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## Studies on anti-inflammatory activity of Vaidyaratnam Recopain Balm (Ayurvedic topical ointment) using carrageen in paw induced edema method in rats

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### Abstract

This study of the anti-inflammatory activity of Vaidyaratnam Recopain Balm using the carrageenin induced edema method in Rat demonstrated results consistent with positive control.

**Keywords:** Vaidyaratnam Oushadhasala, Recopain, *Mahanarayana thailam*, carrageenin induced paw edema

### Introduction

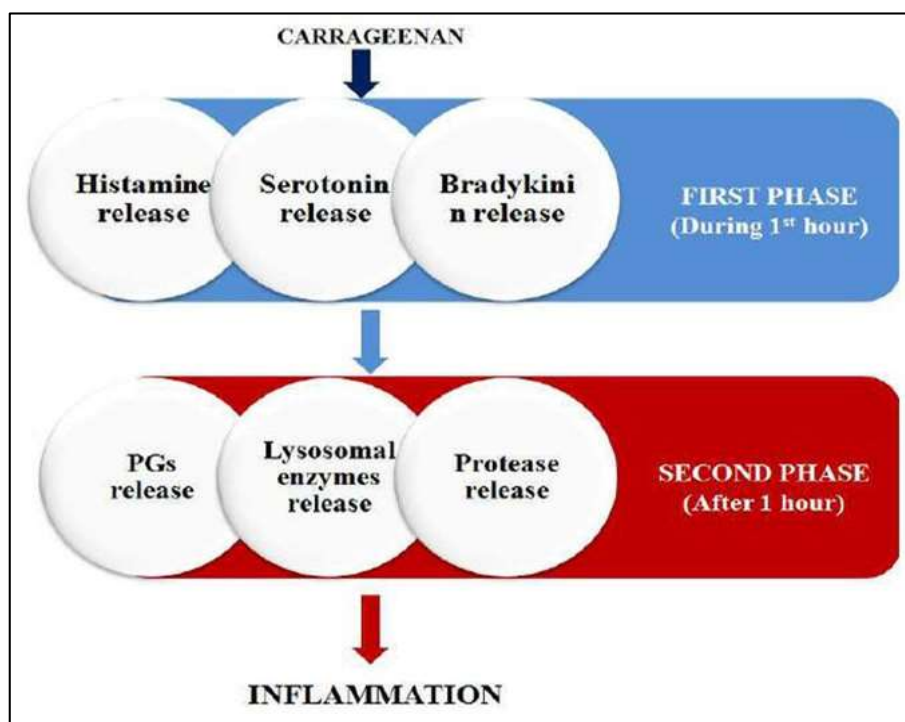
The main symptoms of inflammation are local redness, swelling, pain, warmth and loss of function. Nitric oxide (NO) is a gaseous short-lived free radical that acts as an inflammatory mediator and activates the effects of acute inflammation. Nitric oxide is produced by the oxidation of the nitrogen atom of the terminal guanidine nitrogen atom of L-arginine by the enzymatic activity of nitric oxide synthase (NOS). There are three forms of this enzyme. Two of these enzymes are expressed sequentially, are calcium/calmodulin-dependent, and are classified as constitutive NOS isoforms (cNOS). The third is the cytokine-inducible, calcium/calmodulin-dependent NOS isoform (iNOS), whose genes are regulated by multiple inflammatory mediators. Increased NOS activity or NO release is observed in both acute and chronic inflammation. Administration of L-arginine (precursor to NO synthesis) in subacute arthritis re-aggravates foot inflammation. NSAIDs are widely used throughout the world to treat various inflammatory conditions.

### Background

Inflammation is the body's defense mechanism, removing harmful stimuli and starting the issue healing process. However, the greatest drawbacks of currently available modern drugs are their toxicity and recurrence of symptoms after discontinuation, including their high cost, serious adverse reactions and toxicity, multiple infections, and side effects from currently used drugs. Therefore, the detection and formulation of drugs for their anti-inflammatory activity is a necessity of the hour and there have been many attempts to find anti-inflammatory drugs/formulations from Ayurvedic medicinal plants. Unlike modern medicines which have unique active ingredients that target specific pathways, herbal medicines work on an wholesome approach. Ayurvedic formulations have been a source of biologically active compounds and its formulations for centuries and have been used extensively to treat various ailments.

