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IMMUNOMODULATOR ACTIVITY AND ANTI ARTHRITIC ACTIVITY OF SIDDHA POLYHERBAL FORMULATION MAHAANALURUVA CHOORANAM

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ABSTRACT

Introduction: Mahaanaluruva chooranam (MAC) is a traditional herbal formulation from the siddha system of medicine. It is a polyherbal formulation, meaning a powder blend of several medicinal herbs, specifically used for treating various vaatha dosha imbalances. In siddha, vaatha dosha is associated with functions like movement, circulation, and breathing. Recent research suggests that MAC possess immunomodulatory activity, meaning it could influence the function of the immune system. This property could be beneficial in various ways, such as enhancing immune response and reducing inflammation. Chronic inflammatory conditions are often associated with an overactive immune system. MAC may help modulate the immune response and reduce inflammation. The immunomodulatory properties of MAC suggest its potential applications in a wider range of health conditions. Further scientific studies are needed to validate these claims and explore the mechanisms of action behind MAC's immunomodulatory effects. The study was conducted at CL Baid metha college of pharmacy, Chennai, Tamil Nadu. The study was done by using Wistar albino rats of either sex. The study evaluated MAC's impact on delayed-type hypersensitivity (DTH) using paw pad swelling. Rats receiving MAC at 100mg/kg and 200mg/kg showed increased paw swelling compared to the control group, suggesting a potential stimulation of the cellular immune response. The experiment assessed MAC's effectiveness against arthritis induced by Complete Freund's Adjuvant (CFA). Both MAC doses (100mg/kg and 200mg/kg) significantly reduced paw swelling compared to the untreated control group, indicating an anti-inflammatory effect. The results were comparable to the standard drug, indomethacin. Therefore, the study concludes that Mahaanaluruva chooranam has significant immunomodulator activity which will be helpful for autoimmune diseases like rheumatoid arthritis.

KEYWORDS: Cell mediated immunity, Mahaanaluruva chooranama (MAC), delayed type hypersensitivity (DTH), rheumatoid arthritis, immunomodulator activity.

INTRODUCTION

Rheumatoid arthritis is a debilitating inflammatory and autoimmune disease that affects the joints¹. The condition is remarkably consistent around the world, affecting roughly 0.5-1% of the population². It's caused by the release of a number of pro-inflammatory molecules by macrophages, including reactive oxygen species (ROS) and eicosanoids such as prostaglandins, leukotrienes, and cytokines. Regulating these mediators secreted by macrophages and other immune cells, alongside modulating arachidonic acid metabolism by inhibiting enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), are promising targets for treatment of chronic inflammatory conditions³. The disease is characterised by angiogenesis, synovial hyperplasia, and mononuclear cell infiltration⁴. While various medications have been used to control rheumatoid arthritis, there are numerous reports regarding the side effects of these drugs. Consequently, medical practitioners and scientists are increasingly looking towards medicinal plants and traditional systems of medicine to reduce side effects and toxicity.

A recent WHO survey suggests 80% of the global population uses traditional medicines for primary healthcare. Herbal remedies, reported to possess immunomodulatory properties, are believed to strengthen the body's natural defences. Research indicates anti-inflammatory activity in *Plumbago zeylanica*, *Pongamia glabra*, and *Holoptelia integrifolia*, key herbs in Mahaanaluruva chooranam (MAC).

MATERIAL AND METHODS

The polyherbal preparation, Mahaanaluruva chooranam (MAC), is documented in the classical Tamil text "Agathiyar Vaithiya Vallathi 600^[5]". The ingredients for this formulation are listed in Table -^[6-14]

