ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

ANTI ARTHRITIC ACTIVITY OF HERBO MINERAL SIDDHA PREPARATION *ARUMUGA CHENDURAM* AGAINST TYPE II COLLAGEN INDUCED ARTHRITIS IN EXPERIMENTAL RATS

M. Ramamurthy[1]*, V. Thanigavelan[2], S. Elansekaran[1], V. Srinivasan[3], P. Shanmugapriya[4], G.J. Christian[5]

*[1]Lecturer, [3]Associate Professor, [3]Lecturer, [5]Professor and Head, Department of Noi Naadal, National Institute of Siddha, Chennai, Tamil Nadu, India.

[2]Chief Operating Officer cum Head, R & D, M/s Siddha foods tech, Sithirettipatti PO, Thirumnagalam TK, Madurai district, Tamil Nadu, India.

[4]Associate Professor, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu, India.

ABSTRACT

Siddha system of medicine is the eternal science of life. It is a system that has its extensive bonding with Dravidian culture, language and beliefs. The system of medicine mostly prevailed and prospered in the regions of Dravidian cultures by the great Siddhars. It's unique as one only than other AYUSH traditional systems of medicine across India with its distinctive abundant usage of medicinal plants, metals, minerals and animal products. Siddhars used the steps of Alchemy to prepare various medicines from metallic and mineral origin for attainment elixir and various rare diseases. Siddha medicine is classified into 32 types of internal and external medicine each. Among the 32 types of internal medicine *Chendhuram* is a medicine shelf life of 75 years usually from herbo-mineral combinations. *Arumuga Chendhuram* (ARC) is a herbo-mineral formulation cited in Siddha literature '*Siddha Vaithiya Thirattu'.* ARC was orally administered at higher dose 2gm/kg to the Wistar Albino rats in acute toxicity study and during 28 days of repeated (sub acute) toxicity study, at daily doses of 12, 24 & 48mg/kg of body weight to the Wistar Albino rats. Type II collagen arthritis is another model for developing autoimmune arthritis. The immune pathogenesis mediated by T cell and B cell response to collagen. By this model, nearly 100% arthritis can be achieved. In our study, ARC after 42 days treatment reduced the arthritic swelling significantly and degree of inflammation evident to act against auto immune disorder.

KEYWORDS: Anti Arthritic activity, Arthritis, *Arumuga Chendhuram*, Siddha.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, debilitating, systemic disease that may cause inflammatory changes in tissues and organs, but cardinally attacks synovial joints which are more flexible. It usually affects smaller joints of hands and feet. It is a variety of autoimmune disorder, caused by yet unknown factors. It occurs when the immune system fails to recognize the self antigens and attacks own body's tissues.

Arumuga chenduram is a metallic drug processed by deep oxidation[1] of purified forms of Mercury, Magnetite, Sulphur, Borax, Rock salt and Iron triturated with the juice of *Aloe vera.*

During the process of ARC preparation, the added metallic ingredients converted into the compound oxide forms and it is proved in the study WDXRF studies. Raman spectroscopic analysis warrants the presence of metals in oxide forms. The presence of micron level sized particles influenced the ARC for the better absorption in the intestines when suspended with the adjuvant honey. The size of this particle makes the ARC as efficacious in its lower dosage form. XRD pattern of ARC also show the particles size were in micron levels and crystalline nature. ICP-OES and AAS study revealed that ARC has lower concentration of Arsenic and mercury, and cadmium, lead and vanadium were below the deduction limit. Irons, sodium, Sulphur were in reduced concentration. Mercury (0.13%) was found more than the WHO permissible limit but lead (0.04%) was within the limit. Arsenic and Cadmium was below the detectable level of the instrument in ARC. WHO prescribed the limits of heavy metals only for herbal raw material and food substances which

are not for traditional mineral formulations. So, the therapeutic safety of ARC is validated through the biological samples[2].

The acute toxicity study showed no mortality of rats up to the dosage of 2000mg/kg. No behavioural changes or abnormal clinical signs were observed due to toxicity up to the above dosage throughout the end of 14 day study period. For a period of 28 consecutive days of oral treatment of ARC at 12, 24 & 48mg/kg/day in both sexes of rat, no treatment related toxicity signs or mortality were observed[3]. But no significant change in the body weight gained or lost in the treated test groups were observed compared with control group during the study.

The *Arumuga Chenduram* have been reported to possess anti-inflammatory and analgesic properties. As there was no scientific evidence for its Anti-Arthritic activity, the present study was undertaken to evaluate the Anti-Arthritic activity of *Arumuga chenduram* on Type II Collagen induced Arthritis in Wistar rats. Type II Collagen induced arthritis in rats and mice were well-known to have both clinical and histological similarities to human RA, and this model has been widely used in experiments to study RA.