Vaidyaratnam Recopain Balm is formulated from the Ayurvedic classic *Mahanarayana thailam* mentioned in the book "*Bhaishajya Ratnavali*", which contains a variety of different molecules that work synergistically on targeted elements in complex cellular pathways. In this context, we evaluated the anti-inflammatory activity of a specific Ayurvedic formulation, Vaidyaratnam Recopain Balm, containing a unique combination of Ayurvedic ingredients, using a rat model of carrageenan-induced paw edema. The mechanistic/biochemical changes in this model are as follows

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## Materials and Methods

### Animals

Species - Rat

Strain - Albino wistar

Period of dosing - 6-8 weeks at the time

No. Rats - 24

Sex - Male

Body weight - 150 g

### Trial Drug

#### Recopain Balm

Recopain balm is an Ayush Licensed (under 25 D) (Topical Ointment) Ayurvedic Medicine Manufactured by M/s Vaidyaratnam Oushadhasala, Thrissur - 680 306, Kerala, India.

Composition of Recopain Balm - 10 g

- Mahararayanan thailam* (Ref; Classical Ayurvedic Text – Bhaishajya Ratnavali) - 1.666 g
- Syzygium aromaticum* (Fl. Oil) - 0.333 g
- Mentha arvensis* (Lf. Oil) - 0.5 g
- Eucalyptus globulus* (Lf. Oil) - 0.5 g
- Gaultheria fragrantissima* (oil) - 0.5 g
- Base – QS

Different doses of Vaidyaratnam Recopain balm were prepared at 200 mg, 400 mg 600 mg /paw (approx.) and used to apply topically on the carrageenan induced paw.

### Positive control / Standard Drug

Pharmaceutical grade Diclofenac (topical) cream was used as a positive control drug for comparison. It is a non steroidal anti-inflammatory drug (NSAID) used to treat pain and other symptoms of inflammatory conditions.

### Statistical Analysis

The data are expressed as MEAN  $\pm$  SEM for each treatment group. The data obtained for each response measure were subjected to one way analysis of variance (ANOVA) followed by Dunnet's 't'-test.

### Effect of Carrageenan induced hind paw edema in rat

0.1 ml of 1% (w/v) Carrageenan was injected sub cutaneous into planter region of the hind paws of rat.

### Experimental Procedure

Animals were housed in polypropylene cages with stainless-steel grills, pellet-holding facilities, and bottled water with stainless-steel straws. There are 6 rats in each cage. Animals were divided into 5 groups (6 animals each). Local inflammatory pain was induced in all groups of animals by intraplantar injection of carrageenan (50  $\mu$ l of a 3% suspension). The day before the experiment, record three basal readings of each rat's hind paw. The hind paws of the control group were smeared with normal saline. Animals were housed in polypropylene cages topped with stainless steel grills, pellet food storage facilities, and bottled water with stainless steel straws. There are 6 rats in each cage. Rats had access to drinking water, regular pelleted chow, and rice husk as cage material. Animals were divided into 5 groups (6 animals each). Inflammatory pain was induced in each group of rats by intraplantar injection of carrageenan (50  $\mu$ l of a 3% solution). The day before the experiment, record three basal readings of each rat's hind paw. Apply saline to the limbs of rats in the control group (group 1) and saline in the limbs of rats in the second group (group 2). (Group 1) Rats, Group 2 received the standard drug diclofenac ointment, but Groups 3, 4, 5 received 200 mg, 400 mg, 600 mg/paw of analgesic ointment. Measure paw volume at 0, 1, 2, 3, 4, 5, and 6 h.

The true edema volume was derived from the difference between the initial and subsequent readings after carrageenan injection using a digital chest manometer, while the control (saline) and standard groups were also observed. The edema volume was observed and always compared with positive and standard groups. The preventive effect of Vaidyaratnam Recopain Balm on edema/inflammation was calculated by comparing with the control group.

