persistent damage to the joints and ligaments leading to degeneration.[6]

The incidence of RA increases with age, reaching a peak between 35 and 50 years. In addition, study data indicated that the worldwide prevalence of RA is predicted to be 3 in every 10,000 people.[7] Analgesics, non-steroidal anti-inflammatory medications, disease-modifying anti-rheumatic drugs, and corticosteroids are the usual therapy choices for RA.

Address for correspondence: P. V. Sudhaa Devi, Department of PG Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India.
E-mail: suhaashini65@gmail.com

Received: 01-04-2022
Revised: 21-05-2022
Accepted: 02-06-2022

However, these medications are associated with several side effects, including gastrointestinal irritation, ulceration, and bleeding.[8] On the other hand, phytotherapy is the world's oldest system of medicine, used in rural India for centuries.[9]

The Siddha formulation ChanthirothayaMathirai (CHTM) is listed in classical text of Siddha literature "Veeramamunivararuliseitha Anuboga Vaithiya Sigamani."[10] The active ingredients of CHTM includes purified form of Rasa karpooram (*Hydragyrum subchloride*), purified Manjal (*Curcuma longa*), Purified Vengaram (*Borax*), Lemon (*Citrus lemon*) and the adjuvant composed of purified form of Chukku (*Zingiber officinale*), Milagu (*Piper nigrum*), Thippili (*Piper longum*), Seeragam (*Cuminum cyminum*), and Indhuppu (*rocksalt*). The above ingredients of CHTM possess anti-inflammatory, anti-spasmodic, and anti-oxidant activity.[11-13] This preparation is advocated for treating various arthritic disorders. However, still now, the drug has not been exploredscientifically for the management of RA. As a result, the primary goal of this study is to evaluate the formulation of CHTM for anti-arthritic capability in a CFA-induced arthritic rat model, to prove its efficacy of CHTM in management of RA signs and symptoms.

MATERIALS AND METHODS

Animals

The research employed healthy adult Wistar albino rats weighing between 230 and 250 g. The animals were housed in polypropylene cages and maintained in an air handling unit that was thoroughly aired and supplied 100% fresh air. A 12-h cycle of light and darkness was maintained. The room temperature was kept between 22 ± 2°C and relative humidity of 50–65%. They were fed food (Sai feeds, Bangalore, India) and water on *ad libitum* basis. Before the commencement of the trial, all animals were acclimatized to the laboratory for 7 days. The experimental procedure was authorized by the Sathyabama Institute of Science and Technology's Institutional Animal Ethics Committee in Chennai, Tamil Nadu, India and IAEC no SU/CLATR/IAEC/XVII/183/2021.

Methodology

The animals were divided into three groups, each with six animals. Group I (normal control for right paw and arthritic control for left paw) got normal saline, whereas Group II (low dosage treatment group) received 0.1 ml of freund's adjuvant into the left hind and was administered with 250 mg/kg of CHTM from day 1 to day 21. From day 1 to day 21, animals in Group III (High dosage treatment group) received 0.1 ml of freund's adjuvant into the left hind and were administered 500 mg/kg of CHTM.[14]

Measurement of paw volume

Paw volume and thickness were determined using a plethysmometer and a verniercalliper on the 0th, 7th, 14th, and 21st days. On each day, the mean changes in injected paw edema in relation to the starting paw volume were computed.[15]

Measurement of paw edema

The thickness of the paw was utilized to quantify inflammation-induced edema. To summarize, the dorsoventral thickness of each hind paw was determined using a Calliper positioned at the phalange and metatarsal borders. The measurement was obtained with the calliper's edges barely contacting the hind paw's dorsal and ventral surfaces.[16]

Arthritic score

0 indicates minimal edema or swelling, one indicates minor edema with limited erythema, two indicates minor edema and erythema from the ankle to the tarsal bone, three indicates significant edema and erythema from the ankle to the tarsal bone, and four indicates edema and erythema from the ankle to the whole leg.[17]

Histopathology of paw

After the trial, animals were killed using a high dosage of anesthetic drugs, and the hind paws of control and experimental rats were dissected and fixed in 10% buffered neutral formal saline. Acid digestion was then performed on bone specimens. Following fixation, a section of tissue was embedded in paraffin. Ten-millimeter sections of fixed tissues were cut and stained with hematoxylin and eosin. Light microscopy was used to analyze the slices, and photomicrographs were obtained.

Statistical analysis

ANOVA was used to do the statistical analysis. One-way ANOVA was used to examine the data, followed by Dunnett's multiple comparison test.

