

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *AMAVATARI RASA* BY USING ADJUVANT INDUCED RAT PAW VOLUME METHOD

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ABSTRACT

The disease *Amavata* was first mentioned in *Madhava nidana*. The main Pathogenic factor in the disease is *Ama*, which is formed due to deranged *Agni*. *Amavata* can be compared to Rheumatoid Arthritis; RA is a long lasting auto immune disorder that primarily affects joints. One of the main problems in RA is joint inflammation. RA affects between 0.5 to 3% of adults in the developed world is most frequent during middle age and women are affected 3 times as frequently as men. There are many formulations mentioned in Ayurvedic classics for *Amavata*, among them *Amavatari Rasa* is one of the herbo mineral & *Khalveeya Rasayana*. The purpose of the present study was to observe the effect of *Amavatari Rasa* on inflammation in RA. Freund's Complete Adjuvant (FCA) was used to induce arthritis in rats. Randomly selected 30 Female Albino rats were equally divided into 5 groups of 6 rats each. Rats were injected with 0.1 ml of Freund's Complete Adjuvant (FCA) into the plantar region of the left hind paw. The paw volume of both the hind paws was measured using a Digital Plethysmometer on 1st, 7th, 14th and 21st days of treatment. In Disease Control group, inflammation consistently increased. Where as in Standard (Prednisolone induced) and *Amavatari Rasa* treated (Group IV- TED and Group V- 2 TED) groups showed significant decrease in the inflammation.

Keywords: *Amavata*, Rheumatoid arthritis, *Amavatari Rasa*, *Khalveeya Rasayana*, FCA

INTRODUCTION

Ayurveda, the science of health has its roots from ancient times. This is being evolved in parallel with human race. The supremacy of *Ayurveda* lies in its Holistic approach towards patient care. *Rasa shastra* is a branch of Ayurvedic medicine, which deals with metals and minerals to produce the drugs with higher efficacy in lower doses. In today's scenario, *Amavata* is one of the serious problems among all

joint disorders. A disease resembling clinically Rheumatoid arthritis (RA) is described in Ayurvedic text as *Amavata*.

Amavata as a separate disease entity was described for the first time in detail by *Madhavakara* in his famous treatise called *Madhava Nidana*, dealing with the aetiopathogenesis of the disease in a systematic manner with the signs, symptoms, complica-

tions and prognosis. *Amavata* has been named, taking into account the predominant pathological factors i.e. *Ama* and *Vata*, as they have important place in *Nidana* and *Chikitsa* of this disease.¹

Amavata occurs in all over the world in all races, genders, age and climates and is equated with Rheumatoid Arthritis by the scholars. RA is a long lasting auto immune disorder that primarily affects joints. It typically results in warm, swollen and painful joints. One of the main problems in RA is joint inflammation. In affected joints, the thin synovium grows thicker. The joint swells and the cartilage protecting the ends of the bones in the joints gets damaged. This disease is two or three times more prevalent among females than the males. *Shamana* and *Shodhana Chikitsa* are advised in *Amavata* whereas Analgesics, NSAID and DMARD are required for the management in modern medicines.^{2,3}

The principle aim of the Ayurvedic management in *Shamana chikitsa* is to improve status of *Agni* and thus to break the formation of *Ama* and effective in treating vitiated *Vata dosha* i.e. the drug which fulfils *Deepana*, *Pachana* and *Anulomana* properties.⁴ So the drug selected for this study is *Amavatari Rasa* with the reference of *Bhaishajya Ratnavali*⁵ which is the combination of herbo-mineral ingredients. The main aim of the study is to evaluate the anti-inflammatory activity of *Amavatari Rasa* by using FCA induced rat paw volume method.

OBJECTIVE

To study the anti-inflammatory effect of *Amavatari Rasa* by FCA induced rat paw volume method.

MATERIALS AND METHODS

Source of drug:

All the required drugs were collected from authenticated dealers.

Locale of the work:

The preparation of *Amavatari Rasa* was carried out in Department of PG studies in Rasa Shastra and Bhaishajya Kalpana, Government Ayurvedic Medical College and Hospital, Bengaluru.

The experimental study was carried out in Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bengaluru.

Selection of animals:

The required numbers of healthy female Wistar Albino rats weighing 120-200 gms were procured from Acharya & B.M Reddy College of Pharmacy, Bengaluru.