TABLE - 1 Ingredients of MAC

S.NO	INGREDIENTS	BOTAMNICAL NAME/CHEMICAL NAME	QUANTITY
1	KODIVELI VER	<i>Plumbago zeylanica</i>	1 Palam(35gms)
2	PUNGAN VER	<i>Pongamia glabra</i>	1 Palam(35gms)
3	AAYILIAM PATTAI	<i>Holoptelia integrifolia</i>	1 Palam(35gms)
4	AAYILIAM VER	<i>Holoptelia integrifolia</i>	1 Palam(35gms)
5	VAIVILANGAM	<i>Emblica ribes</i>	1/2 Palam(17.5gms)
6	THIPPILI	<i>Piper longum</i>	1/2 Palam(17.5gms)
7	KADUKKAI	<i>Terminalia chebula</i>	1/2 Palam(17.5gms)
8	KADUGU	<i>Brassica nigra</i>	1/2 Palam(17.5gms)
9	CHUKKU	<i>Zingiber officinale</i>	1/2 Palam(17.5gms)
10	KARUNJEERAGAM	<i>Nigella sativa</i>	1/2 Palam(17.5gms)

COLLECTION, IDENTIFICATION AND AUTHENTICATION OF THE DRUG

All plant materials were sourced from a reputable raw drug shop in Parry's Corner, Chennai, Tamil Nadu, and subsequently verified by botanical and pharmacological experts at the Government Siddha Medical College Hospital, Arumbakkam, Chennai (GSMC/MB 646-655)^[15].

PURIFICATION OF THE DRUGS (16-19)

Preparation and Purification of Plant Materials:

Following the principles outlined in Siddha literature, all plant materials underwent a purification process prior to use in the formulation.

- *Plumbago zeylanica* (kodiveli) root, *Pongamia glabra* (pungan) root, and *Holoptelia integrifolia* (aayiliam) root and stem: The inner vein and outermost bark were meticulously removed from the respective roots. Subsequently, these materials were carefully pulverised into a fine powder. This powder then underwent purification through a traditional Siddha method known as the "pittavial" steam boiling process.
- *Emblica ribes* (vaivilangam): These fruits were meticulously cleaned to eliminate any dust particles. They were then sun-dried for preservation purposes.
- *Piper longum* (thippili) seeds: These seeds were soaked in lemon juice and subsequently dried under sunlight.
- *Terminalia chebula* (kadukkai): The fruits were soaked in water. Following this soaking process, the yellow-tinged water was discarded, the seeds removed, and the remaining fruit material was dried.
- *Brassica nigra* (kadugu) and *Nigella sativa* (karunjeeragam) seeds: These seeds were thoroughly cleaned to eliminate any dust particles and then sun-dried.
- *Zingiber officinale* (chukku): One part of the rhizome was layered with two parts of limestone for a period of three hours. Following this, it was washed, dried, and the outer skin was carefully removed [16-19].

PREPARATION OF THE DRUG

Procedure

The purified plant materials detailed in Table 1 were meticulously pulverised into a fine powder using a mortar and pestle. This resulting powder, designated as Mahaanaluruva Chooranam (MAC) [5], was subsequently transferred to an airtight container for secure storage. Finally, adhering to the principles outlined in a revered Siddha text, the Chooranam underwent a traditional steam purification process known as "pittavial" [20].

Experimental animals:

This study employed healthy adult albino Wistar rats of both sexes, weighing between 150-200 grams. The animals were housed appropriately in polypropylene cages under standard laboratory conditions, adhering to established animal welfare protocols. Group allocation involved dividing the rats into five distinct groups. The Institutional Animal Ethical Committee approved the experimental protocol (Approval no: 10/321/PO/Re/S/01/CPCSEA/dated 12/07/2023).

