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Research Article

ANTI-INFLAMMATORY ACTIVITY OF ROOT AND FRUIT OF *GOKSHURA* (*TRIBULUS TERRESTRIS* LINN.) IN ALBINO RATS

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ABSTRACT

Gokshura Moola (root of *Tribulus terrestris* Linn.) is one of the ingredients of the group of ten medicinal plant roots called *Dasamoola.* It is a major ingredient of Ayurvedic formulations so that the Ayurvedic manufacturing industry is consuming them in abundantly. Instead of roots, the fruits of *Tribulus terrestris* is roughly using in all the preparations of *Dasamoola*. In Ayurvedic classics *Gokshura* is said to be useful in the treatment of dysuria *(Mutrakrichra*), inflammation *(Sopha),* renal calculi *(Asmari),* cardiac diseases *(Hridroga),* rheumatoid arthritis *(Amavata),* rejuvenation *(Rasayana),* aphrodisiac *(Vajeekarana),* etc. Ancient Ayurvedic literature opines that the properties and actions attributed to one part of the plant will be the same for the other parts too. If the fruit of the plant is equally effective as the root then the destruction of the whole plant can be prevented. Hence, the present study is carried to evaluate and compare the anti-inflammatory activity of both root and fruit *Kashaya* (decoction) experimentally by-Carrageenan induced rat paw oedema method with Diclofenac sodium (20 mg/kg) as standard. The results were analysed statistically by ANOVA and LSD post hoc pair wise comparison test. Both root and fruit of *Gokshura*, showed significant anti inflammatory activity in albino rats. But the root of *Gokshura (Tribulus terrestris* Linn*)* showed a greater anti inflammatory action in comparison to its fruit. Thus the current substitution of *Gokshura* roots with fruits can be substantiated by this study but effect may be less compared to root part.

KEYWORDS: *Gokshura, Tribulus terrestris* Linn, Anti- inflammatory, *Sophaghna.*

INTRODUCTION

Gokshura is a well-known Ayurvedic drug that is used in various formulations. Botanically it is identified as *Tribulus terrestris* Linn. of Zygophyllaceae family. *Gokshura Moola* (root of *Tribulus terrestris* Linn.) is a component of *Dasamoola* (group of ten medicinal plants principally comprising of roots as the useful part), a reputed Ayurvedic combination used in the treatment of various diseases. In the commentary of *Madana pala nighantu* [1], it is mentioned that the root is used in *Dasamoola* while the fruit is aphrodisiac (*Vrushya)*. But in practice, instead of roots, fruits of *Tribulus terrestris* is widely being used in all the preparations. About 100 years back, a doubt on the efficacy of this substitution was raised by a traditional *Vaidya* (physician) in one of the oldest Ayurvedic publications of Kerala, Dhanwanthari Magazine, published in 1916 (Kollavarsham 1091. Since *Dasamoola* is a major ingredient of Ayurvedic formulations and has demand of about 100 ton annually there is a great need to discover suitable substitutes for it. According to *Siddha Bheshaja Manimala*[2], therapeutical properties denoted for the part used are to be considered for all the other parts of the same plant. Even though it is a wise decision to avoid the extinction of the drug by the abundant use of root, sufficient therapeutic efficacy needs to be verified.

Gokshura is said to be useful in the treatment of dysuria *(Mutrakrichra*), inflammation *(Sopha),* renal calculi *(Asmari),* cardiac diseases *(Hridroga),* rheumatoid arthritis *(Amavata),* rejuvenation *(Rasayana),* aphrodisiac *(Vajeekarana),* etc.

Dasamoola is a well known *Swayadhuhara Mahakashaya*[3] of *Caraka Samhita,* a classical Ayurvedic text book*. Swayadhu/Sopha* is a disease dealt in Ayurvedic classics, which can be compared to inflammation based on the clinical features. Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. It is a defence mechanism that helps body to protect it against infection, burns, toxic chemicals, allergens or other noxious stimuli. *Swayadhuhara/ Sophaghna* can be interpreted as anti-inflammatory activity. An experimental study was designed, for the pharmacological evaluation and comparison of root and fruit part of *Tribulus terrestris* Linn for their anti-inflammatory effect and to verify whether the fruit of *Gokshura* (*Tribulus terrestris* Linn.) can be a substitute for its root.

MATERIALS AND METHODS

Research design: Carrageenan induced paw oedema model in albino rats [4]

Study setting

Pharmacology laboratory of Department of Dravya guna vijnanam, Govt. Ayurveda College, Thiruvananthapuram.

Experimental animals

Wistar strain albino rats (150-300 g) of either sex were obtained from the pharmacology laboratory of Govt. Ayurveda College, Thiruvananthapuram. They were grouped and housed in poly-acrylic cages (Two animals per cage) and maintained under standard laboratory conditions. They were fed with commercial rat feed and water. The animals were acclimatized 1 week before experimentation period. The animals were exposed to 12h light and 12h dark cycle with the relative humidity at 50-70% and the ambient temperature 22±3ºC during the experimental period. All animals were kept in the same environmental conditions. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC No27/IAEC/AVC/2015).