Test drug – *Arumuga Chendhuram* Ingredients

Common Tamil Name	**Scientific name**
Valai rasam/ Linga rasam	Purest form of Mercury
Purified *Kaantham*	Magnetic oxide of iron
Purified *Gandhagam*	Sulphur
Purified *Venkaaram*	Sodium chloride impure – Rock salt
Ayam powdered	Ferrum - Iron
Katraazhai	*Aloe vera*

Procedure for the preparation of *Arumuga Chendhuram*

Take 5 parts of *Valai Rasam* (purest form of Mercury – *Linga rasam*), 7 parts of purified *Kaantham* (Magnetic oxide of iron), 9 parts of purified *Gandhagam* (Sulphur), 8 parts of purified *Venkaaram* (Sodium biborate- Borax), 4 parts of purified *Indhuppu* (Sodium chloride impure– Rock salt), 12 parts of purified *Ayam* powdered (Ferrum - Iron) and placed in *Kalvam* and make them into fine powdered form which is then rubbed with *Katralai* juice and dried. The above dried formulation is placed in an earthen pot and covered with mud and then started to heat at flame for one day. The finished test drug was stored in an air tight sterile glass container and kept in a dark condition.

MATERIALS AND METHODS

Experimental Animals

Healthy out bred Wistar Albino Rats of either sex weighing about 120 – 160g were obtained from the Animal house of King Institute of Preventive Medicine and Research, Guindy, Chennai and maintained in the Animal house of National Institute of Siddha, Chennai. The female rats obtained were nulliparous and non-pregnant. All the animals were properly maintained and strictly following the "Guidelines on care and maintenance of laboratory animals" that have been framed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Changes, Government of India. The animals received RO water *ad libitum* and fed with Rodent pellet which was purchased from Shri Venkateshwara Traders, Bengaluru. Before the induction of Pharmacological study, all the animals were acclimatized for seven days. The study protocol has got approval from Institutional Animal Ethical Committee of KMCH college of Pharmacy, Coimbatore, India (KMCRET/MD(S)/05/2014-15) and the studies were conducted at the Department of Pharmacology of same college.

Induction of Arthritis

Arthritis was induced using Chicken Sternal Collagen type–II with Incomplete Freund's Adjuvant. Collagen was dissolved in ice-cold 0.1 Macetic acid at a concentration of 2mg/mL, kept overnight and stored at 4◦ C. On day 1, collagen in acetic acid was emulsified with equal volumes of Incomplete Freund's Adjuvant to produce the inducing agent and stored on ice before use. Rats were immunized intradermally with 0.5mL of the emulsion (0.1mL each of the emulsion was injected to five sites; root of tail and regions above each limb). On day 7, after the primary immunization all animals were given booster injection with 0.1mL of chicken collagen emulsified with Incomplete Freund's adjuvant in the same manner.[4]

Experimental Design

Rats were divided into four groups, six animals in each group. After induction of arthritis the animals were grouped and treated for 42 days.

Group I Disease control (Collagen + Vehicle): Treated with 1ml of diluted honey for 42 days

Group II Standard (Collagen + Standard): Treated with standard drug Leflunomide (10mg/kg orally) with 1ml of distilled water for 42 days

Group III Collagen + Test drug at therapeutic dose: Treated with test sample at the dose constituted 24mg/kg ARC for 42 days.

Group IV Collagen + Test drug at high dose: Treated with test sample at the dose constituted 48mg/kg ARC for 42 days

Pharmacological Evaluation

The severity of inflammation in each limb was monitored by set visual criteria for the degree of inflammation, the extent of erythema and edema of the periarticular tissues, and the enlargement, distortion, or ankylosis of the joints.

0 = no inflammation

1 = inflammation of 1 joint

2 = unequivocal inflammation of at least two joints of the limb or moderate inflammation of one joint

3 = severe inflammation of one joint

4 = maximum inflammation of more than one joint in the limb.

The arthritis score was the sum of the scores for all four limbs (maximum score = 16).

Measurement of Paw volume

Paw was marked with ink at the level of the malleoluslaterials and paw volumes were recorded by the plethysmometer immediately after injection and on 7th, 14th, 21st and 28th day. The body weight changes were observed on every week.

Hematological Parameters

At the end of Day 40, blood was collected (under light ether anaesthesia) from the retro-orbital veins of each animal and was placed in a non-heparinized tube for serum separation and it was then kept at −20◦ C for the quantitative determination of the levels of Rheumatoid Factor (RF) and C-reactive protein (CRP) by ELISA (following manufacturer instructions).