### Results and Discussion

Vaidyaratnam Recopain Balm 400 mg and 600 mg visibly reduced paw edema within 4 hours and 5 hours. Higher levels

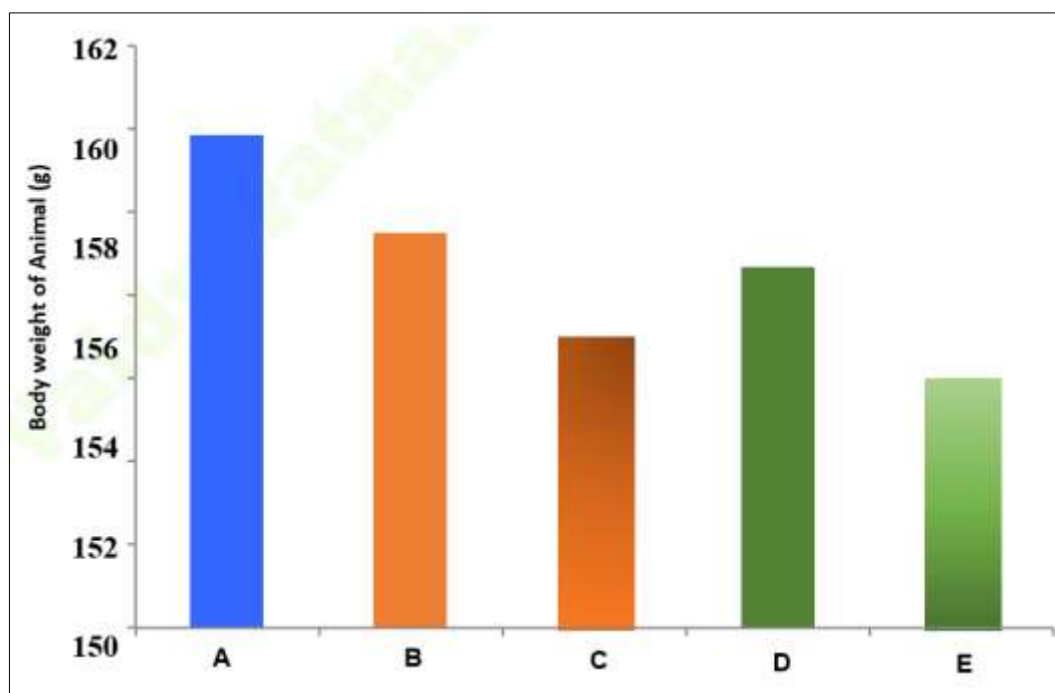
of potential anti-inflammatory activity were observed after 6 hours in the 600 mg group. Edema suppression was observed after continuous use of diclofenac ointment for 4 hours. The inhibitory effect is related to the dose (topical) and time after carrageenan injection. The results of the study showed that Vaidyaratnam Recopain Balm has good anti-inflammatory effects. (Table 1, Figure 2 and animal photos for results). The body weight of the experimental group did not change significantly.

Carrageenan Edema is simple and quick, clinically used with most anti-inflammatory drugs, can provide more accurate,

faster and more reliable results. The fast results of Vaidyaratnam Recopain Balm indicate that the ingredients contained in the unique Ayurvedic formula are absorbed quickly. Its activity is almost equivalent to that of diclofenac ointment (topical), and no relapses were observed in the tests. Anti-inflammatory drugs inhibit lysosomal enzymes, lipoxygenase and cyclooxygenase, which are involved in most anti-inflammatory effects. During testing, the limbs treated with Recopain Balm showed no signs of redness (edema) and skin cracking/flaking (Fig. 1).

