

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SIVATHAI CHOORANAM IN WISTER RATS

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ABSTRACT

Aim: To study the Anti-inflammatory effect of Sivathai Chooranam (SC) in the Male Wister rats of Carrageenan- induced localized inflammation. **Method:** The paw oedema volumes of the test compounds, standard and control groups were measured at 0, 1, 2, 3, 4, 5, 6th hr after Carrageenan treatment with the help of Digital Plethysmometer. Mean increase in paw oedema volume after Carrageenan injection was measured and the percentage of inhibition was calculated.

Drug and solutions

1. Carrageenan (0.1ml of 1%)
2. Diclofenac sodium (10mg/kg IP)
3. Sivathai Chooranam 1gm BD.

Results: The results showed significant ($p < 0.05$) anti inflammatory activity when compared to control group. **Conclusion:** The test drug SC shows potent Anti-inflammatory activity at the concentration of 3.024mg/kg compared to standard drug, Diclofenac Sodium.

KEYWORDS: Siddha medicine, Sivathai Chooranam, Anti-inflammatory activity, Carrageenan induced localized inflammatory pain, Male Wister rats.

INTRODUCTION

An anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signalling to the brain. Prostaglandins are hormone-like substances that affect the body in variety of ways, also regulating inflammatory mediation. An anti-inflammatory diet includes fewer foods that create inflammation-causing

prostaglandins (PGE-2) in the body, and more foods that create anti-inflammatory prostaglandins (PGE-1 and PGE-3).^[1] The rats were acclimatized for 3 days to the laboratory conditions and were identified by a unique tail marking using permanent red marker pen. During the acclimatization, individual animal was subjected to daily general observation and prior to final assignment to the study the animals were subjected to a detailed clinical examination to ensure the selected rats were in a good state of health. Intra plantar injection of carrageenan into the hind paw produces localized inflammation in rats. An intra plantar injection of carrageenan is widely used to produce a model of localized inflammatory pain. In this study the anti-inflammatory effect of Subcutaneous in the rat model of carrageenan-induced localized inflammation.

Ingredients of Sivathai Chooranam

1. Karum shivathai (*Operculina turpethum*), 2. Kadukkai (*Terminalia chebula*), 3. Thandrikai (*Terminalia bellirica*), 4. Kothamalli (*Coriandrum Sativum*), 5. Saathikkai (*Myristica fragrans*), 6. Vaividangam (*Embelia ribes*), 7. Chukku (*Zingiber officinale*), 8. Milagu (*Piper nigrum*), 9. Lavangam (*Syzygium aromaticum*), 10. Nattu sarkkarai (Cane sugar).^[2]

Table: 1.

S.no	Botanical name	Family	Tamil name	Activities
1	<i>Operculina turpethum</i>	Convolvulaceae	Karum sivathai	Anti-inflammatory, Analgesic, Antioxidant, Anti-ulcer, Anti-diabetic, Anti-diarrhoeal, Hepato- protective, Anti-microbial, Anti-cancer ^[3]
2	<i>Terminalia chebula</i>	Combretaceae	Kadukkai	Analgesic, Anti-inflammatory ^[4]
3	<i>Terminalia bellirica</i>	Combretaceae	Thandrikkai	Analgesic, Antioxidant, Antimicrobial, Anti diarrhoeal, Antihypertensive, Hepatoprotective ^[5]
4	<i>Coriandrum sativum</i>	Apiaceae	Kothamalli	Analgesic, Anxiolytic ^[6]
5	<i>Myristica fragrans</i>	Myristicaceae	Saathikkai	Anti-inflammatory, Analgesic, Anti-convulsant, Antibacterial, Antifungal, Antioxidant ^[7]
6	<i>Embelia ribes</i>	Primulaceae	Vaividangam	Analgesic, Anthelmintic, Antianxiety,