Collection and preparation of test drug

The sample drug *Tribulus terrestris Linn*. was collected from beach side of Thiruvananthapuram area and the sample was authenticated by comparing it with different floras. The root and fruit of *Tribulus terrestris Linn*. were separated, cut into small pieces, washed, shade dried and coarsely powdered. The *Kashaya* (decoction) of both root and fruit were prepared by mixing 48g of coarsely powdered drug with 768ml water and reducing to 96ml according to the *Kashaya* preparation procedure mentioned in Sarngadhara Samhitha[5] a classical Ayurvedic text book.

Dose fixation

Human dose of *Kashaya* is 96ml/day. Rat dose calculation was done on the basis of body surface area ratio using the table of Paget and Barnes [6].

Rat dose= 96 x 0.018 (Paget &Barnes conversion factor)

= 1.728 ml/200g rat

Preparation of Standard drug

The standard drug Diclofenac sodium was taken as 20mg/kg [7]. 20mg of the drug was dissolved in 1ml of 2% CMC (Carboxy Methyl Cellulose) solution and administered orally at the dose of 2mg/100gm body weight/ 0.1ml.

Experimental procedure

The acclimatized albino rats were weighed and grouped into 4 with 6 animals in each group. Selection was done randomly so as to assure equal distribution of sex, body weight etc. in each group. Then the animals were marked for proper identification and kept each group in separate cages. Each cage was labelled separately for group identification. The dose for each animal was calculated as per the body weight and was tabulated. The animals were over night fasted without restricting water prior to the experiment. Group I kept as control group received 2% CMC solution and Group II kept as standard group received Diclofenac sodium (20 mg/ 1ml of 2%CMC solution/1000mg). Group III and Group IV kept as test drug treated group and received root decoction and fruit decoction of *Gokshura* (*Tribulus terrestris* Linn) respectively. Initially left hind paw volume of each rat, up to the tibio-tarsal articulation was recorded prior to carrageenan injection, by the mercury displacement method using a plethysmometer. The drugs were administered orally in the calculated doses using a feeding cannula to all groups except group I which was kept as the control group. After 30min, 0.1ml of 1% Carrageenan was injected in the plantar region of the left hind paw of each animal using a tuberculin syringe. Paw volumes were measured after 1st and 3rd hours using a Plethismometer (Fig.1& Fig.2).

The percentage difference of paw volume was calculated. The mean percentage change in paw volume was compared and expressed as percentage oedema inhibition by drug.

Inhibition % = [($C_t - C_0$) control - ($C_t - C_0$) treated] ÷ ($C_t - C_0$) control×100

Where C_1 is paw size after carrageenan injection and C_0 is paw size before carrageenan injection.

Statistical analysis

Descriptive statistics such as Mean and Standard Deviation were calculated and the observed mean difference in change in paw volume (MDPV) during each time period was statistically tested using One-way ANOVA. Then Least Significant Difference (LSD) post hoc pair wise comparison was carried out for mutual comparison of groups. A calculated 'p value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Carrageenan induced rat paw oedema method is a very useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation and is believed to be biphasic. In the early phase of carrageenan-induced inflammation, histamine, serotonine and bradykinin are the first detectable mediators which are not inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). The second phase of swelling has not only been correlated with the elevated production of prostaglandins, but more recently has been attributed to the induction of inducible cyclooxygenase (COX-2) in the hind paw. It can be blocked by the NSAIDs.

In the present study, the root decoction showed marked percentage inhibition (14% and 64.2%) in the development of paw oedema after 1st and 3rd hours of carrageenan injection while the fruit decoction showed percentage inhibition (28.57%) after 3 hour of carrageenan injection (Table 1). When compared with control group, the root decoction ($p<0.001$) and fruit decoction ($p<0.05$) (Group III&IV) showed anti inflammatory effect at 3rd hour. On comparing both the test groups, the root decoction showed more effect in the anti inflammatory action than that of fruit decoction of Gokshura ($p<0.05$).

In this study, significant reduction of the paw oedema was obtained at the 3rd hour after carrageenan injection (second phase). So based on the above reports, it can be inferred that the anti-inflammatory effect of root *Kashaya* and fruit *Kashaya* of *Gokshura* could be due to the inhibition of the enzyme cyclo-oxygenase leading to the inhibition of prostaglandin synthesis.

Thus, the study reveals that, both the root and fruit of *Gokshura (Tribulus terrestris Linn)* possess anti

inflammatory action but the activity of root is more significant than that of fruit.

