

ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECT OF DASHANG LEPA IN ACUTE EXPERIMENTAL MODELS IN ALBINO RATS**Dr. Kumar Ravindra^{*1}, Dr. Gupta S. J² and Dr. Trigunayat Anshuman³**¹Assistant Professor Department of Shalya Tantra, Institute of Ayurveda, Parul University, Limda Vadodara, Gujarat.²Associate Professor Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi 221005.³Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University Varanasi 221005.***Corresponding Author: Dr. Kumar Ravindra**

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ABSTRACT

Background/Purpose: Inflammatory swellings such as – cellulitis, erysipelas, boil, carbuncle etc. are major issue in health sciences. Inflammatory swellings are a part of body's inflammatory process, which helps the body when it becomes injured. Our body undergoes the inflammatory process virtually any time an injury or tissue damage occurs. Along with redness, heat and pain, swelling is a sign of the inflammatory process at work. The principle of management of inflammatory swelling is to localize the infection avoid the toxic substance/bacterial growth, to go in systemic circulation. To study the anti-inflammatory effect of Dashang Lepa in acute, experimental models in albino rats. **Methods:** *Dashang Lepa* contains 10 commonly and easily available and very cost affective indigenous drug which all having good effect in reducing the Vranashopha. Anti- inflammatory effect of Dashang Lepa was studied in carrageenin-induced hind paw oedema in rats and the paw volume was measured plethysmometrically at 5 hour after injection. All the drugs were applied locally. **Results:** The paw volume was significantly reduced in standard (mean = 0.86, p<0.001) and trial Group (mean = 0.82, p<0.001) when compared with control group (mean = 1.00, p=0.15) **Conclusion:** Anti-inflammatory effect of Dashang Lepa against carrageenan-induced inflammation in experimental model significantly reduced the paw volume as compared to the control rats.

KEY WORDS-: Dashang Lepa, Sumag, carrageenan, pedal oedema, inflammation.**INTRODUCTION**

The survival of all organisms requires that they eliminate foreign invaders, such as infectious pathogens, and damaged tissues. These functions are mediated by a complex host response called inflammation. Although inflammation helps clear infections and other noxious stimuli and initiates repair, the inflammatory reaction and the subsequent repair process can cause considerable harm.

In inflammatory swellings, many systemic and local drugs are used. Systemic drugs are effective but not safer from side effect, similarly the local drugs also causes some unwanted effects. The side effects are now so obvious that using these drugs from long duration are not free from severe threats to the health of the patients. It will not be wrong if we say about the long-term use of these drugs, "solution of the problem in itself creates such a big problem for which there is no solution". Here we wish to convey that the medicine is very effective for

fast relief and short term use and long term use of the medicine should be avoided.

Keeping in view all the above facts, now the researchers are searching out for an effective anti-inflammatory medicine having better therapeutic effect with least side effects.

Dashang lepa one of the mixtures of ten very effective and common indigenous drugs for local application in many superficial inflammatory conditions as described in many Ayurvedic texts. *The Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, BHU, Varanasi* use *Dashang lepa* in different superficial inflammatory condition as local applicant, since last one decade. However, it is very effective to reduce the inflammatory process after local application over inflammatory swellings. A clinical study on the role of Dashang Lepa in the management of cellulitis has recently been completed in the department showing its remarkable efficacy.

To the best of our knowledge, there is no scientific report available, which validates the role of *Dashang Lepa* for their anti-inflammatory activity. Therefore, the present study was planned to evaluate the efficacy of *Dashang lepa* for their anti-inflammatory activity on experimental model (Albino Rats) by carrageenan induced pedal oedema in rats through 'mercury displacement method'.

MATERIALS AND METHODS

After getting the approval from institutional ethical committee, inbred Charles foster albino rats of either sex (250 – 300 gm each) were used for performing the anti-inflammatory experiments. For the experiment, following three groups were proposed

- I. Control
- II. Standard
- III. Trial

Six animal were taken for each group and hence 22 animals were needed (18+4; six animals for three groups come to be 18 and 25% of total animal i.e. 4 are taken as extra).

The rats were obtained from Central Animal House, Institute of Medical Sciences, Banaras Hindu University. The rats were housed in healthy and clean surrounding in polypropylene cage with paddy husk as bedding. The male and female rats were housed in separate cages in order to avoid breeding during the entire period of experiment. All the animals had free access to pure drinking water and standard pellet laboratory animal diet. Animal room temperature was set at 24±2 with controlled illumination to provide a light-dark cycle for twelve hours.

All the experiments were performed in Department of Pharmacology, Institute of Medical sciences, Banaras Hindu University.