Arthritic index and body weight change

Rats were examined every week until week 6 after initial sensitization using collagen with Incomplete Freund's Adjuvant. The severity of inflammation in each limb was evaluated every week for the degree of inflammation, the extent of erythema and edema of the periarticular tissues, and the enlargement, distortion, or ankylosis of the joints. Findings were scored on a scale of 0–4, where 0= no inflammation. 1= inflammation of 1 joint. 2= unequivocal inflammation of at least 2 joints of the limb or moderate inflammation of 1 joint 3= severe inflammation of 1 joint 4= maximum inflammation of ≥1 joint in the limb. The arthritic index was calculated as sum of these scores (maximum score = 16). Body weight was measured using digital weighing balance from 1 to 40 days from the day of CIA injection.

Radiographic Analysis

The rats were anaesthetized using Ketamine (50 mg/kg, *i.p.*) and radiograph was recorded on a digital system and seimen's X-ray machine.

Histological Observation

On day 41, one animal from each group was sacrificed by cervical dislocation and the ankle joints of the hind limbs were excised and fixed in Bouin's fluid, subsequently the specimens were decalcified with 10% EDTA for 7 days, dehydrated and embedded in paraffin blocks. Sections of ankle joints (5µm thick) were cut and mounted on slides and stained using haematoxylin and eosin. Grading of cellular infiltration, synovial hyperplasia, pannus formation, joint space narrowing, and cartilage and bone erosion of the ankle joints was blindly investigated by pathologist.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). P Value was calculated using One-way Analysis of Variance (ANOVA) followed by Tukey Kramer Multiple Comparisons Test using GRAPH PAD INSTAT version 3 software programs. Values of $p<0.05$ were considered significant.

RESULTS

Paw volume

Paw volumes of the animals in all groups were recorded on day 1, 14, 18, 22, 28, 35 and 40. Data obtained were depicted in figure 1 and from the results it is clear that, arthritic animals treated with TET exhibited significant reduction in paw volume.

Fig 1.

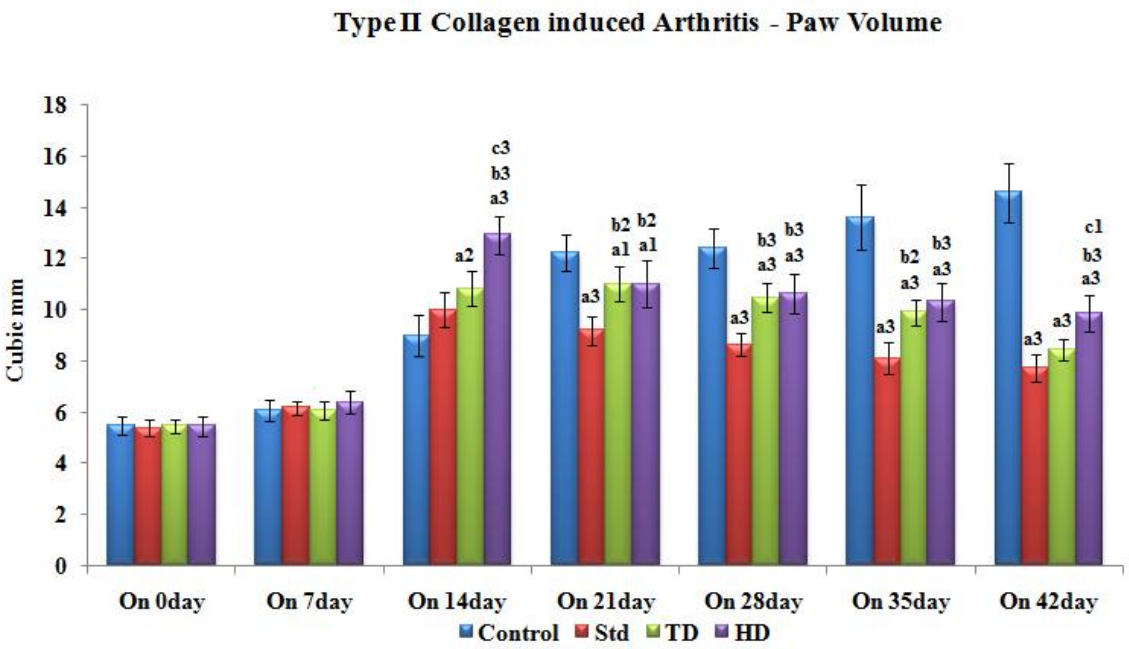


Fig. 1, Effect of *Arumuga Chendhuram* on Paw Volume in Collagen with Freund's adjuvant induced arthritis in Wistar rats of control and experimental groups

[a1]p<0.05 significantly different compared with control rats treated with diluted honey.
[a2]p<0.01 significantly different compared with control rats treated with diluted honey.
[a3]p<0.001 significantly different compared with control rats treated with diluted honey.
[b2]p<0.01 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg
.[b3]p<0.001 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg
[c1]p<0.05 significantly different compared with test group of rats treated with ARC at the dose of 24mg/kg.
[c3]p<0.001 significantly different compared with test group of rats treated with ARC at the dose of 24mg/kg.