RESULTS

Table 1 depicted that the effect of CHTM on paw volume of rats showed that CFA-injected paw volume was significantly increased than the normal control paw, indicating the induction of arthritis characterized by swelling and edema, whereas the treatment with CHTM at both dosage levels CFA+ 250 mg/kg and CFA+ 500 mg/kg elicited the substantial reduction in paw volume and edema from 14th to 21st day of peak threshold time.

Table 1: Effect of CHTM on paw volume of rats

Group	Paw Volume in ml			
Control Paw	**0th Day**	**7th Day**	**14th Day**	**21st Day**
Mean	0.8217	0.8317	0.8583	0.805
Std. Deviation	0.07705	0.1332	0.08819	0.112
Std. Error	0.03146	0.05437	0.036	0.04573
CFA–Arthritic Control Paw	**Paw Volume in ml**			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	0.8717	2.233	2.853	3.117
Std. Deviation	0.05565	0.3077	0.09913	0.3168
Std. Error	0.02272	0.1256	0.04047	0.1293
CFA+250 mg/kg CHTM	**Paw Volume in ml**			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	0.88	2.018	2.433	1.96
Std. Deviation	0.07563	0.205	0.09331	0.2665
Std. Error	0.03088	0.08368	0.03809	0.1088
CFA+500 mg/kg CHTM	**Paw Volume in ml**			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	0.8633	1.987	2.288	1.748
Std. Deviation	0.06713	0.2368	0.1132	0.2258
Std. Error	0.02741	0.09667	0.04622	0.09217

Table 2: Arthritic score of arthritis control and CHTM drug-treated rats using assessment scale

CFA–Arthritic Control Paw	7th Day	14th Day	21st day
Mean	3.167	3.5	3.833
Std. Deviation	0.7528	0.8367	0.4082
Std. Error	0.3073	0.3416	0.1667
CFA+250 mg/kg CHTM	**Arthritic Score**		
	7th Day	**14th Day**	**21st Day**
Mean	3	3.167	2.5
Std. Deviation	0.8944	0.9832	0.8367
Std. Error	0.3651	0.4014	0.3416
CFA+500 mg/kg CHTM	**Arthritic Score**		
	7th Day	**14th Day**	**21st Day**
Mean	2.5	3.333	2.333
Std. Deviation	1.225	0.8165	1.506
Std. Error	0.5	0.3333	0.6146

Table 3: Effect of CHTM on Freund's adjuvant-induced paw edemausingvernier caliper

Arthritic Control Paw	Paw Thickness in mm			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	2.933	5.05	6.7	7.233
Std. Deviation	0.4131	0.4324	0.4336	0.2805
Std. Error	0.1687	0.1765	0.177	0.1145
CFA+250 mg/kg CHTM	**Paw Thickness in mm**			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	3.967	4.25	6.383	5.4
Std. Deviation	0.4412	0.505	0.5565	1.374
Std. Error	0.1801	0.2062	0.2272	0.561
CFA+500 mg/kg CHTM	**Paw Thickness in mm**			
	0th Day	**7th Day**	**14th Day**	**21st Day**
Mean	3.55	3.917	6.117	4.95
Std. Deviation	0.6834	0.371	0.3061	0.1871
Std. Error	0.279	0.1515	0.1249	0.07638

As per Tables 2 and 3, the arthritic score and paw edema of ratsshowed significant increase in the arthritic score and paw thickness of CFA-injected paw, indicating swelling and edema of CFA paw when compared to the normal control paw. Meanwhile, the treatment with CHTM at both doses showed thesignificant decrease in the arthritic score and also the substantial reduction in paw edema and swelling was also seen.

The X-ray radiographical image revealed that the control paw of rats integrated joints showed normal morphology, whereas subchondral erosion of joints was observed in the arthritic control paw. The paw with treatment of trial drug CHTM at dose 250 and 500 mg/kg showed significant reduction in joint swelling associated with reversal of bone and tissue morphology [Figure 1].

The sample belonging to control paw revealed, the normal morphology of synovium with regular arrangement of cartilage, bone, and muscle architecture on rat paw. The sample of arthritis control paw showed, high margination of inflammatory cells with induction of arthritis and with signs of synovial hyperplasia associated with degeneration and destruction of joint. In sample of low-dose, drug-treated rats exhibited mild cartilage destruction with restoring histology of synovium and bone morphology, followed by the marginal signs of rejuvenation. The microscopic observation of high-dose drug treatment evidenced normal histomorphology of synovial membrane and regularly alignedarrangement of cartilage seenin tibia and femur [Figure 2].

