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Anti-Inflammatory Activity of Proportions of Triphala Ointment in Wister Albino Rats

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ABSTRACT

Background: Inflammation is a reaction of living tissues towards injury. The complex biological reaction to harmful agents such as microbes, pathogens, damaged cells that contain vascular responses, activation of leukocytes and various systemic reactions causes inflammation. So, number of herbal medicine is recommended for the treatment of inflammation that has no side effects. *Triphala*, an Ayurvedic formula composed of three different drugs. Terminalia chebula Retz, Terminalia bellerica Roxb and Emblica officinalis Linn is widely used for various microbial infections and inflammation.

Aims and Objectives: To evaluate anti-inflammatory activity of *Triphala* ointment.

Methods: *Triphala* is now evaluated for its anti-inflammatory action. *Triphala* is formulated into a ointment of different concentrations and is evaluated for in-vivo anti inflammatory activity by 1:1:1 and 1:2:4 combination, In-vivo evaluation is done by Carrageenan induced paw edema method. The results obtained are compared with standard NSAID Diclofenac ointment.

Results: When test drug treated groups are compared with standard group, *Triphala* ointment 1:1:1 and *Triphala* ointment 1:2:4 showed significant effect 3 hours onwards to 5 hours with a mean value 0.07833±0.06555 (89.34%), 0.1±0.105 (86.39%) and 0.08333±0.06501 (88.70%) respectively.

Conclusion: In this study, *Triphala* ointment with 1:2:4 combinations showed better anti-inflammatory activity (88.70%) when compared to *Triphala* ointment 1:1:1(86.39%) in the reduction of rat paw volume.

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INTRODUCTION

Inflammation is a reaction of living tissues towards injury. The complex biological reaction to harmful agents such as microbes, pathogens, damaged cells that contains vascular responses, activation of leukocytes and various systemic reactions causes inflammation. Inflammation is the local response of living tissue to injury due to any agent. Signs of inflammation are rubor (redness), callor (heat), dolor (pain), tumor (swelling) and functio laesa[2]. According to WHO 1 in 6 people and 1 in 3 families suffer from inflammation due to various causes[3]. According to Bhaishajya Ratnavali, *Triphala Kwatha is* indicated in *Shotha Chikitsa* explained in *Shotha Rogadhikara Adhyaya*[4]. *Vranashopa* (Inflammation) has 3 progressive stages *Amavastha, Pachyamanavastha* and *Pakwavastha* respectively[5]. *Amavastha* (Early inflammatory stage) *Lakshanas* mentioned in Sushruta Samhita[6] and Bhavaprakasha[7] are *Mandoshamata, Twaksavarna, Sheetashopha, Sthairya, Mandavedana* and *Alpashopha* resembles to inflammatory swelling. *Triphala* is consisting fruits of three plants such as *Haritaki, Vibhitaki* and *Amalaki. Acharya Sushruta* in the context of *Dravyasangrahaniya Adhyaya* explained about

Triphala as *Haritaki*, *Vibhitaki* and *Amalaki* but the proportion of this is not mentioned. So we take them in equal proportion (1:1:1).[8] In *Sharangadhara Samhita, Churna Kalpana Adhyaya* mentioned about unequal proportion of *Triphala* that is 1 part *Haritaki*, 2 parts *Vibhitaki* and 4 parts *Amalaki* (1:2:4)[9]. The qualities of each drugs, *Haritaki* is *Kashaya Pradhana Pancharasa Rasa, Laghuguna, Ushnavirya* and *Madhura Vipaka*[10] and *Vibhitaki* is *Kashaya Rasa, Rukshaguna, Ushnavirya, Madhura Vipaka*[10] and *Amalaki* is *Amla*, *Madhura*, & *Kashaya Rasa, Laghuguna*, *Sheetavirya*, *Madhura Vipaka.*[11] *Triphala* is having *Kaphapittagna, Vranaropaka*, *Twakroganashaka* property.[11] Till date there is only few scientific work carried out and less publication of 1:2:4 combination forms of *Triphala*. Hence, the present study is undertaken to assess anti-inflammatory activity on carrageenin induced rat paw edema model by in-vivo study of *Triphala* ointment.

MATERIALS AND METHODS

Collection of the raw material: *Triphala Kwatha Churna* weighing total of *Triphala* 1:1:1 - 90 gm and *Triphala* 1:2:4 – 210 gm was collected from B.V.V.S Pharmacy, Bagalkot.

