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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SIDDHA HERBO-MINERAL FORMULATION RATHINAGARA RASA MEZHUGU IN ANIMAL MODEL

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ABSTRACT The usage of Nonsteroidal and steroidal drugs in the treatment of Musculoskeletal disorders often results in adverse effects such as renal damage, gastric irritation, etc. Alternative therapies with less or no side effects are the need of the hour. Siddha system offers a lot of Anti-inflammatory drugs that are polyherbal or Herbo-mineral in nature and devoid of the above-mentioned adverse effects. This research work deals with the Anti-inflammatory screening of a Siddha Herbo-mineral Formulation Rathinagara rasa mezhugu (RNM) documented in classic Siddha literature Anuboga vaidhaya naveneetham Part 5 indicated for inflammatory disease conditions. Anti-inflammatory activity was evaluated by Carrageenan-induced paw edema in Wistar rats. Indomethacin (10 mg/kg, orally) was used as standard. The formulation in doses of 20mg/kg, 45mg/kg, and 90 mg/kg showed 56%, 61.53%, and 90.47% inhibition of paw edema, respectively at the end of 5 h. The result of the study showed that RNM at all dose levels significantly($p<0.5$) reduced the inflammation in a dose-dependent manner when compared to the control. RNM at 90mg/kg was found to be significant (**$p<0.01$) and effective in the reduction of paw edema after 5hrs.From the results, it can be concluded that Rathinagara rasa mezhugu (RNM) was found to exhibit high anti-inflammatory potential which confirmed the traditional use of the drug.

KEYWORDS : Musculoskeletal disorders, Siddha medicine, Rathinagara rasa mezhugu, Carrageenan, Anti-inflammatory activity

1. INTRODUCTION

Musculoskeletal disorders (MSDs) are one of the commonest causes of long-term pain and disability, affecting millions of people. It is the second most common cause of disability in terms of Years Lost Due to Disabilities (YLD's) with low back pain being the most frequent condition. These disorders cause pain and discomfort which interfere with daily activities (Storheim K et al.,2014; Vos T et al., 2012). There is an increase in the incidence of MSD's that affects globally. The global prevalence of MSDs ranges from 14% to as high as 42%; on the other hand, in India, epidemiological studies indicate a community-based prevalence of about 20% (Woolf AD et al.,2003) and occupation-specific prevalence found to be as high as 90% in various studies. In addition to this, the World Health Organization (WHO) also estimates that 40% of people over the age of 60 years suffer from MSD and about 80% of the people have had low back pain at some point in their life (Sharma R .2012). For these ailments, analgesics, anti-inflammatory, and corticosteroids are the drugs of choice. Inflammation is a biologically occurring reaction inside vascular tissues when exposed to any destructive stimuli (White M J .1999).The process of inflammatory reactions can be classified as acute or chronic, wherein acute inflammation is of short duration with sudden onset. Acute inflammation starts with initial transient arteriolar constriction followed by vasodilation, increased vascular endothelial permeability, exudation of fluid and plasma proteins, and transmigration of leukocytes from vessels into the injured tissues. Chronic inflammation starts after 2-4 days of onset of the acute response and is mainly associated with tissue destruction and inflammation that occurs at the same time or granuloma formation(Dr Nishant S Jain.2021).In general, Steroidal, and nonsteroidal medications are employed in the treatment of inflammation. Conventional or synthetic drugs used in the treatment of diseases are sometimes inadequate and can have serious adverse effects. Prolonged use of higher doses of NSAIDs produces analgesic nephropathy, salt, water retention, and Gastric irritation and many of these drugs produce hepatotoxicity and nephrotoxic chronic usage (Padmaja udhyakumar 2021; Brunton LL et al.,2006). Therefore, it is necessary to find anti-inflammatory substances that will work and have a better safety profile. It is a known fact that Traditional medicines play a significant role in meeting international healthcare needs with less or no adverse effects. They are continuing now and will continue to play a significant role in the future (Ravishankar B et al.,2007). Efforts must be made to introduce new medicinal plants to develop cheaper drugs (Ikram M.1983). Thousands of plant-based formulations are used in the Indian traditional system (Verma S et al.,2008). The Siddha medicines are efficient, time-tested, and devoid of severe side effects. This treatment branch is wholly based on biotic medium; natural, herbal (*Thavaram*), Inorganic (*Thathu*), and animal products (*Jeevam*)as innovative medicinal resources (Ranga R S et al.,2004). This research work deals with the In vivo Anti-inflammatory activity of Siddha Herbo-mineral formulation *Rathinagara rasa mezhugu* (RNM) documented in Classic Siddha literature '*Anuboga vaidhaya naveneetham Part 5* (Hakeem. Pa. Mu. Abdullasayub,2014). Even though this medicine has several therapeutic uses and few research studies have been conducted on its therapeutic potential, no systematic experimental studies were conducted to delineate its effect on inflammation and associated pain. So, an attempt had been taken to study the Anti-inflammatory activity of the drug in experimental animals.