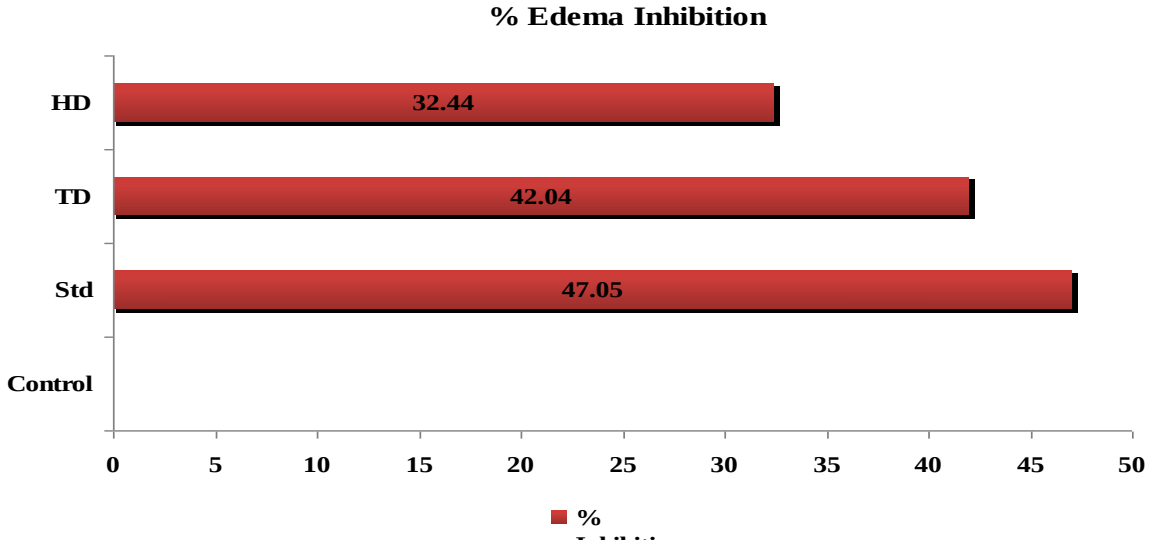


Fig. 2, Effect of *Arumuga Chendhuram* on inhibition of Arthritis index in Collagen with Freund's adjuvant induced arthritis in Wistar rats of experimental groups

Among the experimental groups, ARC at the dose of 24mg/kg exhibited better inhibition but less effective than Leflunomide.

Effect of *Arumuga Chenduram* on Arthritic Index and Body Weight

Arthritic index and body weight of animals in all groups were recorded on day 1, 7, 14, 21, 28, 35 and 42. Data obtained were depicted in figure

Fig.3,:

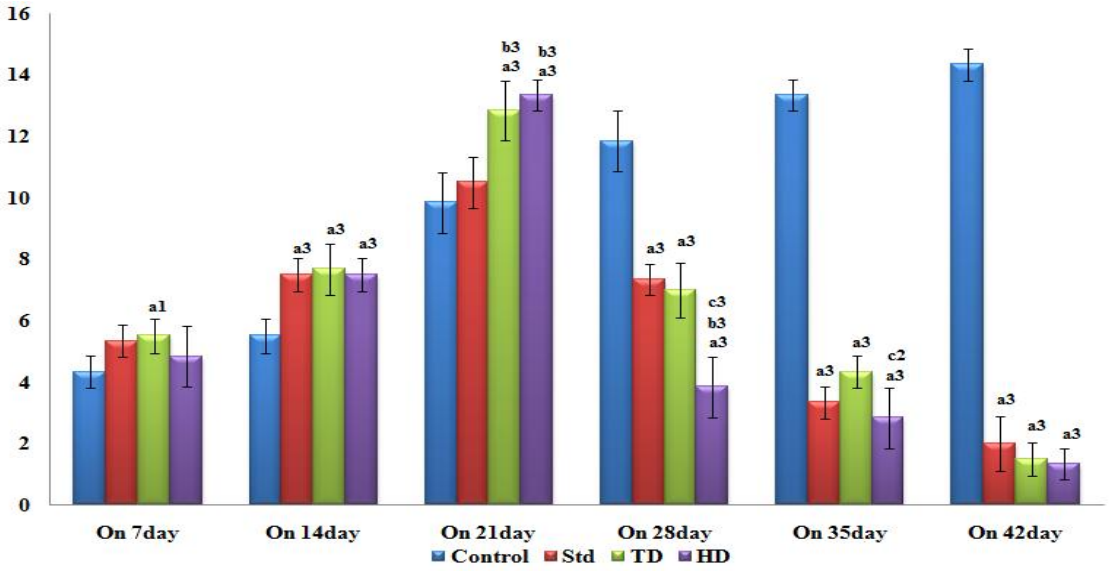


Fig. 3, Effect of *Arumuga Chendhuram* on Arthritis index in Collagen with Freund's adjuvant induced arthritis in Wistar rats of control and experimental groups

^{a1}p<0.05 significantly different compared with control rats treated with diluted honey.