Equipment's and instruments: Plethysmometer, UV spectrophotometer,

Procurement of Animals: Animals were well maintained under identical condition of place light, temperature, food and other condition at the Animal house attached to HSK College of Pharmacy, Bagalkot. All animals were kept under standard husbandry conditions (Temperature 22-28^0 C and Relative Humidity 65±10%) for 12hr dark and 12hr light cycle respectively in standard propylene cages. The animals were fed with standard food (Pranav Agro Industries, Sangli, and Maharashtra) and water ad libitum. All the experiments were conducted in accordance with direction of Institutional Animals Ethics Committee.

Table No 1: Composition of *Triphala Kwatha*

SL NO	Ingredient	Ratio(1:1:1)	Quantity	Ratio(1:2:4)	Quantity
1	*Haritaki*	1 part	30 gm	1 part	30gm
2	*Vibhitaki*	1 part	30 gm	2 parts	60gm
3	*Amalaki*	1 part	30 gm	4 parts	120gm
4	Total	1:1:1 ratio	90 gm	1:2:4 ratio	210 gm
5	Water	16 parts	1440 ml	16 parts	2010 ml
6	*Kwatha* obtained	1/8th	180 ml	1/8th	251ml

Preparation of *Triphala* Ghana (Solid extraction)

The *Triphala Kwatha* obtained (180ml) was subjected to mild heat till it becomes semi solid in consistency and the remnant (30g) was used as Ghana for the preparation of ointment. Same preparation for both 1:1:1 and 1:2:4 proportion of *Triphala* ointment.

Preparation of *Triphala* ointment

Ointment was prepared with the concentration of 33.3% drug. Base was prepared with bees wax (36gm) and petroleum jelly (60gm). Initially bees were melted on water bath at 70^0 c. Then petroleum jelly was added with continuous stirring to form a homogenous mixture. This mixture was then added with *Triphala Ghana* (24gm) and mixed properly by using *Kalwa Yantra* (Mortar pestle) to obtain chocolate/brown colored ointment. It was then stored in air tight containers. Same preparation for both 1:1:1 and 1:2:4 proportion of *Triphala* ointment.

Table No 2: Composition of *Triphala* ointment

Ingredients	*Triphala*-1:1:1	*Triphala*-1:2:4
Triphala Ghana	24 gm	24 gm
Bees wax	36 gm	36 gm
Petroleum Jelly	60 gm	60 gm
Preservatives (Methyl paraben)	0.1%	0.1%

EXPERIMENTAL STUDY

Table No 3: Grouping of Wister Albino rats for experimental study

Groups	Treatment
Control group (n=6)	Receives Normal Saline
Standard group (n=6)	Topical application of Diclofenac ointment
Test group (n=6)	Topical application of *Triphala* ointment 1:1:1
Test group (n=6)	Topical application of *Triphala* ointment 1:2:4

Carrageenin-induced rat paw edema.

Inflammation in the left hind paw of rat was induced by an injection of 0.1mL of 1% (w/w) of carrageenan in saline subcutaneously in the plantar side of the left hind paw of rat. The paw volume was measured using Plethysmometer before the carrageenan injection paw volume was measured and after 15 mins the ointment was applied to the left hind paw by gently rubbing 50 times with the thumb finger and after at intervals of time at 0, 0.5, 1, 3, 5 hr. The same experiment was repeated with the test drugs.

The percentage of inhibition of paw edema volume in each treatment group is calculated by the equation;

$$\text{Percentage of inhibition} = \frac{\text{volume of -volume of control test}}{\text{Volume of control}} \times 100$$