				Antibacterial, Anticancer, Anticonvulsant, Antidepressant, Antifungal, Antihistamic, Antidiabetic ^[8]
7	Zingiber officinale	Zingiberaceae	Chukku	Analgesic, Anti-inflammatory ^[9]
8	Piper nigrum	Piperaceae	Milagu	Analgesic ^[10]
9	Syzygium aromaticum	Myrtaceae	Lavangam	Antimicrobial, Analgesic, Antioxidant, Anticancer, Anthelmintic, Antiulcer, Anti-inflammatory, Anti-depressant, Bone preserving, Antipyretic, Antithrombotic ^[11]

The raw drugs were collected at Tirunelveli, Tamilnadu. It was then purified by to remove dust and impurities and made into fine powder.

Study Guidelines: The study plan was developed based on the guidelines of Vogel^[12] and also it has reference to Chao Ma and Jun-Ming Zhang^[13] and Walker et al.^[14] Winter CA, Risley EA, Nuss GW. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med., 1962; 111: 544–7.

Requirements of Animals

- ❖ Species: Rat
- ❖ Strain: Wister
- ❖ Age: 6-8 weeks at the time of dosing
- ❖ Total no. of Rats: 30
- ❖ Sex: Male
- ❖ Drugs and chemicals: 0.1ml of 1% Carrageenan, Diclofenac sodium (standard),
- ❖ Apparatus: Digital plethysmometer.
- ❖ Test compound: Sivathai Chooranam (SC).

Acclimatization

The rats were acclimatized for 3 days to the laboratory conditions and were identified by a unique tail marking using permanent red marker pen. During the acclimatization, individual animal was subjected to daily general observation and prior to final assignment to the study

the animals were subjected to a detailed clinical examination to ensure the selected rats were in a good state of health.

MATERIALS AND METHODS

In this study, Male Wister rats are selected totally 30 in numbers and the age is 6-8 weeks at the time of dosing. Male Wister rats were housed in polypropylene cages with stainless steel top grills having facilities for holding pellet food and drinking water in bottle with stainless steel sipper tube. Each cage contained 6 rats. All rats had free access to potable water and standard pelleted laboratory animal diet ad libitum. Paddy husk was used as bedding material. Approval No: KMCRET/ MD(S)/ 02/ 2016-17 by the Institutional Animal Ethical Committee (IAEC) of KMCH College of Pharmacy, Coimbatore (685/PO/Re/S/2002/CPSCEA) Dated 21st August 2002 constituted in accordance with the guidelines of the CPCSEA, Government of India.

Drugs and chemicals

The animals were housed in polypropylene cages with stainless steel top grills having facilities for holding pellet food and drinking water in bottle with stainless steel sipper tube. Each cage contained 6 rats. All rats had free access to potable water and standard pelleted laboratory animal diet ad libitum. Paddy husk was used as bedding material. The animals were divided into 5 groups (6 rats/ group). Localized inflammatory pain was induced in all groups of animals by intra plantar injection of carrageenan (0.1ml of 1%). Group 1 received vehicle orally, Group 2 received a standard analgesic drug, Diclofenac sodium (10 mg/kg IP), whereas groups 3, 4 and 5 received Sivathai Chooranam 3.024mg, 15.12mg and 75.6mg b.wt. The doses of Sivathai Chooranam were prepared in Honey, where as Diclofenac sodium was dissolved in normal saline.

One day before the experiment, three basal readings of hind paw in each rat were recorded. Group I received (0.1ml of 1% carragennan), Group II animals received Diclofenac sodium (10 mg/kg IP). Group- III, IV and V animals received the Sivathai Chooranam 3.024mg, 15.12mg and 75.6mg b.wt. After 30 min, the rats were challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan into the sub plantar region of left paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The paw volume was measured at 0, 1, 2, 3, 4, 5 and 6th hr after carrageenan injection using Digital Plethysmometer. The difference between initial and subsequent reading gave the actual oedema volume.