Housing and feeding condition:

Animals were kept in polypropylene cages with paddy husk bedding. The temperature in the experimental room was around 25°C. Lightening was natural sequence being 12 hours dark, 12 hours light. They were provided standard food pellets and water *ad libitum*.

All the experiments were performed in the morning according to the guidelines for the care of laboratory animals. The experimental procedures were carried out in accordance with the ethical guidelines for animals proposed by the government of India. The approval of the ethical committee was obtained before performing the experiments. Ethics Committee (IAEC) with reference no: IAEC/ABMRCP/2016-2017/23

Grouping:

Randomly selected 30 Female Albino Rats were equally divided into 5 groups of 6 rats each. In each group the animals were marked with yellow colour to permit individual identification i.e. at the region of head, body, tail, front leg, back leg and no mark. Each group of 6 rats were kept in separate polypropylene cages denote the number from 1 to 5 respectively, for one week prior to dosing to allow acclimatization of them to laboratory conditions.

Table 1: Showing the Details of Grouping

Group	Purpose	Drugs	No. of animals
Group I	Normal control	Distilled water	6
Group II	Disease control	FCA induced + 1% Gum acacia	6

Group III	Standard group	FCA induced+ 1% Gum acacia+ Prednisolone	6
Group IV	Trail group I	FCA induced + 1% Gum acacia+ <i>Amavatari Rasa</i> (TED)	6
Group V	Trail group II	FCA induced + 1% Gum acacia+ <i>Amavatari Rasa</i> (2 TED)	6

Drug and Chemicals:

Picric Acid, FCA, Gum Acacia, Prednisolone, *Amavatari Rasa* and Distilled Water.

Equipments:

Tuberculin Syringe (1ml Capacity), Gavage Needle, Gloves, Digital Plethysmometer, Mortar and Pestle.

Injection of disease: FCA induced arthritis

Rats were injected with 0.1 ml of Freund’s Complete Adjuvant (FCA) into the plantar region of the left hind paw. A mark was put on the leg at the maleous region of the rat to facilitate the dipping of the leg into the Plethysmometer. The paw volume of both the hind paws was measured using a Digital Plethysmometer and body weight was recorded on the day of adjuvant injection considered as 0th day.

Administration of doses:

Animals were kept for 12 hours of fasting. Then assigned drugs were administered orally using a gavage needle in a calculated dose.

Dosing

Table 2: Comparison between drugs and days

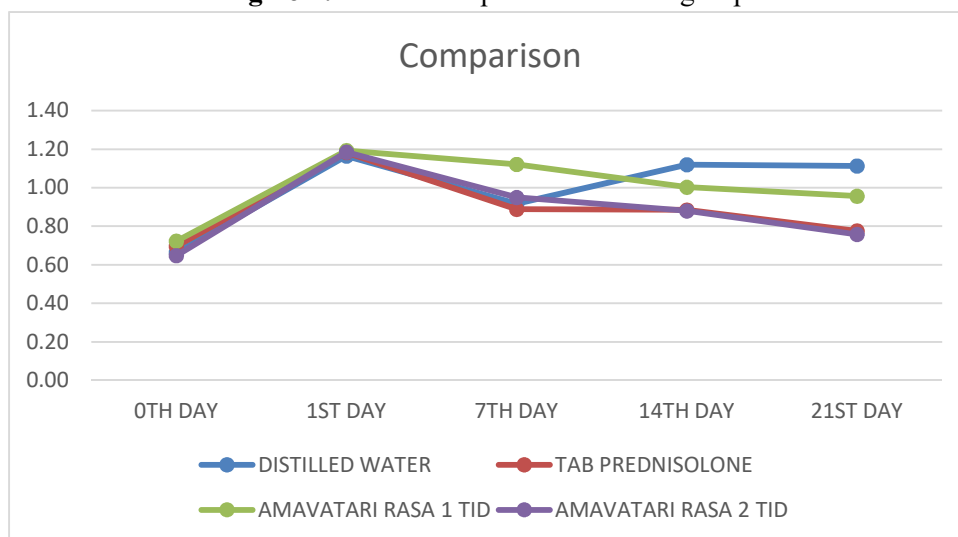
Group	Drugs	0 th Day	1 st Day	7 th Day	14 th Day	21 st Day
Group II	Distilled water	0.67	1.17	0.92	1.12	1.11
Group III	Tab Prednisolone	0.70	1.18	0.89	0.89	0.78
Group IV	<i>Amavatari Rasa</i> (TED)	0.72	1.19	1.12	1.00	0.96
Group V	<i>Amavatari Rasa</i> (2 TED)	0.65	1.19	0.95	0.88	0.76