Table 1. Descriptive Statistics and Test of Significance (ANOVA) for the comparison of MDPV (Mean Difference in Paw Volume), among four groups in different time periods -1st hour, and 3rd hour

Group	MDPV 1hour Mean ± SD	% Inhibition	MDPV 3hour Mean ± SD	% Inhibition
I	0.5±0.167		0.56±0.150	
II	0.3±0.109	40	0.13±0.103	76.8
III	0.43±0.150	14	0.2±0	64.2
IV	0.5±0.109	0	0.4±0.219	28.57
p value	0.091ns		.000**	

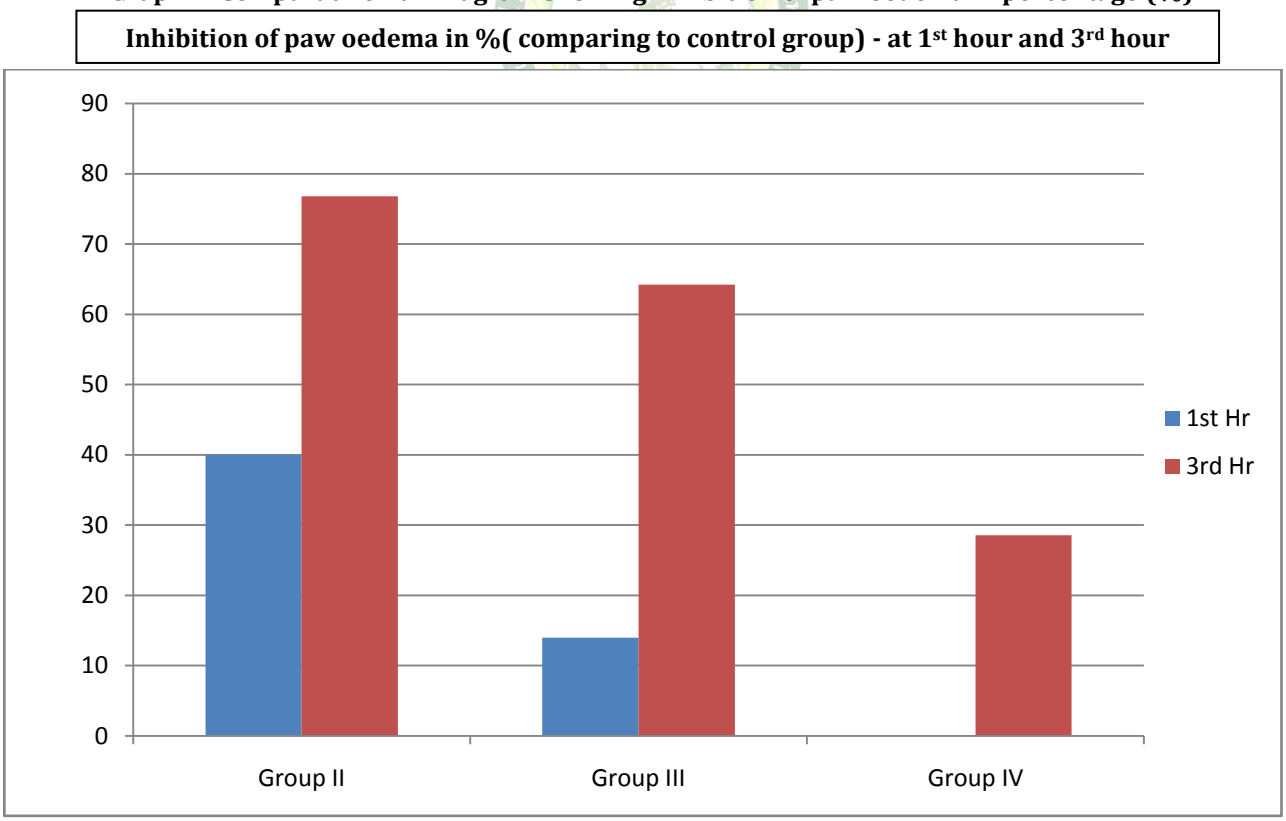
MDPV- Mean Difference in Paw Volume, SD- Standard deviation, Values are expressed in centimetres. Ns: not significant (p>0.05), **: significant at 1% level (p<0.01).

Table 2. Comparison of difference in mean paw volume among each group during 1st hour and 3rd hour

		p value	
	Comparison	**1st hour**	**3rd hour**
1	I (control) vs II (standard)	**	***
2	I (control) vs III (root decoction)	Ns	***
3	I (control) vs IV (fruit decoction)	Ns	*
4	II (standard) vs III (root decoction)	Ns	Ns
5	II (standard) vs IV (fruit decoction)	**	**
6	III (root decoction) vs IV (fruit decoction)	Ns	*

*Ns: not significant (p>0.05), *: significant at 5% level (p<0.05), **: significant at 1% level (p<0.01) ***: significant at 0.1% level (p<0.001).*

Graph 1. Comparative Bar Diagram showing Inhibition of paw oedema in percentage (%)



Group II - Standard group
Group III –*Tribulus terrestris* Root decoction
Group IV - *Tribulus terrestris* Fruit decoction

CONCLUSION

Both root and fruit of *Gokshura*, showed significant anti inflammatory activity in albino rats. However, *Gokshura* root has more pronounced anti inflammatory activity in comparison to *Gokshura* fruits. Considering the non availability of *Dasamoola* drugs, the current substitution of *Gokshura* roots with fruits can be substantiated by this study.

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