METHODOLOGY

Immunomodulatory Activity: Delayed-Type Hypersensitivity (DTH) Reaction

The effect of the Maha Analuruva Chooranam (MAC) on antigen-specific cellular immune response in rat was evaluated from DTH levels using the foot pad swelling test. Three groups of five rats each were constituted comprising a control group and two treated groups. The control group received 2% Dimethyl Sulfoxide (DMSO), whereas the treated groups received Maha Analuruva Chooranam (MAC) orally for 14 consecutive days at 100 and 200 mg/kg body weighted reactions were induced using bovine serum albumin (BSA, 1 mg/mL) as antigen. Animal was sensitized by subcutaneous injection of 50 μ L of BSA solution emulsified with Freund's complete adjuvant on day 7 following extract treatments in the plantar region of the right hind foot paw, and challenged on day 14 by subcutaneous injection of 50 μ L of antigen emulsified in Freund's incomplete adjuvant on the same foot pad. At the same time, the contralateral foot pad received an equal volume of phosphate buffer saline emulsified in

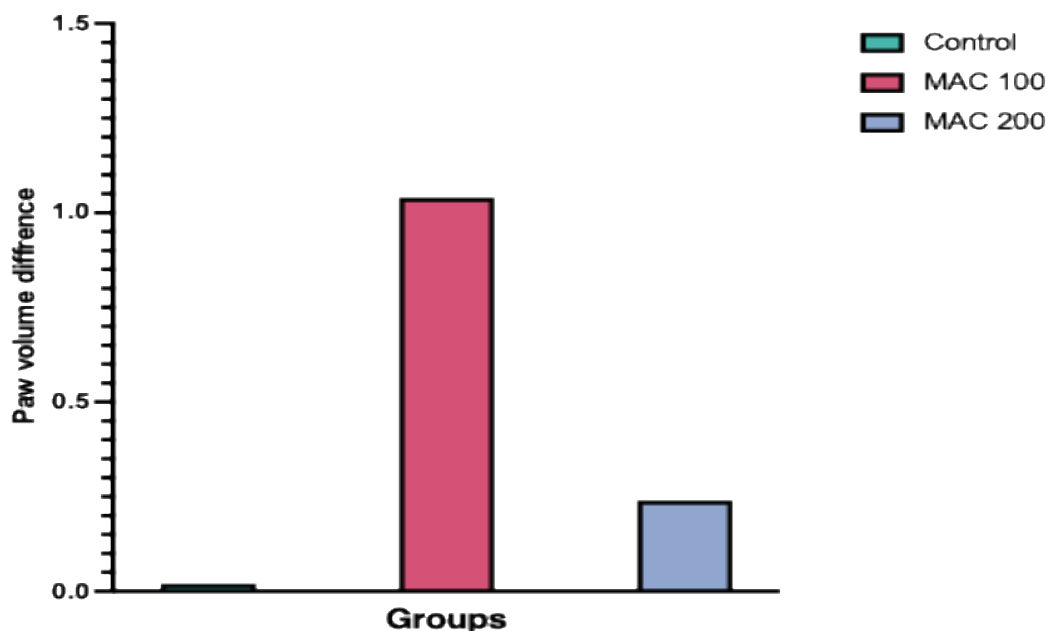
Freund's adjuvant. Increase of foot pad thickness was measured with a Digital plethysmograph, The difference of the right hind pad and the left hind pad was used as DTH.

TABLE -2 Delayed-type hypersensitivity (DTH) reaction of MAC

S.no	Groups	Dose	Volume of Paw		Difference in paw volume
			Initial	Final	
1	Control		8.33±1.42	8.31±1.21	0.02
2	MAC	100mg	9.20±1.24	10.24±1.26	1.04
3	MAC	200mg	9.58±1.24	9.82±1.24	0.24

Effect of Maha Analuruva Chooranam (MAC) on delayed-type hypersensitivity reaction (DTH) in mice immunized with bovine serum albumin. Values represent mean \pm SD of foot pad thickness. Group bearing different superscript letters are significantly different according to Waller–Duncan test (at $p < 0.05$) $n=6$

Difference in paw volume in DTH study



Complete Freund's adjuvant (CFA) induced arthritis in rats:

Rheumatoid arthritis is a long term auto immune multisystem illness in which the body's immune system attacks the body's tissues and joints mistakenly causing an inflammatory synovitis which often progresses the destruction of joint ankylosis and articular cartilage. An autoimmune disease is a condition which arises from an abnormal response to our normal immune system. The immune system is a host defence mechanism comprising complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body. The synovium (inside of joints) is a thin delicate lining serves as an important source of nutrients for cartilage which thickens during RA resulting in inflammation and pain in and around the joints. Albino Wistar rats weighing 150-200 g were selected for the study. They were divided into five groups each containing six animals. All groups except control received 0.1 ml of CFA emulsion injected on sub plantar surface of right hind paw

Group 1 was negative control group, received vehicle p.o.

