



## Research Article

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### A COMPARATIVE STUDY ON THE ANTI-INFLAMMATORY EFFECTS OF TRIVIDHA PAKA OF KSHEERABALA TAILA

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Received on: 16/06/15 Revised on: 09/07/15 Accepted on: 08/08/15

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DOI: 10.7897/2277-4343.066129

#### ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAID) owing to its exceedingly speckled side effects and adversities are in the verge of a moribund reliance. Ksheerabala Taila; a sage old Ayurveda remedy remarkably emphasised in the treatment of Vata Vyadhi and Vatarakta is well used since ages as an anti-inflammatory medicine. Developing researches have demonstrated its efficacy to counter neuro toxicity and subsequently validated its neuro protective effect. Though, the much acclaimed traditional wisdom regarding its anti-inflammatory action is neither scientifically appraised nor compared with any standards. The aim of the study was to scientifically establish the anti-inflammatory effect of Ksheerabala Taila by comparing it with the standard anti-inflammatory drug Diclofenac and to assess the specific Paka (stage) that exhibits it more. The study confirmed oral anti-inflammatory effect of Ksheerabala Taila which significantly ( $P < 0.01$ ) inhibited the (6 h) late onset carrageenan-induced rat paw oedema in Wistar strain Albino rats. This activity was found to be more in Mrdu and Madhya Paka of Ksheerabala Taila. The study could also conclude that the anti-inflammatory effect exhibited by Ksheerabala Taila was comparable to that of Diclofenac and Ksheerabala Taila could be an appropriate alternative to the potentially harmful NSAID's.

**Key Words:** NSAID, Ksheerabala Taila, Anti-inflammatory activity, carrageenan, Mrdu Paka and Madhya Paka.

#### INTRODUCTION

Sneha kalpana (Oleagenous preparation) is a secondary formulation in which Taila (Sesame oil) or Ghrta (ghee) are used as the media into which the active principles of the drugs are extracted. It is a pharmaceutical process to prepare oleaginous medicaments in which the kalka, kwatha and drava dravya <sup>1</sup> taken in specific proportions, are subjected to unique heating pattern and duration. It is mainly aimed at the mass transfer of the aqueous and lipid-soluble active principles. The Trividha Paka (Three stages) i.e. Mrdu, Madhya and Khara are the imperative stages<sup>2</sup> of a sneha Kalpana which possesses specific therapeutic utility<sup>3</sup> and administration routes. This specificity is due to the difference in the concentration of drugs in different Pakas.

Ksheerabala is one among the sneha kalpana prepared by using Go Ksheera (Cow's milk), Balamoola Kalka (paste of *Sida rhombifolia* root), Balamoola kashaya (decoction of *Sida rhombifolia* root) and Tila Taila (Sesame oil). It has a Rasayana (rejuvenation) property and is used in therapeutics both topically and systemically. It is indicated in all Vata Vyadhis, mainly in Vatarakta<sup>4</sup>; an inflammatory condition.

Inflammation is defined as the reaction of vascularised living tissue to injury. It is closely intertwined with the process of repair.<sup>3</sup> Inflammation can be classified as either acute or chronic. The increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues as an initial response of the

body against the harmful stimuli results in Acute Inflammation.

The present study was intended to experimentally evaluate and establish the anti-inflammatory activity of Ksheerabala Taila by comparing it with Diclofenac, a standard anti-inflammatory drug in acute inflammation models of experimental rats. The study was also projected to identify the specific Paka of Ksheerabala taila that serves the purpose more effectually.

#### MATERIALS AND METHODS

##### Animals

Wistar strain albino rats of either sex weighing between 180-280 g were obtained from the animal house of S.D.M centre for research in Ayurveda and allied sciences. The animals were fed with rat pellet feed (Amrut brand) and tap water was given ad libitum. Animals were maintained under normal ambient conditions. The protocol was approved by the Institutional Animal Ethics Committee with Approval number SDM - CAU- 13-14-16.