2.MATERIALS AND METHODS

Evaluation of Anti-Inflammatory Activity of *Rathinagara rasa Mezhugu* by Carrageenan induced hind paw edema method in Wistar Albino Rats

The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and oedema.

The anti-inflammatory activities of *Rathinagara rasa mezhugu* at 20mg/kg 45mg/kg& 90mg/kg doses were evaluated using carrageenan-induced paw edema method.

Procurement and rearing of experimental animal

a. Species/Common name	: Rattus norvegicus/ Wistar albino rat
b. Age / weight / size	: 140-160gms, 6-8 weeks
c. Gender	: both sexes
d. Acclimatization Period	: 7 days prior to dosing
e. Housing	: Polypropylene cages
f. Husbandry	: 12-h light/12-h cycle photoperiod 22°c (±3°) and relative humidity 30–70%
g. Feed and water	: Rodent pellet feed , RO Purified water ad libidum
h. Identification	: Animals were kept in individual cages and identified/marked using Picric acid

Experimental Animals Healthy adult Wistar albino rats weighing

between 140-160 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 + 2 0C and relative humidity 50–65%. They were provided with food 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 National Institute of Siddha (NIS/IAEC-22/R02/16112021/E4) Chennai, Tamil Nadu, India.

The experimental protocol:

Animal grouping:

The animals were divided into 5 groups of 6 animals each. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan with normal saline, in the right hind paw of the rats. In which group I served as control was administrated with vehicle (Palm jaggery) + Carrageenan (0.1ml of 1% w/v). The second group receives Indomethacin (5mg/kg, p.o) + Carrageenan (0.1ml of 1% w/v). The third group receives test drug RNM at the dose of 20mg/kg, p. o+ Carrageenan (0.1ml of 1% w/v). animal belongs to group IV received test drug RNM at the dose of 45mg/kg, p.o + Carrageenan (0.1ml of 1% w/v). Animal belongs to group V received test drug RNM at the dose of 90mg/kg, p.o 'at 1hr before inducing paw edema.

Evaluation of Anti-inflammatory potential:

Paw edema was induced by injecting 0.1ml of 1% carrageenan into sub plantar tissues of right hind paw of rats. Rathinagara rasa Mezhugu at the dose of 20 mg/kg, 45mg/kg, 90mg/kg were administrated orally 30 min prior to carrageenan administration. The paw volume was measured 1,2,3,4,5 hrs after carrageenan injection by the mercury displacement method using plethysmograph. Percent inhibition (%IE) of edema is calculated according to the following formula,

$$\%IE = Vc\text{-}Vt / Vc \times 100$$

Where,

Vc is the inflammatory increase in paw volume in control group of animals and Vt is the inflammatory increase in paw volume in drug treated animals. Inhibition of Paw volume in drug – treated group was compared with the control group (Group 1), where indomethacin (10mg/kg. p.o) was used as reference drug.

Data analysis:

The data are expressed in mean ± SEM. Results were analysed using one way ANOVA followed by Dunnett's test. Differences were considered as statistically significant if p□ 0.05. when compared with control.