Group 2 was positive control group(untreated)

Group 3 received 100 mg/kg of Maha Analuruva Chooranam (MAC)

Group 4 received 200 mg/kg of Maha Analuruva Chooranam (MAC)

	Group - 1		Group - 2		Group - 3		Group - 4		Group - 5	
Time (Day)	Control volume		CFA volume		MAC 100mg volume		MAC 200mg volume		Standard volume	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	8.32±1.42	8.33±1.22	8.34±1.14	9.13±1.20	8.45±1.23	8.56±1.22	8.46±1.20	8.54±1.25	8.47±1.43	8.51±1.21
7	8.30±1.38	8.30±1.24	9.71±1.12	10.11±1.22	9.21±1.33	10.87±1.34	9.57±1.32	9.85±1.28	9.65±1.44	9.49±1.19
14	8.31±1.36	8.31±1.21	10.32±1.08	10.89±1.32	9.20±1.24	10.24±1.26	9.58±1.24	9.82±1.24	9.39±1.34	9.31±1.21
21	8.32±1.08	8.32±1.08	10.65±1.06	11.12±1.24	9.16±1.22	10.14±1.37	9.95±1.20	9.80±1.29	9.46±1.24	9.18±1.28

Group 5 STD group, received indomethacin (10mg/kg).

Assessment of inflammation and arthritis:

Test drug was administered p.o. once a day from the day of injection of induction and continued up to 14 days after treatment. The assessment of inflammation and arthritis was done on following parameters:

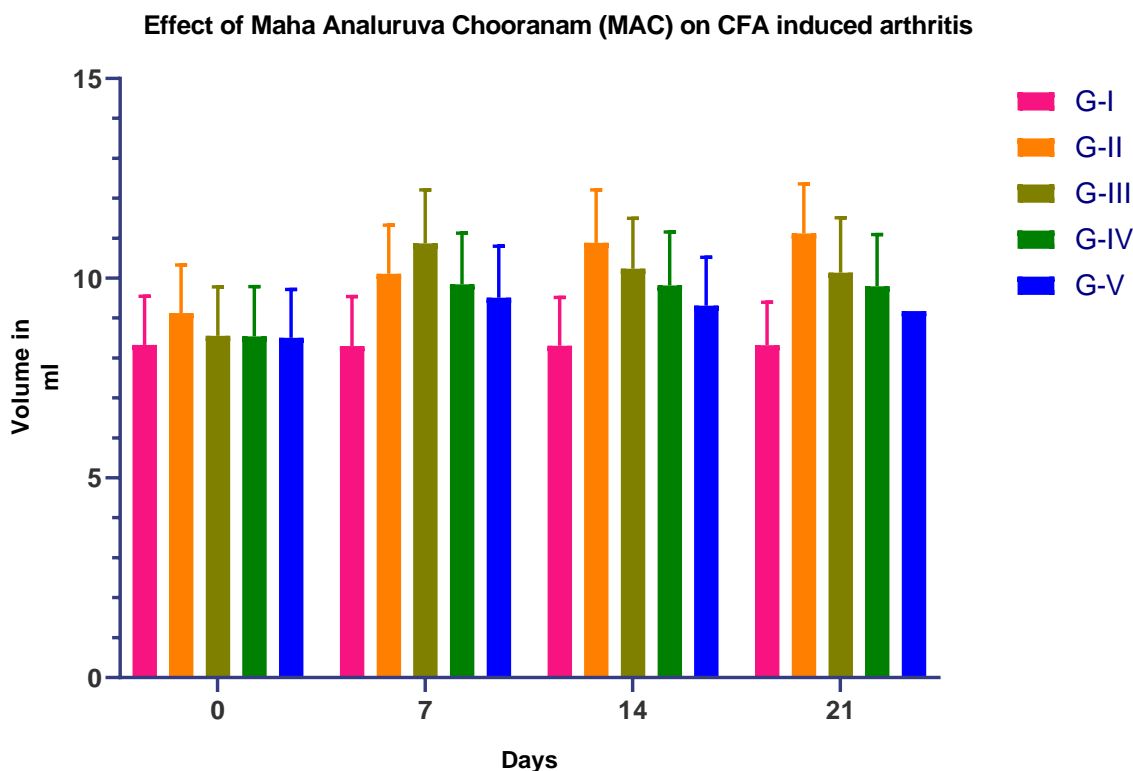
Paw volume: The change in the inflammatory reaction was measured using Digital plethysmograph on 0, 7, 14, and 21 days from the day of induction.