Dosage schedule

The required dose for mice/rat will be calculated by using the standard dose calculation procedure from recommended clinical dose.

Conversion formula

- ❖ Human dose is 1g day
- ❖ Total clinical dose (a) x conversion factor (b) 0.018 = (c) per 30 gm of mice
- ❖ 1000 mg x 2(a) x 0.018 (b) = 18 (c) /140gms of mice
- ❖ 108/1000x140 = 15.12 mg

Experimental Doses Calculated as per the standard procedures are:

Table: 2.

S.no	Groups	Dose /kg, weight	Dose /30 gms weight	Volume of administration
1	Vehicle Control	--	--	1 ml
2	Therapeutic Dose	15.12 mg	3.024mg	1 ml
3	Middle Dose	75.6mg	15.12mg	1 ml
4	High Dose	378mg	75.6mg	1 ml

Treatment received by each group - Experimental design

Group-I: Served as a negative control (0.1ml of 1% Carrageenan)

Group-II: Served as standard received Diclofenac sodium (10mg/kg, IP) + (0.1ml of 1% Carrageenan)

Group-III: Received Sivathai Chooranam (3.024mg/kg) + (0.1ml of 1% Carrageenan)

Group IV: Received Sivathai Chooranam (15.12mg/kg) + (0.1ml of 1% Carrageenan)

Group V: Received Sivathai Chooranam (75.6mg/kg) + (0.1ml of 1% Carrageenan)

Table: 3 Effect of Sivathai Chooranam on Carrageenan-induced paw oedema in rats (body weight).

Group	Only Carrageenan	Carrageenan + Standard	Carrageenan+ SC- LD	Carrageenan + SC- MD	Carrageenan + SC- HD
Initial body weight	123.17±0.90982	129.17±1.0462	127.5±1.4549	133.33±1.6055	122.33±0.95452

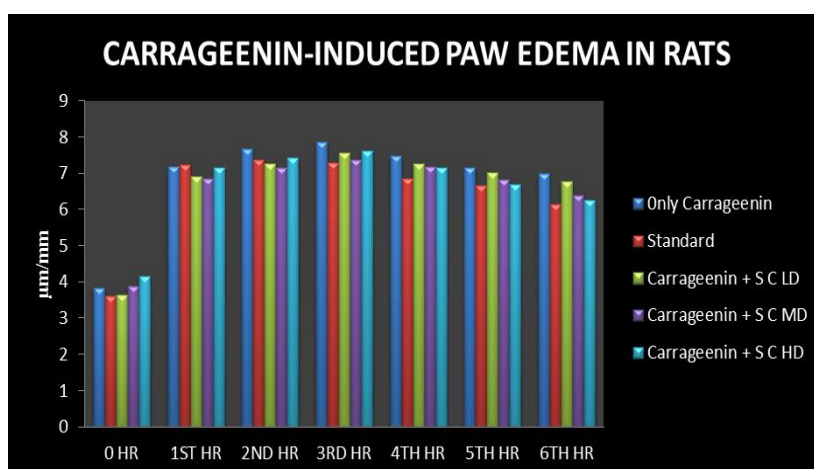
Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- Not significant **P< 0.05 calculated by comparing treated group with control group.

Table 4: Effect of Sivathai Chooranam on Carrageenan-induced paw oedema in rats.