**Table 1:** Effect of Vaidyaratnam Recopain Balm on Body weight

Carrageenin control (Mean ± SEM)	Diclofenac Ointment	Carrageenin + Diclofenac Ointment	Recopain Balm	Carrageenan + Recopain Balm
162.3±2.11	160.8±0.70	157.5±1.00	159±1.5	156±1.00



- A - Carrageenin
- B - Diclofenac
- C - Carrageenin + Diclofenac
- D - Recopain
- E - 1g Carrageenin + Recopain

**Fig 1:** Effect of Vaidyaratnam Recopain Balm on Carrageenan induced paw edema in rats (body weight)

**Table 2:** Anti inflammatory activity Vaidyaratnam Recopain Balm using Carrageenin induced Paw Edema Method

Treatment mg/paw	Paw Edema Volume (µm/mm) in different time interval (Mean ± SEM)							
	Time Interval (hrs.)							
	0 min	0.5 h	1h	2h	3h	4h	5h	6h
Carrageenin Control	3.59±0.09	5.84±0.07	7.12±0.25	8.15±0.02	8.78±0.002	9.11±0.08	9.12±0.11	8.59±0.13
Diclofenac Ointment	3.59±0.09	8.69±0.06	8.08±0.27	7.51±0.15	7.083±0.24	6.24±0.24	5.25±0.14	4.42±0.13
Recopain Balm 200	3.59±0.09	8.52±0.06	7.49±0.13	7.17±0.09	7.21±0.06	7.08±0.09	6.39±0.14	5.32±0.13
Recopain Balm 400	3.5±0.09	8.31±0.34	8.07±0.07	7.40±0.49	7.08±0.09	6.08±0.09	5.39±0.14	5.17±0.22
Recopain Balm 600	3.59±0.09	8.25±0.18	8.08±0.04	7.04±0.02	6.54±0.25	6.08±0.09	5.39±0.14	4.42±0.13

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by Dunnett's 't' test not significant\*\* P< 0.05 calculated by comparing treated group with control group.