The anti-inflammatory activity of *Dashang Lepa* was studied by Carrageenan induced pedal oedema in rats.

DRUGS AND CHEMICALS

All the drugs of *Dashang Lepa* which is mentioned in many Ayurvedic texts like: Chakradutta in Visarpa-Visphota Chikitsa 23; Sharangdhar, Madhyam Bhag, Uttarkhand 11/4-6; Bhavprakash, Uttrardh, Dwitiya Bhag 56/32; Yogaratnakar, Uttrardh, Visarpa Chikitsa; Bhaishjyarnavali 57/18, were taken from the local market of *Gola-Dinanath* of Varanasi and medicinal garden of Department of *Dravya Guna*, Institute of Medical Sciences, Banaras Hindu University. There were 9 phytochemicals determined in methanolic extract of *Dashang lepa*. These phytochemicals are alkaloids, glycosides, flavonoids, tannins, phenolics compounds, carbohydrates, saponins, phytosterol and triterpenes¹. This is showing that *Dashang lepa* has multiple properties but here aim is to evaluate anti-inflammatory and analgesic activity by local application of drug in

Vranashopha. Dashang Lepa is a mixture of ten indigenous drugs, which are as mentioned in different Ayurvedic literature as:

1. Shirisha [*Albizia lebbbeck*] - Stem bark
2. Madhuyashti [*Glycyrrhiza glabra*] - Root
3. Tagara [*Valeriana wallichii*] - Rhizome
4. Raktachandnam [*Pterocarpus santalinus*] - Heartwood
5. Ela [*Eletteria cardamomum*] - Seeds
6. Jatamansi [*Nardostachys jatamansi*] - Rhizome
7. Haridra [*Curcuma longa*] - Rhizome
8. Daruharidra [*Berberis aristata*] - Stem/root
9. Kushta [*Saussurea lappa*] - Root
10. Hrivera [*Pavonia odorata*] - Root

The anti-inflammatory activity of *Dashang Lepa* was evaluated by Carrageenan induced pedal oedema in rats. For inducing the pedal oedema, the rats were injected with 0.1 ml of a 1% Carrageenan solution in saline in to the sub-planter of the left hind paw. The Carrageenan solution was made by mixing 10 mg of carrageenan into 100 ml of saline then heating it to get a homogenous mixture. Sumag (i.e. combination of dried magnesium sulphate 58%, urea 1%, sulphacetamide sodium 2.5%, proflavine and glycerine) was used in this experiment as standard drug.

EXPERIMENTAL PROCEDURES

Mercury displacement method test for anti-inflammatory activity evaluation

Three groups' viz. control standard and trial were decided to evaluate the anti-inflammatory activity of *Dashang Lepa*, where sumag was taken as standard drug for local application.

A 1% Carrageenan solution in saline was used for inducing the inflammation. The Carrageenan solution was made by mixing 10 mg of Carrageenan into 100 ml of saline and then heating it to get a homogenous mixture.

Now the experimental procedures of all the three groups are being discussed one by one

Control group: The six rats were taken and marked as C1, C2, C3, C4, C5, C6.

Before inducing inflammation, the paw volume of each rat was measured by mercury displacement method plathysmographically. Three readings were taken for each rat and the average value (V0) was taken as the original paw volume before inducing inflammation.

Now, with the help of insulin syringe, the rats were injected 0.1 ml of a 1% carrageenan solution in saline into the sub-planter region of the left hind paw. The rats were allowed to relax for half an hour so the inflammation could manifest completely.

Now, the rat's paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured thrice for each rat

by mercury displacement method plathysmographically. The average value was taken as the paw volume after inflammation was fully manifested in all Groups (V1). The paw volume was again measured in the same way after 5 hour (V2).

Standard group

The six rats were marked as S1, S2, S3, S4, S5, S6.

The sumag was applied over the fully inflamed paw of all the six rats and then the volume was measured by mercury displacement method plathysmographically after 5 hour (V2).