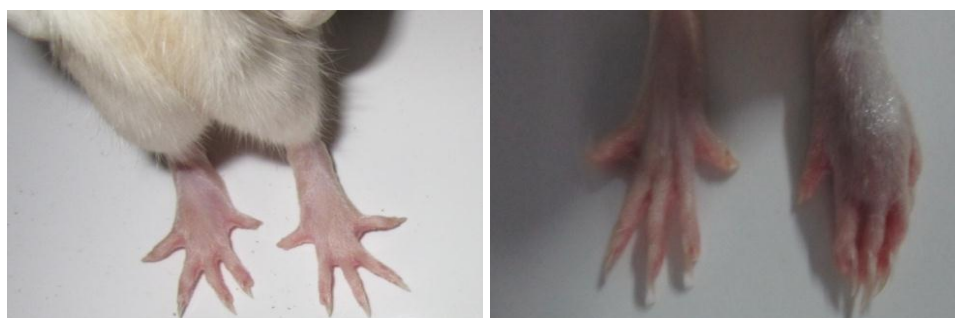
Group	Paw Volume after induction with carrageenan. Increase in paw volume (Mm) after Carrageenan injection (mean \pm SEM)/Percent inhibition of oedema			
	Initial Paw volume(mm)	Final Paw volume(mm)	Difference	Percentage protection (%)
Control	3.88 \pm 0.19836	3.88 \pm 0.19836	–	–
Only carrageenan	3.83 \pm 0.189367	7.00833 \pm 0.0838418	3.17	82.76 %
Carrageenan+ Standard	3.60833 \pm 0.125391	6.15167 \pm 0.162448***	2.55	70.83 %
Carrageenan+ SC- LD	3.64 \pm 0.116304	6.76333 \pm 0.0783865 ^{ns}	3.12	85.71 %
Carrageenan+ SC- MD	3.89667 \pm 0.169188	6.39833 \pm 0.0964509 ^{ns}	2.5	64.26 %
Carrageenan+ SC- HD	4.17333 \pm 0.166907	6.25 \pm 0.177144**	2.08	49.88 %

Values are expressed as the mean \pm S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- Not significant **P< 0.05 calculated by comparing treated group with control group.

Effects of Sivathai Chooranam on Carrageenan-induced paw oedema in rats

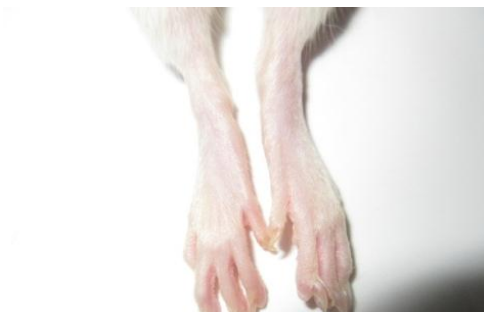
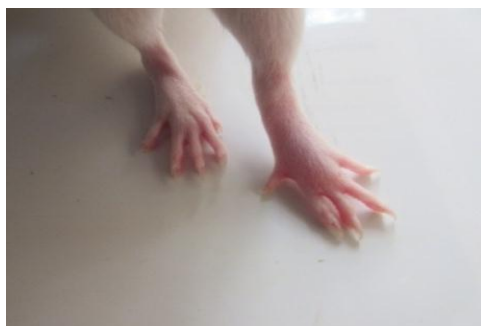
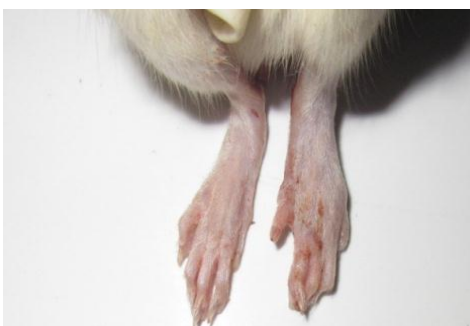


Effect Of Sivathai Chooranam On Carrageenan-Induced Paw Edema In Rats



Group: I- Control

Group: II Only Carrageenan

**Group: III- Carrageenan + STD****Group: IV- Carrageenan + LD****Group: V- Carrageenan+ MD****Group: VI- Carrageenan+ HD**

CONCLUSION

To conclude, the Sivathai Chooranam having an anti-inflammatory activity at low dose level (Percentage of protection 85.71%) when compared to the standard drug Diclofenac sodium (Percentage of protection 70.83%). Therefore Sivathai Chooranam is used for the treatment of pain and inflammation at low dose level and it is found that it useful for inflammatory disorders.

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