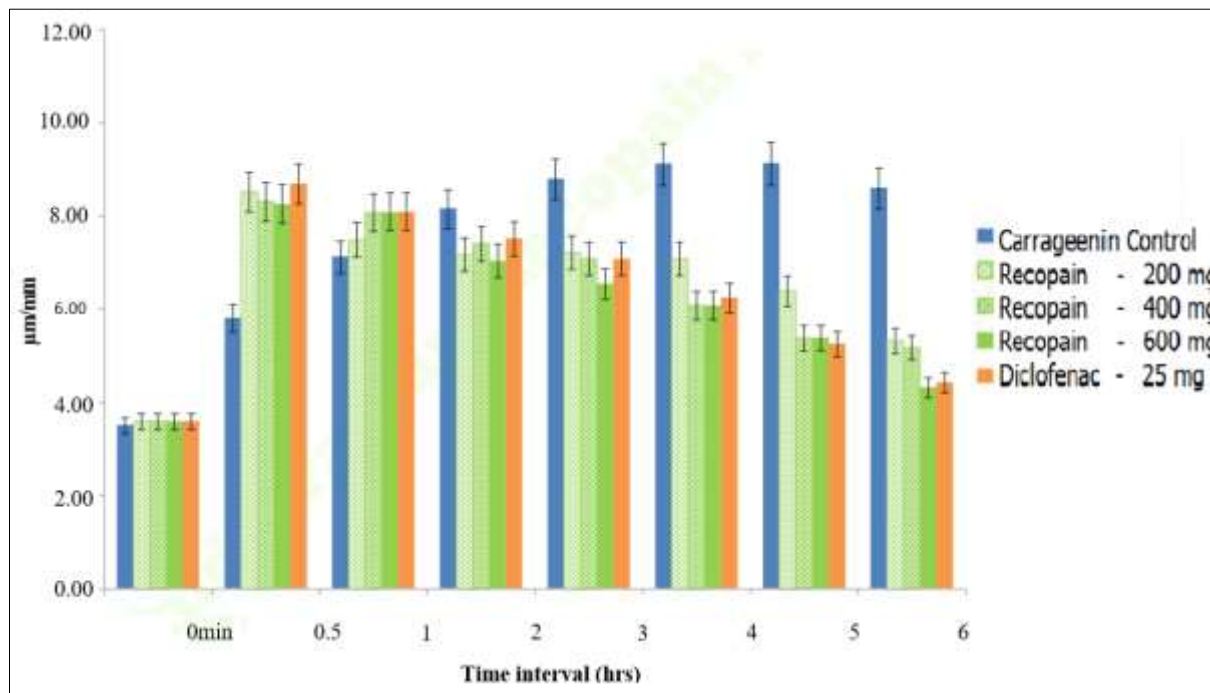
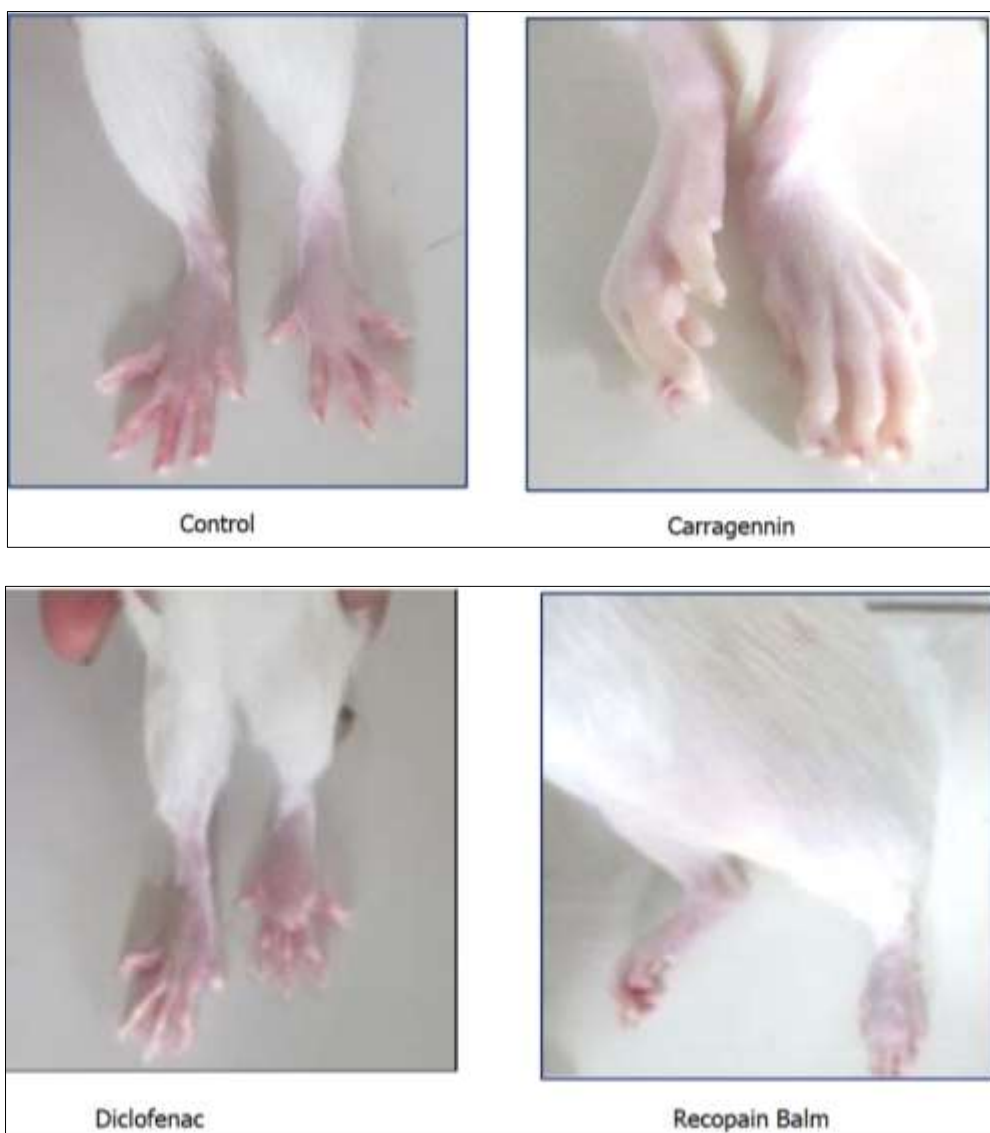


Fig 2: Effect of Vaidyaratnam Recopain Balm on Carrageenin induced paw Edema

Photographs of Test Animals





Recopain Balm showing reduced Paw edema



### Conclusion

The study supports that Vaidyaratnam Recopain Balm can be used as a topical anti-inflammatory agent for all sorts of inflammation.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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