DISCUSSION

RA is a chronic, systemic, and immune-mediated inflammatory illness that manifests as joint swelling, synovial inflammation, and cartilage loss. In addition, RA is linked with shortened life expectancy and lower quality of life. Increased inflammatory mediator synthesis and activation of T- and B-cells and macrohages are thought to be responsible for illness development.[5,6] CFA-induced arthritis is a widely explored module for evaluating the immunomodulatory potential of the drugs in rodents. Since it was evidenced that injection of CFA accelerates the inflammatory cytokines by aggravating the T lymphocytes and macrophage

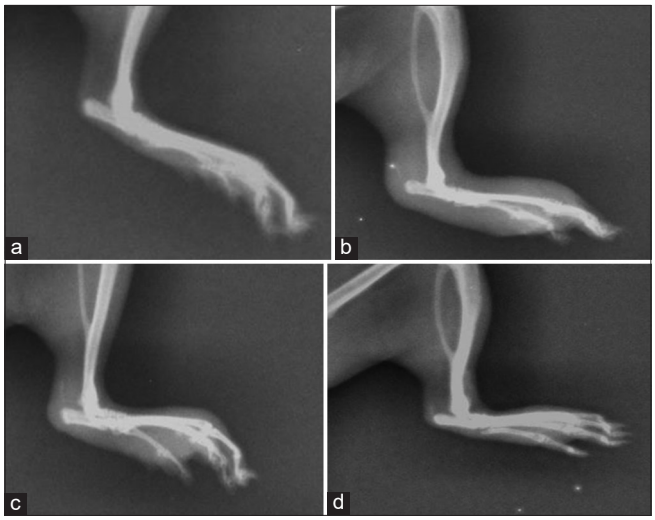

Figure 1: X-ray radiography assessment (a) normal control, (b) CFA arthritic control, and (c) CFA+ 250 mg/kg of CHTM and (d) CFA+ 500 mg/kg of CHTM

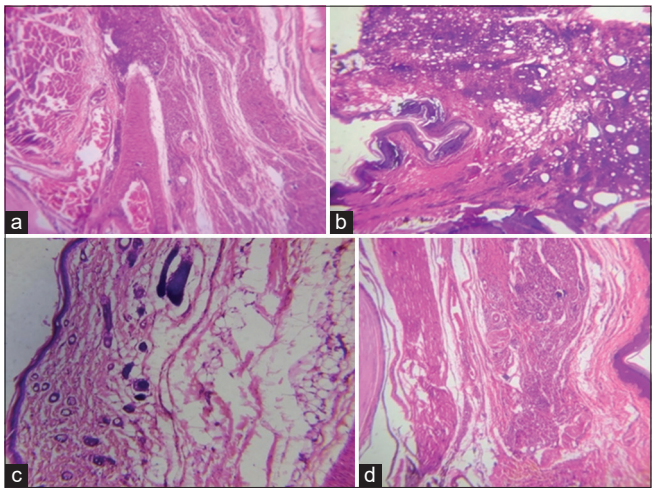

Figure 2: Histopathology of rat paw (a) normal control paw, (b) CFA arthritic control paw, and (c) CFA+ 250 mg/kg of CHTM (d) CFA+ 500 mg/kg of CHTM

immune cells. Increased production of cytokines (IL-6, TNF-, IL-1, and IL-1) and transcription factors involved in the pathogenesis of arthritis is one way by which joints are destroyed in arthritis.[7]

The present study showed that CFA-injected paws had increased paw volume with induction of arthritis compared to the normal control paw, whereas substantial reduction in paw volume was observed when treated with CHTM at both dosage levels from 14th to 21st day of peak threshold time. These findings were in accordance with earlier reported studies of Newbould BB.[18]

The arthritic assessment score of the present study revealed that the severity of arthritis in CFA-injected paws were with the maximum score of 3.833. The treatment with CHTM at both the dose level had shown a significant decrease in arthritic score of 2.5 at 250 mg/kg and 2.33 at 500 mg/kg, which signifies the anti-arthritic potential of CHTM formulation in the experimental animals. Kumar *et al*. also reported similar findings in their study.[19]

The study reported that the CFA-injected paws showed, increase in thickness with the induction of edema and swelling than the normal control paws, whereas the CHTM-treated pawsat both dosage levels markedly reduced the paw edema. Petchi *et al*. also showed similar findings in their study.[20]

The study results manifested that the low dose of CHTM-treated rats had mild cartilage destruction with marginal signs of rejuvenation; meanwhile, the high dose of CHTM-treated rats showed almost normal histomorphology of bone and synovium. Subchondral erosion of joints was observed in the arthritic control paw. This histopathological findings were found to be consistent with the study of Vijayalaxmi *et al*.[16] Added to this, a study had been previously reported for the standardization of CHTM[21] for RA, to prove its safety, quality, and benefits of the phytoconstituents that pose antirheumatic property.