##### Investigational Drug and Dosage Preparation

The preparation of Ksheerabala Taila was carried out as per the reference of Sahasra yoga<sup>5</sup> and Ayurvedic Formulary of India. The Taila Paka was assessed in par with the pharmaceutical parameters explained in Sharangdhara Samhita<sup>6</sup>. The drug source of Bala was identified as *Sida rhombifolia* by the Dravya Guna Department of S.D.M. Ayurveda College Udupi. The test drugs viz. Mrdu, Madhya and Khara Paka of Ksheerabala Taila were prepared in the Department of Rasa Sastra and Bhaishajya

Kalpana, S.D.M. College of Ayurveda, Udipi and were used for the experimental study.

The dose of the test drugs were calculated by the Paget and Barnett's standard table for conversion which is:  $0.018 \times 5 \times$  Human dose. Human dose of Taila is 1 Pala (48 ml)<sup>7</sup>. Hence the dose for each animal was made out by multiplying 0.00432 with the weight of animal. The test drug was administered by oral route with the help of feeding needle.

#### Experimental Protocol

Animals (n=30) were allocated to five main groups with six animals each. To the first group, tap water was administered to serve as control. Second, third and fourth groups were treated with the Mrdu, Madhya and Khara Paka of Ksheerabala Taila respectively. The fifth group was taken as standard and administered with the standard drug Diclofenac (100mg/kg). The test drugs were administered once daily for five consecutive days.

#### Oedema Assay

Anti-inflammatory activity of a drug can be measured by noting the reduction in oedema produced by the local injection of substances like formaldehyde, carrageenan, histamine, dextran and ovalbumin. One of the most commonly used methods is based on the ability of such drugs to inhibit the oedema produced in the hind paw of the rat by the injection of a phlogistic agent (irritant). The method of carrageenan induced inflammation as introduced by Winter et al<sup>8</sup> (1962) was adopted in this study

owing to its benefits like high specificity, lack of drawbacks, easy availability of albino rats and carrageenan and easy measurability of paw volume of rats.

#### Procedure

The test drug was administered once daily for five consecutive days. On 5<sup>th</sup> day prior to Carrageenan injection the initial paw volume of left hind paw was measured using a Plethysmometer and this was considered as initial paw volume.

An hour after drug administration; 0.1 ml of freshly prepared 1% Carrageenan in sterile saline solution was injected to the sub-plantar Apo neurosis of the left hind limb to produce the paw oedema. The rats were administered with the tap water in the dose of 2 ml/100g body weight to ensure uniform hydration. This is supposed to minimize the variation in oedema formation. The intensity of oedema formation was recorded after 2<sup>nd</sup> hour, 3<sup>rd</sup> hour, 6<sup>th</sup> hour and 24<sup>th</sup> hour after Carrageenan injection. Results were expressed as percentage increase in paw volume in comparison to the initial values. Percentage increase in paw volumes were calculated by subtracting the initial paw volumes from the paw volumes obtained after the injection of the phlogistic agent. The figure was divided by the initial paw volume and multiplied by hundred.

The data obtained were statistically analysed using one way ANOVA followed by Dunnet's multiple 't' test as Post hoc test with 'p' value <0.05 considered as statistically significant.