Statistical analysis:

The data were expressed as mean ± SEM for each of the parameters studied and were analysed using One-Way ANOVA by Graph pad 9, and Post hock analysis were done followed by Dunnet's test. P < 0.05 was statistically significant.

TABLE -3 Effect of Maha Analuruva Chooranam (MAC) on CFA induced arthritis

The data is expressed as mean±SEM; n=6, p<0.05



RESULT

The results, presented in the table, indicate that treatment with MAC at both dosages (100 mg/kg and 200 mg/kg) significantly increased the DTH response compared to the control group ($p < 0.05$) as determined by the Waller–Duncan test. The paw volume in the MAC-treated groups showed a statistically significant increase compared to the control group, suggesting an enhanced cellular immune response. The table summarizes the paw volume data for each group across the measurement time points. As expected, the control group (no CFA injection) maintained a consistent paw volume throughout the experiment. In contrast, the CFA-induced groups (Groups 2-5) exhibited a significant increase in paw volume compared to the control group, indicating the development of arthritis. When comparing the CFA-induced groups, treatment with MAC at both dosages (100 mg/kg and 200 mg/kg) showed a statistically significant reduction in paw volume compared to the untreated arthritic group (Group 2) at all time points ($p < 0.05$). This suggests that MAC treatment helped to alleviate inflammatory paw swelling. The effect was comparable to the standard drug, indomethacin (Group 5), indicating the potential effectiveness of MAC in managing CFA-induced arthritis in rats. Therefore, the results suggest that Maha Analuruva Chooranam (MAC) may possess immunomodulatory properties and exhibit anti-arthritic effects.

DISCUSSION

Immunomodulatory Activity: The DTH reaction assay indicated that MAC treatment (100 and 200 mg/kg) induced a statistically significant increase in paw pad thickness compared to the control group. This suggests that MAC may stimulate cell-mediated immunity, a key aspect of the immune response. However, further investigation is necessary to elucidate the specific mechanisms underlying this effect. **Anti-arthritic Activity:** The results from the CFA-induced arthritis model revealed promising anti-arthritic properties of MAC. CFA injection successfully induced arthritis in rats, as evidenced by increased paw volume compared to the control group. Notably, treatment with both doses of MAC (100 and 200 mg/kg) significantly reduced paw volume compared to the untreated CFA group. This suggests that MAC may possess anti-inflammatory properties that can alleviate joint inflammation associated with

arthritis. The observed effect was comparable to the standard drug, indomethacin, indicating the potential therapeutic efficacy of MAC. This study employed a limited sample size (n=6 per group) for both experiments. Further studies with larger sample sizes would strengthen the generalizability of the findings. Additionally, the specific bioactive components in MAC responsible for the observed effects remain unidentified. Future research should focus on isolating and characterizing these bioactive compounds to gain a deeper understanding of the mechanisms of action.