CONCLUSION

Many siddha formulations pose anti-arthritic activity, but their efficacy was not scientifically proven nor validated to explore their hidden potential benefits. Therefore, the herbomineral formulation CHTM showed significant anti-arthritic activity against CFA-induced arthritis in wistar rats. The study findings also concluded that, the Siddha medicine CHTM showed a considerable improvement in relieving arthritic swelling and a drop in arthritic score was noted. The radiological and histopathological findings also justified the above results. Thus, CHTM would be a great beneficial alternative drug for treating chronic inflammatory diseases such as RA, and further, research studies are required to prove its efficacy and use in clinical level.

ACKNOWLEDGMENT

The publication is a part of author's MD program. The authors acknowledge Government Siddha Medical College and the Tamil Nadu Dr. MGR Medical university, Guindy, Chennai, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

The author Dr. P. V. Sudhaa Devi conceptualized, executed the study, and written the draft, Dr. S. M. Chitra designed the study and edited the manuscript, Dr. N. Anbu edited and approved the study.

REFERENCES

1. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
2. Nicholson LB. The immune system. Essays Biochem 2016;60:275-301.
3. Adaji EE, Ahankari AS, Myles PR. An investigation to identify potential risk factors associated with common chronic diseases among the older population in India. Indian J Community Med 2017;42:46-52.
4. Neogi T. The epidemiology and impact of pain in osteoarthritis. Osteoarthritis Cartilage 2013;21:1145-53.
5. Winter CC, Brandes M, Müller C, Schubert T, Ringling M, Hillmann A, *et.al* Walking ability during daily life in patients with osteoarthritis of the knee or the hip and lumbar spinal stenosis: A cross sectional study. BMC Musculoskelet Disord 2010;11:233.
6. Wheaton MT, Jensen N. The ligament injury-osteoarthritis connection: The role of prolotherapy in ligament repair and the prevention of osteoarthritis. J Prolother 2011;3:790-812.
7. Abhishek A, Doherty M, Kuo CF, Mallen CD, Zhang W, Grainge MJ. Rheumatoid arthritis is getting less frequent-results of a nationwide population-based cohort study. Rheumatology (Oxford) 2017;56:736-44.
8. Benjamin O, Goyal A, Lappin SL. Disease modifying Anti-Rheumatic drugs (DMARD). Treasure Island (FL): Statpearls Publishing; 2022.
9. Pandey MM, Rastogi S, Rawat AK. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evid Based Complement Alternat Med 2013;2013:376327.
10. Swami SA. Veerama Munivar Aruli Seitha Anuboga Vaithiya Segaman. Chennai, 26: Thamarai Nulagam; p. 30-31.
11. Meena R, Ramaswamy RS. Preclinical, pharmacological and toxicological studies of Karpoora Chindhamani Mathirai (KCM) for analgesic, anti-inflammatory, antipyeretic effects in rats. Int J Ayurved Med 2012;3:11-5.
12. Jacob A, Wu R, Zhou M, Wang P. Mechanism of the anti-inflammatory effect of curcumin: PPAR-gamma activation. PPAR Res 2007;2007:89369.
13. Srinivasan K. Antioxidant potential of spices and their active constituents. Crit Rev Food Sci Nutr 2014;54:352-72.
14. Kore KJ, Shete RV, Desai NV. Anti-arthritic activity of hydroalcoholic extract of *Lawsonia innermis*. Int J Drug Dev Res 2011;3:217-24.
15. Nair V, Singh S, Gupta YK. Evaluation of disease modifying activity of *Coriandrum sativum* in experimental models. Indian J Med Res 2012;135:240-5.
16. 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. J Bone Rep Recomm 2015;1:9.
17. Abdin AA, Abd El-Halim MS, Hedeya SE, El-SaadanyAA. Effect of atorvastatin with or without prednisolone on freund's adjuvant induced-arthritis in rats. Eur J Pharmacol 2012;676:34-40.
18. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J Pharmacol Chemother 1963;21:127-36.
19. Kumar RN, Anbu N, Sivaraman D. Pharmacological evaluation of anti-arthritic potential of siddha formulation theer andhaskhan mathirai on freund's adjuvant induced arthritis in wistar rats. World J Pharm Res 2021;10:1992-2001.
20. Petchi RR, Parasuraman S, Vijaya C, Gopala Krishna SV, Kumar MK. Antiarthritic activity of a polyherbal formulation against freund's complete adjuvant induced arthritis in female wistar rats. J Basic Clin Pharm 2015;6:77-83.
21. Sudhaadevi PV, Chitra SM, Anbu N. Standardization of siddha herbo mineral formulation chanthirothaya tablet (CHTM) for rheumatoid arthritis (RA). Int J Health Sci 2022;6:2579-88.

Source of Support: Nil. **Conflicts of Interest:** None declared.