## OBSERVATIONS & RESULTS

Table 1: Effect of Trividha Paka of KBT in % Change in Paw Volume in First Hour

Group	% increase in paw volume	% Change
Control	35.58±4.72	-
Standard	29.57±6.21	16.89↓
Mrdu paka	26.73±4.50	24.87↓
Madhya paka	21.71±6.50	38.98↓
Khara paka	52.70±8.95	48.11↑

Data: Mean±SEM

Table 2: Effect of Trividha Paka of KBT in % Change in Paw Volume in Third Hour

Group	% increase in paw volume	% Change
Control	81.80±7.02	-
Standard	42.96±10.55	47.48↓
Mrdu paka	35.80±5.66	56.23↓
Madhya paka	48.47±13.49	40.70↓
Khara paka	83.70±15.88	2.32↑

Data: Mean ±SEM

Table 3: Effect of Trividha Paka of KBT in % Change in Paw Volume in Sixth Hour

Group	% increase in paw volume	% Change
Control	93.38±8.47	-
Standard	32.85±7.48**	64.82↓
Mrdu paka	42.24±4.83**	54.76↓
Madhya paka	25.20±6.65**	73.01↓
Khara paka	60.89±9.20**	34.79↓

Data: Mean±SEM \*\* P<0.01

Table 4: Effect of Trividhapaka of KBT in % Change in Paw Volume in 24<sup>th</sup> Hour

Group	% increase in paw volume	% Change
Control	16.41±1.76	-
Standard	22.01±8.75	34.12↑
Mrdu paka	4.19±2.35	74.46↓
Madhya paka	9.25±1.63	43.63↓
Khara paka	21.29±5.99	29.73↑

Data: Mean±SEM



Plethysmometer



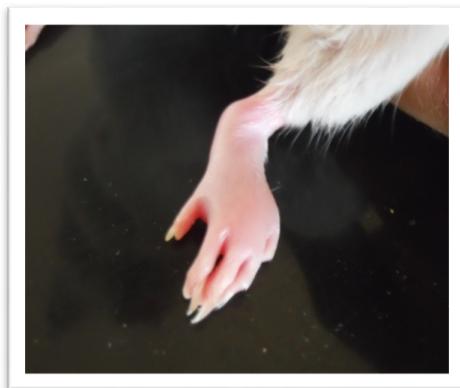
Marking Hind Paw



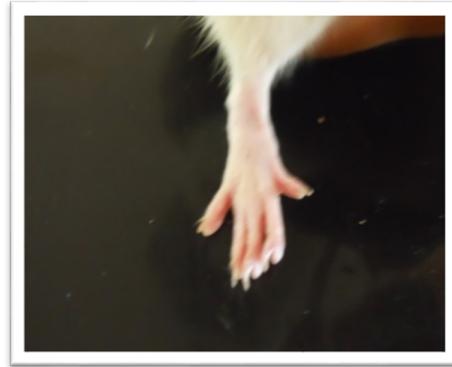
Injecting Carrageenan Solution



Measuring Paw volume



Paw oedema before Administration of Ksheerabala Taila



Paw oedema after Administration of Ksheerabala Taila After

The effect of Trividha paka of Ksheerabala Taila on paw volume during 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 24<sup>th</sup> hour has been depicted in Tables 1, 2, 3 and 4 respectively. During 1<sup>st</sup> hour and 3<sup>rd</sup> hour Mrdu and Madhya paka of Ksheerabala taila along with standard group, though non-significant showed a decrease in % increase in paw volume while Khara paka caused an increase of the same. While in the 6<sup>th</sup> hour all the four groups produced a statistically significant decrease in % increase in paw volume. Again in the 24<sup>th</sup> hour Mrdu and Madhya paka resulted in a decrease while khara paka and standard group caused an increase in % increase in paw volume.

## DISCUSSION

Carrageenan induced paw oedema test is commonly used as a primary test for acute inflammation and to screen the ability of the test drug to reduce local oedema induced by carrageenan. The particular inflammation created by carrageenan was found to be biphasic. The early phase lasting for 1 to 2 hours after carrageenan injection is mainly mediated by histamine serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandins released and mediated by bradykinins, leukotrienes secreted by polymorphonuclear cells and tissue macrophages.