^{a3}p<0.001significantly different compared with control rats treated with diluted honey.

^{b3}p<0.001 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg

^{c2}p<0.01 significantly different compared with test group of rats treated with ARC at the dose of 24mg/kg.

^{c3}p<0.001 significantly different compared with test group of mice treated with ARC at the dose of 24mg/kg.

Body Weight Analysis

Table: 1 Effect of *Arumuga Chendhuram* total body weight in Wistar Rats – Collagen with Freund's adjuvant induce

Treatment	Collagen+Vehicle	Collagen+STD	Collagen+T.D	Collagen+HD
On 0th Day(g)	112.56±2.51	125.59±4.65 ^{a2}	147.84±9.31 ^{a3c3}	130.23±5.11 ^{a3c3}
On 7 rd Day(g)	112.53±2.14	116.89±7.59	150.07±12.60 ^{a3b3}	130.99±9.32 ^{a2c2}
On 14th Day(g)	118.36±5.19	125.18±14.40	147.03±14.33 ^{a2b1}	133.05±7.88
On 21th Day(g)	128.77±9.45	133.30±18.05	143.98±9.98	133.77±8.25
On 28th Day(g)	132.90±15.51	133.47±18.67	146.81±11.74	130.95±8.00
On 35th Day(g)	148.68±12.45	140.10±22.36	147.82±13.42	133.59±9.98
On42th Day(g)	153.54±18.25	146.51±21.52	140.87±11.87	129.57±10.02

^{a2}p<0.01 significantly different compared with control rats treated with diluted honey.

^{a3}p<0.001 significantly different compared with control rats treated with diluted honey.

^{b1}p<0.05 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg.

^{b3}p<0.001 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg

^{c2}p<0.01 significantly different compared with test group of rats treated with ARC at the dose of 24mg/kg.

^{c3}p<0.001 significantly different compared with test group of rats treated with ARC at the dose of 24mg/kg

Hematological Parameters: C - Reactive protein, Rheumatoid Factor

Table: 2 Effect of *Arumuga Chendhuram* on RA Factor & C- Reactive Protein in Wistar rats of Disease control and experimental groups

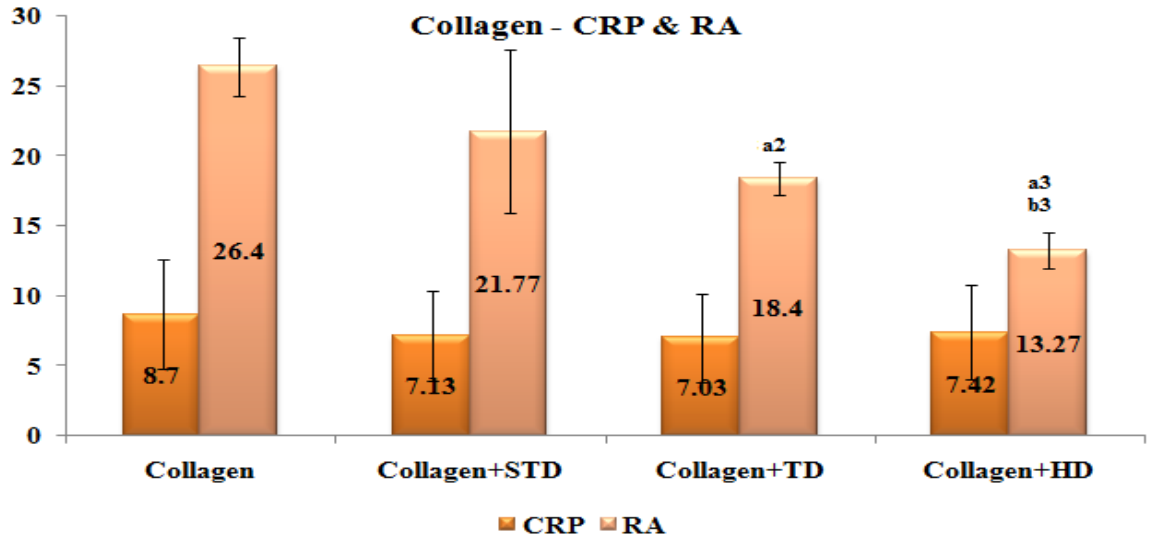
Group	Only Collagen	Collagen+ Standard	Collagen+ Therapeutic Dose	Collagen+ High Dose
C-Reactive protein	8.7±3.89	7.13±3.22	7.033±3.15	7.42±3.42
Rheumatoid Faetor	26.4±2.063	21.77±5.83	18.4±1.19 ^{a2}	13.27±1.29 ^{a3b3}

^{a2}p<0.01 significantly different compared with control rats treated with diluted honey.