CONCLUSION

This study investigated the immunomodulatory and anti-arthritic effects of Maha Analuruva Chooranam (MAC) in rats. The findings suggest potential immunomodulatory properties and a protective effect against Complete Freund's Adjuvant (CFA)-induced arthritis. This study provides preliminary evidence for the immunomodulatory and anti-arthritic properties of Maha Analuruva Chooranam. The observed effects warrant further investigation to explore the therapeutic potential of MAC in managing inflammatory conditions like rheumatoid arthritis. Future research should focus on elucidating the specific mechanisms of action, conducting in vitro studies to identify bioactive components, and evaluating efficacy in larger pre-clinical models.

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REFERENCES

1. Jaijesh Paval, Srinivasan Keloth Kaitheri, Bhagath Kumar Potu, Sreejith Govindan, Raju Suresh Kumar, Sareesh Naduvil Narayanan, Sudheer Moorkoth, Anti-arthritic potential of the plant *Justicia Gendarussa* Burm F. *Clinical Science*, 2009, 64(4): 357-60.
2. Shruti Srivastava, Pradeep Singh, Keshri K. Jha, Garima Mishra, Sourabh Srivastava, Ratan L. Khosa, Evaluation of Anti-arthritic potential of the methanolic extract of the aerial parts of *Costus speciosus*. *Journal of Ayurveda & Integrative Medicine*, 2012, Vol 3 (4): 204-208.
3. S Tripathy, D Pradhan, M Anjana, Anti-inflammatory and Anti-arthritic potential of *Ammania baccifera* Linn. *International journal of Pharma and Biosciences*, 2010, Vol 1 (3): 1-7.
4. S Meera, N S Kumar, V S S S Guptayam, Screening of Anti-arthritic, Anti-inflammatory and Analgesic activity of a Polyherbal Formulation. *Int. J. Pharmacol*, 2008, 4(5): 398- 402
5. Agathiyar vaithiya vallathi 600, R.C. Mohan-thamarai noolagam 7, N.G.O. colony, vadapalani, Chennai 26, Pg 113.
6. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, *Indian Medicine and Homeopathy*, Second Edition 2006, Page no:113
7. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, *Indian Medicine and Homeopathy*, Second Edition 2006, Page no:248
8. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, *Indian Medicine and Homeopathy*, Second Edition 2006, Page no:431
9. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, *Indian Medicine and Homeopathy*, Second Edition 2006, Page no:39
10. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, *Indian Medicine and Homeopathy*, Second Edition 2006, Page no:39

11. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, Indian Medicine and Homeopathy, Second Edition 2006, Page no:500
12. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, Indian Medicine and Homeopathy, Second Edition 2006, Page no:336
13. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, Indian Medicine and Homeopathy, Second Edition 2006, Page no:115
14. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, Indian Medicine and Homeopathy, Second Edition 2006, Page no:113
15. K.S. Murugesu Mudhaliyar, Gunapadam- Mooligai Vaguppu, Indian Medicine and Homeopathy, Second Edition 2006, Page no:303
16. Gamble JS. Flora of the presidency of madras by J.S Gamble [Internet]. West, Newman and Adlard; 1970 [cited 2023 Jul 25]. Available from: https://openlibrary.org/books/OL7240405M/Flora_of_the_Presidency_of_Madras#:~:text=Flora%20of%20the%20Presidency%20of%20Madras%20was%20published,south%20India%20%28%22Madras%20Presidency%22%20under%20the%20British%20Rule%29
17. Kannusamipillai; Sikitcha Rathna Deepam Ennum Vaidhiya Nool; B. Rathna nayakkar and sons; edition 2014, page.no.28
18. Kannusamipillai; Sikitcha Rathna Deepam Ennum Vaidhiya Nool; B. Rathna nayakkar and sons; edition 2014, page.no.29
19. Kannusamipillai; Sikitcha Rathna Deepam Ennum Vaidhiya Nool; B. Rathna nayakkar and sons; edition 2014, page.no.31
20. Dr. P. Parthipan MD(S) (2008) Sarakku - suththi seimuraigal. Chennai-106, Tamilnadu: Indian medicine and homeopathy department, page no: 14