Anti-inflammatory activity

Figure1: Application Triphala ointment

Figure2: Measuring the Paw volume

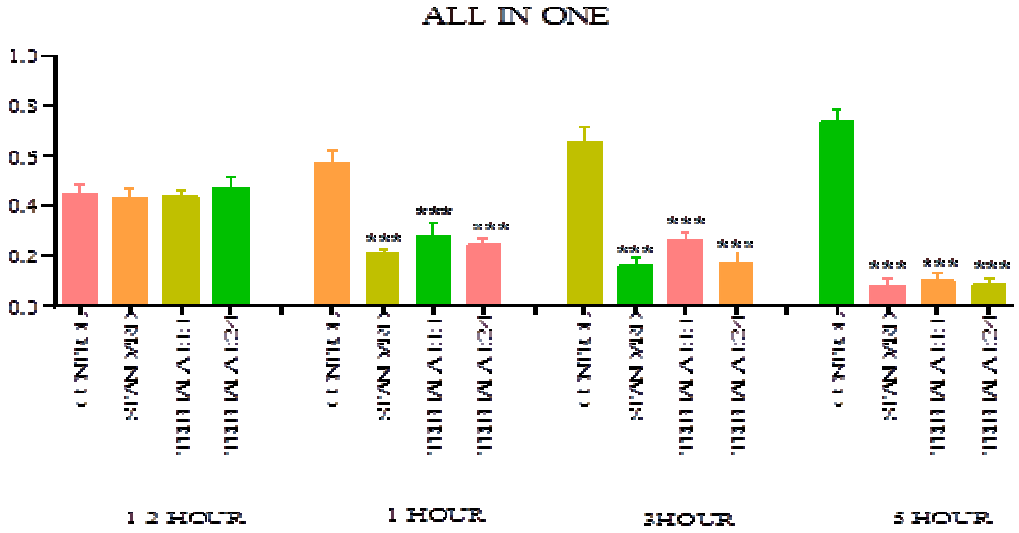
OBSERVATION AND RESULTS

Table No 4: Mean value of Experimental study

SL NO	GROUP	Paw volume in ml(% of edema inhibition)			
		½ hour	1 hour	3 hours	5 hours
1	GROUP-A (CONTROL)	0.4467±0.09309	0.5717±0.08641	0.655±0.05958	0.735±0.1058
2	GROUP-B (STANDARD)	0.4333±0.1295 (2.91%)	0.21±0.05367 (63.22%)	0.1583±0.1209 (75.87%)	0.07833±0.06555 (89.34%)
3	GROUP-C *TRIPHALA* 1:1:1	0.435±0.1427 (2.46%)	0.2817±0.08472 (47.81%)	0.2683±0.05981 (59.08%)	0.1±0.105 (86.39%)
4	GROUP-D *TRIPHALA* 1:2:4	0.47±0.1191 (5.82%)	0.2483±0.07935 (50.78%)	0.1683±0.06841 (74.35%)	0.08333±0.06501 (88.70%)

Results of Anti-inflammatory activity showed. All value are expressed as Mean ± SEM, n=6, ***p<0.001***p<0.001 as compared to control group (0.655±0.05958). Standard group 0.07833±0.06555 (89.34%) and *Triphala* 1:2:4 proportion 0.08333±0.06501 (88.70%) showed significant reduction in paw volume when compare to test groups *Triphala* 1:1:1 (86.39%)

Graph: Showing Histogram of Effect of *Triphala* ointment on Carragenin induced paw edema volume in rats.



Discussion

It contributes to the balance of all the three *Doshas Vata, Pitta* and *Kapha, Amalaki* for *Pitta*, *Vibhitaki* for *Kapha* and *Haritaki* for *Vata. Triphala* is rich in active ingredients like tannins, carbohydrates, saponins, ellagic acid, sorbitol and ascorbic acid. Till date there is only one scientific work carried out and less publication of 1:2:4 combination forms of *Triphala*.

When test drug treated groups are compared with standard group, *Triphala* ointment 1:1:1 and *Triphala* ointment 1:2:4 showed significant effect 3 hours onwards to 5 hours with a mean value 0.07833±0.06555 (89.34%), 0.1±0.105 (86.39%) and 0.08333±0.06501 (88.70%) respectively. The results obtained show that *Triphala* ointment 1:1:1 and *Triphala* ointment 1:2:4 has significant anti inflammatory activity which is nearly comparable to the standard drug Diclofenac (89.34%). The't' value obtained is significant indicating that the drug *Triphala* ointment in both 1:1:1 and 1:2:4 has better results in reducing paw edema in Wister Albino rats. In this study, *Triphala* ointment with 1:2:4 combinations showed better anti-inflammatory activity when compared to *Triphala* ointment 1:1:1 in the reduction of rat paw volume.

Probable mode of action

In-vitro anti-inflammatory activity of *Triphala* might be attributed to the presence of the various secondary metabolites like tannins, saponins, steroids, alkaloids, reducing sugars, terpenoids and flavonoids. These experimental findings support the traditional use of this plant for the treatment of various ailments especially against pain and inflammatory conditions. However, further investigations are required to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action responsible for the anti inflammatory activities of *Triphala* ointment. *Triphala* ointment composition of *Triphala Gana*, Bees wax and petroleum jelly. There is no reference to this formulation but *Triphala* having anti-inflammatory activity, *Triphala* 33.3% drug was used in the study based on the *Yukti*. Bees wax contains Tricontanol it helps in reduction of cholesterol level and also prevents infection.

Conclusion

The't' value obtained was significant indicating that the drug *Triphala* ointment in both 1:1:1 and 1:2:4 ratios helped in reducing paw edema in Wister Albino rats. In this study, *Triphala* ointment

with 1:2:4 combinations showed better anti-inflammatory activity (88.70%) when compared to *Triphala* ointment with 1:1:1 ratio (86.39%) in reduction of rat paw volume.

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