^{a3}p<0.001 significantly different compared with control rats treated with diluted honey.

^{b3}p<0.001 significantly different compared with standard group of rats treated with Leflunomide at 10mg/kg

Fig.4,

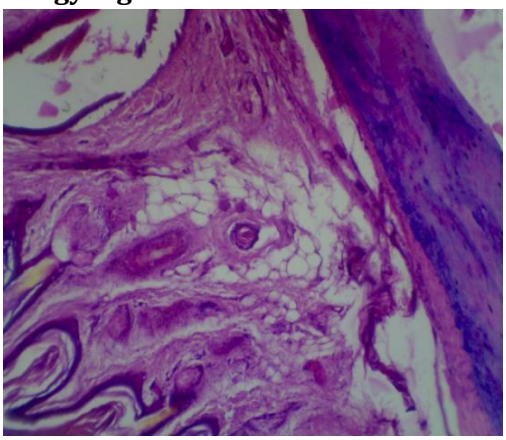


Effect of *Arumuga Chendhuram* on CRP & RA in Wistar rats of control and experimental group

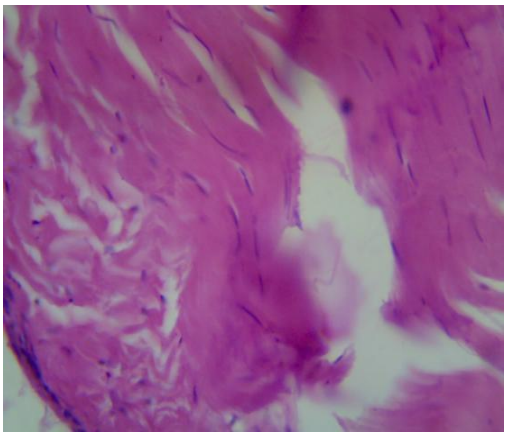
Effect of *Arumuga Chenduram* on Histopathology

Histopathological evaluation of hind paws (Figure 7) joints of normal group rats showed intact articular cartilage and normal synovial lining with absence of inflammation and infiltration of inflammatory cells. Cartilage damage, synovial thickening, congestion hemorrhage, infiltration of large number of lymphocyte, neutrophils, plasma cells and pannus formation were observed in Collagen with Freund's adjuvant induced arthritis induced rats.

Histopathology Fig.1



10X shows skin with normal epidermis



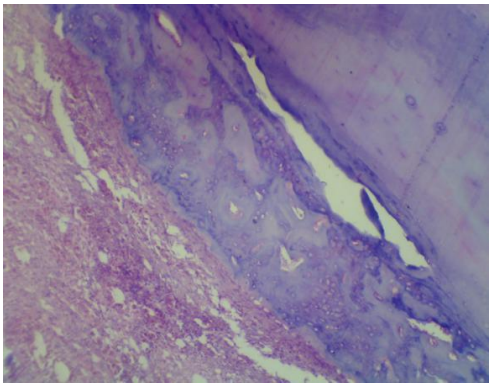
40x shows fibro collagenous stroma & dermis

Plate: 1 Effect of ARC on the inflamed joints induced by Type II Collagen with Incomplete Freund's adjuvant – Control group.

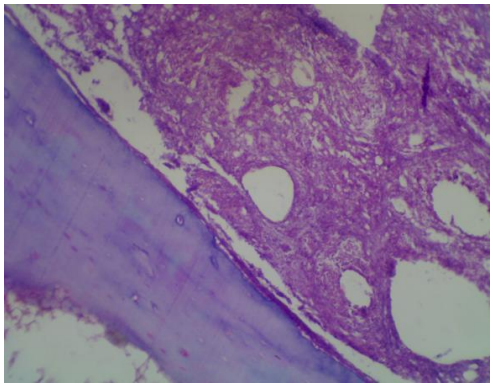
Microscopic appearance

Section studied shows normal epidermis. Dermis shows normal fibro collagenous stroma and synovium and mature bony trabeculae. There is no evidence of inflammation granuloma/necrosis in the section studied.