3. RESULTS AND DISCUSSION

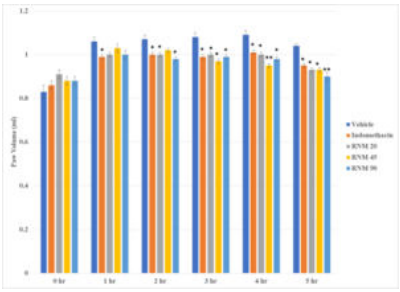
The time of course of edema development in carrageenan-induced rat paw edema model in rats is generally represented by Mean±SEM (n=6)1.04±0.01 in the control group, treatment with drug RNM at 20mg/kg, 45mg/kg,90mg/kg has 0.93±0.01,0.93±0.01, 0.90±0.02, 0.95±0.01 in a standard group at 5 hrs. The effect of Rathinagara rasa mezhugu was tabulated in table no.1 and graph no. 1

Table no.1: Effect of Rathinagara rasa mezhugu by Carrageenan-induced Paw edema in Rats.

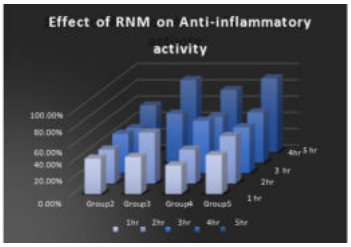
Treatment	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Vehicle	0.83±0.03	1.06±0.02	1.07±0.02	1.08±0.02	1.09±0.02	1.04±0.01
Indomethacin	0.86±0.02	0.99±0.01*	1.00±0.01*	0.99±0.01*	1.01±0.01*	0.95±0.01*
RNM 20	0.91±0.02	1.00±0.01	1.00±0.01*	1.00±0.01*	1.00±0.01*	0.93±0.01*
RNM 45	0.88±0.02	1.03±0.02	1.02±0.01	0.97±0.01*	0.95±0.01**	0.93±0.01*
RNM 90	0.88±0.02	1.00±0.02	0.98±0.01*	0.99±0.01*	0.98±0.01*	0.90±0.02**

Values are in Mean±SEM (n=6)
*P<0.05, **P<0.01 & ***P<0.001 Vs Vehicle

The data are expressed in mean ± SEM. Results were analysed using one-way ANOVA followed by Dunnett's test. Differences were considered statistically significant if p□ 0.05. when compared with the control. p value ***P<0.001 is extremely significant, **P<0.01 and *P<0.05 were considered as significant and P>0.05 indicates non-significant.



Graph no.1 Effect of RNM on carrageenan induced paw edema in Rat. Mean of paw edema volume(ml) is measured in 0,1,2,3,4,5 hrs. Indomethacin (10mg/kg) is standard drug is compared with RNM at 20mg/kg,45mg/kg,90mg/kg. Data are expressed in Mean±SEM (n=6)



Graph no.2 - Effect of RNM carried out by Carrageenan induced paw edema in Rat.Inhibition of paw edema is measured in 0, 1, 2, 3 , 4 , 5 hrs in percentage (%). Data are expressed in percentage.

Inflammation is a systemic and local response of living tissues after injury to it. The factors that can cause inflammation are bacteria, viruses, fungi, parasites, antigen-antibody reactions, mechanical trauma, organic and inorganic poisons, and foreign bodies. These defence inflammatory reactions help the body prevent the spread of injurious agents and to remove dead or damaged cells and tissues. Acute inflammation has three components. They are increased blood flow, structural changes in microvasculature that allows plasma protein and leucocytes, and emigration of leucocytes from microcirculation and their accumulation in the focus of injury. Inflammation is regarded as the main cause of the development of many human diseases including neurological, intestinal, cardiovascular, dental, and renal disorders (Lumeng C. N et al.,2011; Kuek et al.,2006; Marchant D. J et al.,2012).The current study aimed at the evaluation of the effect of the Siddha Herbo-mineral formulation Rathinagara rasa mezhugu (RNM)by Carrageenan-induced paw edema in Wistar albino rats. Edema induced by phlogistic agents is a widely accepted model for the evaluation of the anti-inflammatory effect of drugs (Winter CA et al,1962; El-Shenawy SM et al.,2002). Carrageenan-induced rat paw model in rats is known to be sensitive to cyclo-oxygenase inhibitors. Cyclooxygenase is an important enzyme in the production of prostaglandins, prostacyclin, and thromboxane which are involved in inflammation, pain, and platelet aggregation. Steroidal and non-steroidal anti-inflammatory drugs are currently the most used drugs in the treatment of acute inflammatory disorders(Sunita Verma.2016).These drugs obstruct cyclooxygenase enzyme activity results to prevent prostaglandin production. Prostaglandins play a major role in the development of second phase of inflammatory reaction. Carrageenans are a family of linear sulphated polysaccharides that are extracted from red edible seaweeds. Carrageenan induces rat paw edema in three distinct phases associated with various biochemical events. The first phase initially (1 h) involves the degranulation of mast cells with histamine and serotonin release followed by the second phase (60 to 150 min) with bradykinin release and induction of pain. The late phase (3-4 h) is then characterized by eicosanoid production. Thus, the phase-wise manner effectiveness of the test anti-inflammatory agent can reveal the mechanism by which it acts using this test(Vinegar R et al.,1969).