The initial reading was taken before the injecting FCA. After one day of injection, dosing was started. *Amavatari Rasa* in TED (45mg/kg bodyweight) and 2 TED (90 mg/kg bodyweight) dose was administered orally for Group IV and V respectively. Prednisolone (10mg/kg) was used as standard drug. The paw volume was measured on 1st, 7th, 14th and 21st day of treatment. The percentage inhibition of paw volume in drug treated group was compared with the control group.

RESULTS:

In Standard Group (Group III) though it showed statistically insignificant result at the end of 21st day, experimentally significant result has been found. Statistical insignificance may be because of higher standard deviation value on 21st day results. In *Amavatari Rasa* treated Group IV and V Statistically & Experimentally significant result has been found.

Fig no 1: Linear Comparison between groups



STATISTICAL RESULTS

Table 3: Showing statistical results.

	0 th Day	1 st Day	7 th Day	14 th Day	21 st Day
Control	0.665±0.102	1.165±0.027	0.917±0.073	1.120±0.068	1.113±0.054
Standard	0.697±0.106	1.183±0.029	0.937±0.175	0.885±0.201	0.777±0.154
% Inhibition	4.545	1.549	2.135	26.554	43.348
Trial I	0.723*±0.073	1.193*±0.035	1.122*±0.075	1.003*±0.063	0.957*±0.071
% Inhibition	8.065	2.374	18.276	11.628	16.376
Trial II	0.648*±0.112	1.185*±0.037	0.950*±0.154	0.880*±0.204	0.758*±0.088
% Inhibition	2.571	1.688	3.509	27.273	46.813
F Value	0.676	0.808	3.283	3.431	17.031
DF	3,20	3,20	3,20	3,20	3,20
P Value	0.577	0.504	<0.05	<0.05	<0.05

DISCUSSION

In the present study an attempt has been made to evaluate the anti-inflammatory activity of *Amavatari Rasa* by FCA induced rat paw volume method. The FCA induced arthritis model is widely used for evaluating anti-arthritis activity of drugs. It shares a number of clinical and immunological features with human arthritis; therefore FCA model has a high degree of validity.

In the present study randomly selected 30 rats weighing between 120-200gms were equally divided into 5 groups of 6 each. The Group I of Normal Control, Group II of Disease Control was administered 1% Gum acacia in rat dose of 5ml/kg body weight,

In Group III of Standard was Prednisolone in 10mg/kg body weight, in Group IV of Trail drug 1 (*Amavatari rasa TED*) was treated with 45mg/kg body weight and lastly Group V of Trail drug 2 (*Amavatari rasa 2 TED*) was treated with 90mg/kg body weight PO.

The study revealed that *Amavatari rasa* possess significant anti-inflammatory activity in experimental animals at the dose of 45mg/kg body weight and highly significant at the dose of 90mg/ kg body weight PO.

CONCLUSION

The reference of the present study was adopted from *Bhaishajya Ratnavali, Amavata rogadhikara. Shodhana, Bhavana, Mardana, Swedana and Vati nirmana* are the important pharmaceutical procedures involved in the preparation of *Amavatari Rasa*. *Amavatari Rasa* comes under *kharaleeya rasayana* and a *Vati kalpana*, which is one of the easy and widely used dosage forms.

Normal Control Group showed no change in their physiological conditions. Disease Control Group

showed continued inflammation over the period of experiment. Standard drug showed experimentally significant and statistically insignificant results.

Experimental study proved that *Amavatari Rasa* in Group V (2 TED) is more significant when compared to *Amavatari Rasa* in Group IV (TED). With the factual evidence obtained by the above experimental data, it has been concluded that the *Amavatari Rasa* is having significant anti-inflammatory activity.



Injection of FCA into



Inflammation after injection



Digital plethysmometer Left plantar

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