Histopathology Fig.2



10x shows dense inflammation with mature bone



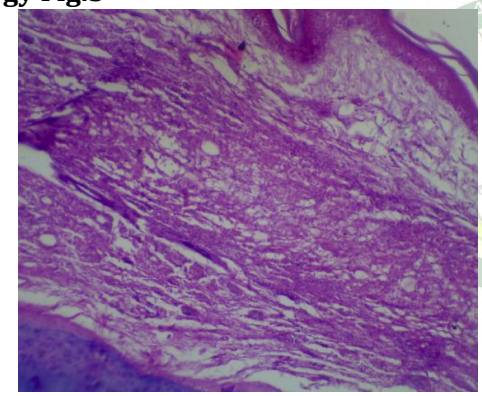
40x shows bone with Inflammatory infiltrates

Plate: 2 Effect of ARC on the inflamed joints induced by Type II Collagen with Incomplete Freund's adjuvant – Control group

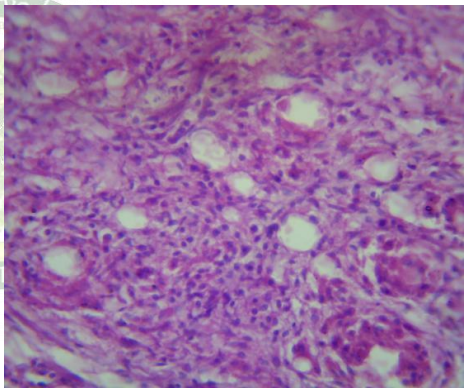
Microscopic appearance

Section studied shows normal epidermis. Dermis shows dense inflammation of neutrophils, lymphocytes and macrophages with areas showing necrosis and edema seen mainly in the periarticular region.

Histopathology Fig.3



10x shows skin with mature bone



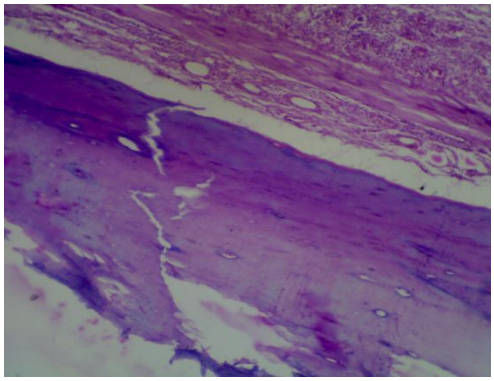
40xshowsdenseinflammationwith necrosis

Plate: 3 Effect of ARC on the inflamed joints induced by Type II Collagen with Incomplete Freund's adjuvant – Control group.

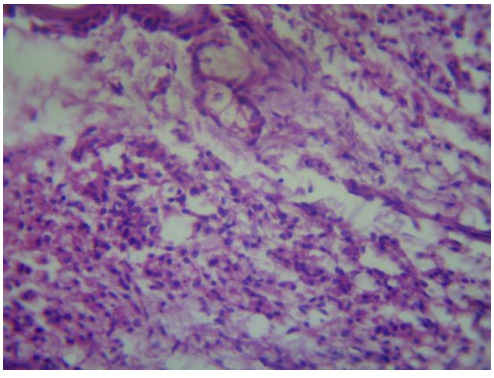
Microscopic Appearance

Section studied shows normal epidermis. Superficial dermis shows diffuse scattered collection of neutrophils, lymphocytes and plasma cells. There is no evidence of toxicity.

Histopathology Fig.4



10x shows deep dermis with mononuclear



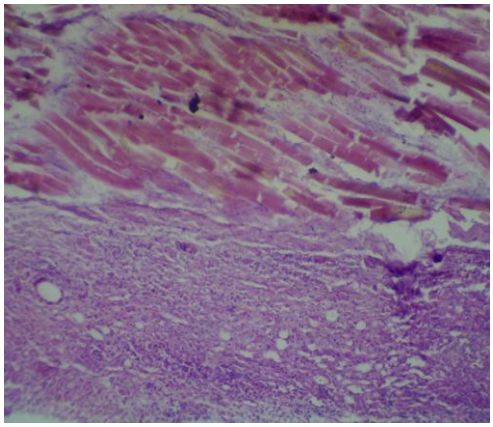
40x shows inflammatory infiltrates

Plate: 4 Effect of ARC on the inflamed joints induced by Type II Collagen with Incomplete Freund's adjuvant – Control group.

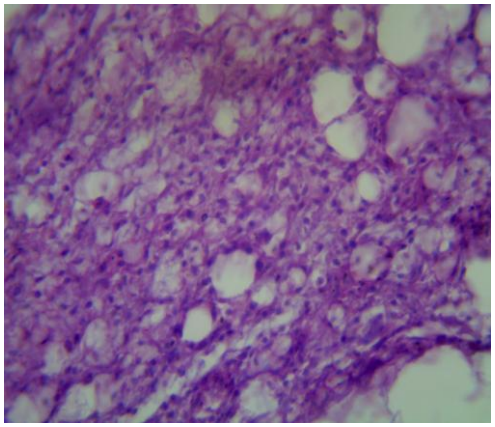
Microscopic Appearance

Section studied shows normal epidermis. Dermis shows mononuclear infiltration with karyorrhectic debris (necrosis). The bony trabeculae are normal. Muscular layer shows dense inflammatory infiltrates.