Trial group

RESULTS

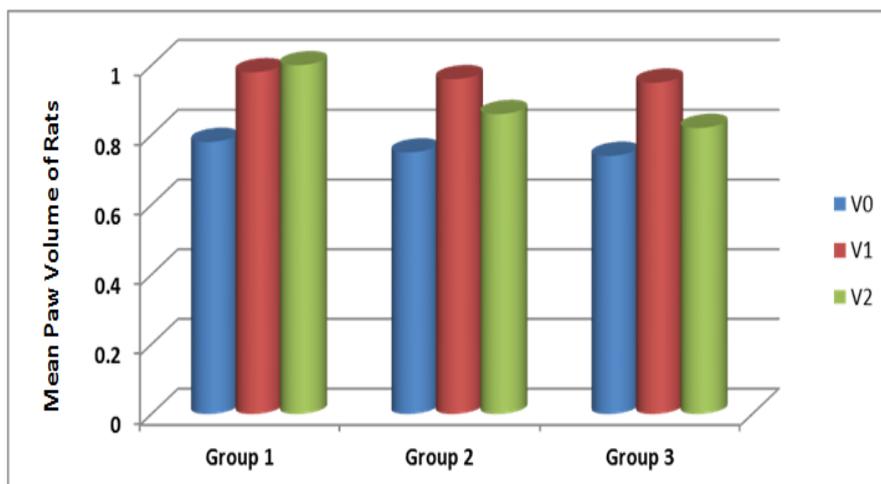
Table: 6.31 Statistical analysis of Rats' paw volume in experimental study.

RATS PAW VOLUME (Mean ± SD)				
Group	Volume before carrageenan inj. (V0)	Volume after 30 min of carrageenan inj. (V1)	Volume after 5 hr of drug application (V2)	Within the group comparison paired t-test (V1-V2)
Gr.-1 Control	0.78 ± 0.04	0.98 ± 0.04	1.00 ± 0.05	-0.01 ± 0.02 t= 1.58 p=0.15 (NS)
Gr.-2 Standard	0.75 ± 0.42	0.96 ± 0.04	0.86 ± 0.06	0.10 ± 0.03 t=6.52 p<.001 (HS)
Gr.-3 Trial	0.74 ± 0.05	0.95 ± 0.05	0.82 ± 0.06	0.12 ± 0.01 t=19 p<.001 (HS)
Between the group comparison One-way ANOVA	F=0.821 p=0.559	F=0.772 p=0.479	F=13.39 p<0.001	
Post Hoc test Dunnett t-test Gr.-1 Vs Gr.-2 Gr.-1 Vs Gr.-3	-	-	p=0.003 p<0.001	

The six rats were marked as T1, T2, T3, T4, T5, T6.

Dashang Lepa was applied over the fully inflamed paw of all the six rats and then the volume was measured by mercury displacement method plathysmographically after 5 hour (V2).

1. Mean and Standard deviations (SD) of all variable calculated.
2. Within the group paired t-test was done of all groups and t and p value was calculated
3. With the help of p value significances were noticed of the groups.
4. One way ANOVA test and post Hoc test between the group comparison.



Graph: 6.14 Statistical analyses of carrageenan induced pedal oedema in rats

After 5 hour, of drug application the decreased mean paw volume was found to be 0.10 in Standard Group treated with Sumag and 0.12 in Trial Group treated with *Dashang Lepa* locally. The mean paw volume was found to be -0.01 after 5 hour in the Control group, which was

not treated by any drug that indicates it was slightly increased. The paw volume was significantly reduced in standard (mean = 0.86, p<0.001) and trial Group (mean = 0.82, p<0.001) when compared with control group (mean = 1.00, p=0.15) (Table 6.31).

The present study showed that the trial drug (*Dashang Lepa*) possessed significant effect against acute inflammation in comparison to control. The trial drug however, produced a more significant effect as compared to the standard drug, which is evident from the differences in p value in between group comparison by Post Hoc test namely Dunnett t-test. (p=0.003 Gr.-1 Vs Gr.-2 p<0.001 Gr.-1 Vs Gr.-3)

DISCUSSION

The observations of the present study suggest significant anti-inflammatory activity of *Dashang Lepa* against Carrageenan induced paw oedema in rats. The carrageenan-induced paw oedema test is widely accepted as a sensitive phlogistic tool for investigating potential anti-inflammatory agents.

The time course of oedema development in carrageenan induced paw oedema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and is partly due to the trauma of injection and partly due to the release of histamine, 5-HT and kinins (*Crunkhorn P et al*). Platelet activating factor and arachidonic acid metabolites also play a role during this phase (*Boughton-Smith NK et al*). Prostaglandins (PGs) play a major role in the development of the second phase of reaction, which is measured around 3 hour times (*Di Rosa M et al*).

The presence of PGE2 in the inflammatory exudates from the injected foot can be demonstrated at 3 hour and period thereafter. It has been reported that the second phase of oedema is sensitive to the most clinically effective anti-inflammatory agents (*Smucker E et al*). In This experimental study, it was observed that the test drug produced a significant reduction of oedema after 5 hours of carrageenan injection. It provided a lead regarding the mechanism of anti-inflammatory activity of test drug i.e. by blocking the prostaglandin synthesis that is the mediator of the late phase of inflammation induced by carrageenan (*Vane J and Booting R et al*).

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