The anti-inflammatory activity was expressed as percentage increase in paw volume by Ksheerabala Taila prepared in three different Pakas Viz. Mrdu, Madhya and Khara. The present study reported potent anti-inflammatory activity by Mrdu and Madhya Paka of Ksheerabala Taila during 3<sup>rd</sup> and 6<sup>th</sup> hour after carrageenan injection in the plantar region of rats paw. The Mrdu and Madhya Paka of Ksheerabala Taila exhibited significant anti-inflammatory activity (\*\*p<0.01, \*p<0.05) by reducing the paw volume.

Thus the test drug has a potential to inhibit the release of inflammatory mediators such as prostaglandins, leukotrienes and bradykinins from polymorphonuclear cells and tissue macrophages. In conclusion the present study supports the therapeutic use of Ksheerabala Taila and its potential role in the inflammatory disease conditions. It would be interesting to study and elucidate the mechanism of action involved in the anti-inflammatory activity reported above.

To focus on the influence of Paka on the expression of anti-inflammatory activity has shown that Mrdu and Madhya Paka samples produce moderate activity by 3<sup>rd</sup> hour (Table 2) and significant activity was reached at the 6<sup>th</sup> hour (Table 3). This is indicative of its influence over the second phase of carrageenan oedema formation.

In contrast to this; in Khara Paka sample there was a pro-inflammatory moderate response at first hour (Table 1) indicating early phase changes, nil effect at third hour and weak to moderate activity at 6<sup>th</sup> hour was observed. This shows that Khara Paka has a marked influence over the expression of the desired pharmacological activity. The reason why reduced activity was observed required to be probed.

Further in Mrdu Paka and Madhya Paka models, though non-significant, anti-inflammatory activity persisted even at 24<sup>th</sup> hour (Table 4) where as in Khara Paka sample a mild pro-inflammatory effect was observed. Inter group analysis of the test sample response indicated significantly better effect in Madhya Paka sample at 3<sup>rd</sup> and 6<sup>th</sup> hour in comparison to the Khara Paka sample. This clearly shows the influence of correct preparatory procedures on the quality of the product with respect to expression of pharmacological effect.

It is important to note that; the degree of reduction in acute inflammation achieved by Mrdu and Madhya Paka of Ksheerabala Taila was comparatively greater than that of the standard drug Diclofenac; however, this difference was statistically insignificant. Interestingly, in the 24<sup>th</sup> hour, Mrdu and Madhya Paka of Ksheerabala Taila continued the anti-inflammatory action, while the standard drug, Diclofenac showed mild pro inflammatory influence. This difference though statistically non-significant, ascertains the fact that Mrdu and Madhya Paka of Ksheerabala Taila is equally effective to Diclofenac in reducing inflammation.

## CONCLUSION

This study was designed and carried out to assess the anti-inflammatory activity of Trividha Paka of Ksheerabala Taila against acute inflammation represented by carrageenan oedema. In addition to this effect of these preparations were also compared to the standard anti-inflammatory drug; Diclofenac. Significant anti-inflammatory activity with late onset was observed in the Mrdu and Madhya Paka of Ksheerabala taila. The effect was good and significant. However, In Khara Paka of Ksheerabala taila, only moderate anti-inflammatory activity at 6<sup>th</sup> hour was observed. This clearly indicates that Khara Paka reduces the therapeutic efficacy of Ksheerabala taila. In addition, Mrdu and Madhya Paka of Ksheerabala Taila were found to be effective anti-inflammatory agents which can be comparable to Diclofenac. Further clinical and experimental studies are required to explore and establish the effectiveness of Ksheerabala Taila as a safe and effective alternative for harmful NSAID's.

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## Cite this article as:

Chithra G. Nair, R. R. Geethesh P., Sudheendra Honwad, Ravi Mundugaru. A comparative study on the anti-inflammatory effects of trividha paka of ksheerabala taila. Int. J. Res. Ayurveda Pharm. 2015;6(6):692-695 <http://dx.doi.org/10.7897/2277-4343.066129>

Source of support: Nil, Conflict of interest: None Declared

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