Histopathology Fig.5



10x shows deep dermis with mononuclear Inflammation



40x shows mononuclear infiltration

Plate: 5 Effect of ARC on the inflamed joints induced by Type II Collagen with Incomplete Freund's adjuvant – Control group

Microscopic Appearance

Section studied shows normal epidermis. Dermis shows fibrocollagenous stroma with diffuse scattered inflammatory infiltrates composed of neutrophils, lymphocytes and macrophages. Deep dermis shows scattered inflammatory infiltrates. Muscular layer and bony trabeculae are normal.

DISCUSSION

In Siddha system of medicines herbo mineral formulations plays an important role in treating chronic diseases. Among that metallic drugs exhibit better efficacy in lower dosage and have long period of stability. *Arumuga chendhuram*[8] is a metallic drug processed by the process of deep oxidation of purified forms of Mercury, Magnetite, Sulphur, Borax, Rock salt and Iron triturated with the juice of *Aloe vera.*[5] Moreover ARC is in brick fine red colour supports that the elements found in the oxide forms predominantly ferric oxide.

Type II Collagen induced Arthritis in rats is a well established experimental model for the study of the pathophysiology of various types of human arthritis, especially Rheumatoid Arthritis (RA). It results in marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption and periosteal proliferation and inflammation. Considering these advantages we utilized the Type II Collagen induced arthritic model in rats to assess the potential effects of *Arumuga chenduram* upon inflammatory parameters.[6]

In Rheumatoid arthritis, pain and inflammation are the predominant symptoms that the patients suffered. The drug used for the treatment should possess anti-analgesic, anti-inflammatory and anti-arthritic activities. In such manner, the study was designed and ARC was evaluated for those activities in animal models to substantiate potential anti-arthritic drug.

Type II collagen arthritis is model for developing autoimmune arthritis. The immunopathogenesis mediated by T cell and B cell response to collagen[7]. By this model, nearly 100% arthritis can be achieved. In our study, ARC after 42 days treatment reduced the arthritic swelling significantly and degree of inflammation evident to act against auto immune disorder.

CONCLUSION

From the results of above study, it is validated that *Arumuga Chendhuram* possesses anti-arthritic activity at its therapeutic dose of 130mg/kg/dose.

REFERENCES

1. Kuppusamy mudaliyar. K.N, Siddha maruthuvam (General], 6th ed (2004), Department of Indian medicine and Homeopathy, Chennai Page 609.
2. M.Ramamurthy, V. Thanigavelan, K. Manickavasakam. (2016). Physico-chemical characterization of siddha sastric formulation *Arumuga Chendhuram,* International Journal of

Ayurveda and Pharma Research. International Journal of Ayurveda and Pharma Research. 2016;4(4):27-31.

3. Ramamurthy. M, Thanigavelan. V, Manickavasakam. K (2016). Toxicological Study of a Siddha Sastric Formulation Arumuga Chendhuram in Rat Model. Journal of Applied Pharmaceutical Science Vol. 6 (03], 081-087.
4. David D Brand, Kary A Latham1, Edward F Rosloniec. (2007). Collagen-induced arthritis :2(5):1269
5. Kapoor RC. (2010). Some observations on the metal based preparation in the Indian system of Medicine. Indian J Traditional Knowledge; 9:562-75.
6. Krishna Das Madhu, Jyoti Harindran Antiarthritic potential of Ocimum gratissimum L. in collagen induced arthritic Sprague- Dawley rats. Biomedicine & Aging Pathology 2014; 4:191–196.
7. N. Rasenack and B.W. Müller, Micron-size drug particles: common and novel micronization techniques, Pharmaceutical Development and Technology 9(1), 2004, 1-13.
8. R. Thiyagarajan, L.I.M, Gunapadam 2 & 3 (Thaathu Jeevam Vaguppu], 8th edition (2013), Department of Indian medicine and Homeopathy, Chennai. Page 42.

Cite this article as:
M.Ramamurthy, V.Thanigavelan, S.Elansekaran, V.Srinivasan, P.Shanmugapriya, G.J. Christian. Anti Arthritic Activity of Herbo Mineral Siddha Preparation Arumuga Chenduram Against Type II Collagen Induced Arthritis in Experimental Rats. International Journal of Ayurveda and Pharma Research. 2020;8(7):28-36.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr M. Ramamurthy
Lecturer,
Department of Noi Naadal,
National Institute of Siddha,
Chennai, Tamil Nadu, India.
Email: ramsnis@gmail.com
Cell: 9443178112