As shown in Graph no. 1 Rathinagara rasa mezhugu (RNM) at 20 mg/kg dose showed significant Anti-inflammatory activity at (p<0.5) in the 3rd, 4th, and 5th hour when compared to the control group. RNM at

45mg/kg showed significance ($p<0.01$) in the 4th hour. RNM at 90mg/kg showed significant activity at ($p<0.5$) in the 2nd,3rd, and 4th hour and ($p<0.01$) in the 5th hour when compared to the control group. It caused 56%, 61.53%, and 90.47% inhibition as compared to that of 10mg/kg of indomethacin and the control group. Therefore, it can be inferred that the inhibitory effect of Rathinagara rasa mezhugu on carrageenan-induced inflammation may be due to the inhibition of cyclo-oxygenase leading to the inhibition of prostaglandin synthesis. RNM at 90mg/kg was found to be significant (**$p<0.01$) and effective in the reduction of paw volume after 3 hrs. So, from the above points, this study proved the test drug Rathinagara rasa mezhugu has Anti-inflammatory activity.

It is also evident from the review of literature, that the ingredients in the formulation are proven to be effective anti-oxidant immune-modulators, and anti-inflammatory agents based on the pharmacokinetics of their bioactive compounds. The phytoconstituents of ingredients (Ricinus communis, Semecarpus anacardium) Such as alkaloids, phenolic compounds, bioflavonoids, sterols and glycosides, triterpenoids, demonstrated anti-inflammatory activity in various experimental models. Ilavarasan et al., 2005 studied the free radical quenching activity and anti-inflammatory properties of the methanolic extract of Ricinus communis on Wistar albino rats. In this study methanolic extract inhibited lipid peroxidation which was induced due to the ferrous sulphate and carbon tetrachloride in rat kidney and liver homogenates (Ilavarasan R et al.,2006). Anil Kumar et al. studied the anti-inflammatory activity of R. communis methanolic extract due to the presence of flavonoids because the flavonoids have a protective effect against carrageenan-induced paw edema in rats (Anil Kumar Saini edt L.,2010).M J Bhitre et al., investigated the anti-inflammatory effects of SA nut extract on developing and developed adjuvant arthritis. Semecarpus anacardium significantly decreased the carrageenan-induced paw edema and cotton pellet granuloma. These results indicate the potent anti-inflammatory effect and therapeutic efficacy of SA Nut extract against all phases of inflammation is comparable to that of indomethacin (Bhitre MJ et al.,2008). Da Yeon Lee et al., studied that the Sulphur compounds inhibited the production of nitric oxide (NO) and prostaglandin E2 (PGE2) and the expression of the pro-inflammatory cytokines tumor necrosis factor-α, interleukin-1β, and interleukin-6 in lipopolysaccharide (LPS)-activated macrophages(Da Yeon Lee et al.,2012).The result obtained from the previously established data in the literature shows that the ingredients of RNM Showed significant Anti-inflammatory activity in various scientific studies. By considering all the factors, it can be suggested that Rathinagara rasa mezhugu (RNM)has remarkable medicinal value in treating life-threatening inflammatory conditions. Results and discussion give the necessary justification to prove the effectiveness of the drug as an anti-inflammatory agent.

4. CONCLUSION

The Drug Rathinagara rasa mezhugu (RNM) showed dose dependant anti-inflammatory activity in Carrageenan-induced edema in Wistar rats. The effect may be attributed to the presence of anti-inflammatory agents through the inhibition of COX-2. More specific animal models and Clinical trials are required to understand the exact molecular mechanisms of action. So that it can be used as a safe, cost-effective treatment for inflammatory disease conditions.

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Conflict of interest: Nil

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