Research Article

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AN EXPERIMENTAL EVALUATION ON THE ANTI INFLAMMATORY ACTIVITY OF LEAVES OF KARANJA (*PONGAMIA PINNATA* LINN. PIERRE)

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ABSTRACT

Karanja (*Pongamia pinnata* Linn. Pierre) of Papilionaceae family grows throughout India. It is easily available, cost effective and have been described in various Samhitas and Nighantus. Indication of Karanja patra in Shotha is seen in Bhavaprakasha Nighantu and Kaiyadeva Nighantu, thus indicating its Anti inflammatory activity. Many of the drugs we are using as Shothahara is the roots, hence to avoid the destruction of the whole plant the present study of evaluating the Anti inflammatory activity of Karanja patra which is easily available throughout the country has been taken up. In vivo anti inflammatory activity of the drug was evaluated in Wistar albino rats using Carrageenan induced paw edema for acute inflammation and Cotton pellet implanted granuloma formation for Chronic inflammation. The experimental study reveals that the drug Karanja (*Pongamia pinnata* Linn. Pierre.) at 4.32 ml rat dose possess anti inflammatory activity especially in acute inflammation. Conclusion: Karanja (*Pongamia pinnata* Linn Pierre) leaves in 4.32 ml /kg rat dose is said to have Shothahara (Anti inflammatory) activity especially in acute inflammation is justified.

Keywords: Karanja, Shotha, Anti inflammatory, Shothahara

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind affecting the majority of population, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants.¹

Inflammation is one among the major condition associated with various threatening diseases. It is a complex reaction in tissues that consists mainly of responses of blood vessels and leucocytes. The vascular and cellular reactions of inflammation are triggered by soluble factors that are produced by various cells or cell derived from plasma proteins and is generated in response to the inflammatory stimulus.² A comprehensive list of inflammatory diseases would run over hundred, which include diseases such as Alzheimer's, Rheumatoid arthritis, Inflammatory bowel disease, SLE (Systemic Lupus Erythematosus), Multiple sclerosis, Diabetes and many more.³

The disease Shotha as explained by classics shows the signs and symptoms of shotha as that of inflammation such as davathu (dolor/pain), sira ayama (dilatation of veins), siratanutva (increased vascular permeability), ushma (calor/heat), angavivarnata (erythema or discolouration of the affected site), gaurava (heaviness due to exudation of protein rich fluid into extravascular spaces), utsedha (tumor/swelling).⁴⁻¹⁴

Karanja (*Pongamia pinnata* Linn. Pierre) of Papilionaceae family is found throughout India in tidal and beach forests and even cultivated as avenue trees¹⁵ easily available, cost effective and have been described in various Nighantus and Samhitas. Mainly its seeds are used but its leaves are leaves are mentioned in Bhavaprakasha Nighantu, Kaiyadeva Nighantu in Shotha thus indicating its anti-inflammatory activity.

MATERIALS AND METHODS

Source of data

The leaves of the drug Karanja (*Pongamia pinnata* Linn. Pierre) were collected from their natural habitat of Karnataka from the botanical garden of Shree Dharmasthala Manjunatheshwara college of Ayurveda and Hospital, Hassan and authenticated from the Department of Dravyaguna, Shree Dharmasthala Manjunatheshwara college of Ayurveda and Hospital, Hassan

Place of work

Centre – SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi.

Animals: Healthy wistar albino rats of either sex weighing between 150-250 g were obtained from the animal house of SDM Research Centre, Udupi. The rats were fed with normal rat diet obtained from Sai Durga feed, Bangalore and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided had the following conditions: controlled lighting of 12:12 h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%. The experimental protocol was approved by the institutional ethical committee under the approval no.6 SDMCAU/IAEC/2015-16.

Dose Fixation

The dose was 4.32ml/kg weight of the rat according to the reference of Paget and Barnes (1964) formula.

Drug Preparation: The swarasa (juice) of the drug was prepared at the Research Centre, SDMCAU, Udupi. The swarasa was prepared by adding 8 parts of water in one part coarse powder of the drug and reducing it to 1/4th part by boiling in low flame.¹⁵

On each day of experimentation, the drug was weighed over accurate digital weighing machine in the quantity of ten grams. Then 80 ml of distilled water was added to it and kept for boiling over low flame reducing to 1/4th to obtain the quantity of 20 ml of swarasa.

Experimental Design

Healthy adult Wistar albino rats of either sex weighing 150 to 250 g were divided into three different groups consisting of six rats in each group. Control group animals were administered with normal tap water at a dose of 5ml /kg in 0.5% gum acacia, animals of standard group were administered with Diclofenac sodium at a dose of 5 mg/kg for acute anti-inflammatory activity and chronic anti-inflammatory activity. The test group was administered with the test drug at a dose of 4.32 ml/kg for rats.

Route of administration: The drugs were administered orally by using rat feeding tube fixed to syringe.

Methodology

a) Acute anti - inflammatory activity: The acute anti-inflammatory activity was evaluated by Carrageenan induced hind paw oedema test in Wistar albino rats by method of Winter *et al.*¹⁶ The group specific drugs were administered orally for seven consecutive days and 1 hour before the Carrageenan injection on 7th day. Acute inflammation was produced by injecting 0.1 ml of 1% Carrageenan solution into sub plantar surface of rat's hind paw. The paw volume up to the tibio-tarsal articulation was measured using a Plethysmometer at basal, 1 hour, 3 hour and 6 hour after Carrageenan injection.

The anti-inflammatory activities were expressed as percentage decrease in paw oedema using the following formula:

$$\text{Percentage change in paw oedema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c is the paw volume of control group and V_t is the paw volume of test group.

b) Chronic anti-inflammatory activity: The effect of test drug on cotton pellet implanted granuloma formation in rats was studied as per the method described by D'arcy *et al* (1960). The rats were anaesthetised under ketamine (80 mg/kg, i.p). The dorsum was shaved and swabbed with 70% (v/v) alcohol. Mid-line incision of 1cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of small blunt forceps. One sterile cotton pellet weighing 100 mg (prepared by rolling of a cotton piece of 100 mg and sterilized by autoclaving for 30 min under 15 lbs pressure) was inserted per tunnel and the incision was closed with interrupted sutures after expelling the air from the tunnel. Group specific drugs were administered for seven consecutive days starting from the day of implantation. The rats were sacrificed on 8th day and dissected for collection of spleen and adrenal glands. Implanted cotton pellets were removed and cleaned of extraneous tissues and dried by placing them in a hot air oven overnight at 80°C and then weighed. The difference between the initial weight and the final weight of the pellet after drying was taken as the weight of granuloma tissue. The result was expressed as mg of granulation tissue formed per 100 g body

weight. The weight of spleen and adrenal gland were noted. The dissected organs were preserved in 10% formalin and sent for histopathological examination. In addition, blood was withdrawn from retro- orbital plexus with the help of capillary tubes and the samples were collected to estimate biochemical parameters.

Procedure for histopathological examination

a. Fixation: The tissue would be excised out immediately after sacrificing the animals, cleaned of extraneous tissue, cut into pieces of appropriate thickness and would be transferred into 10% formalin solution. The tissues were allowed remaining in it till they would take up for processing.

b. Tissue processing: Tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. Tissue should be thoroughly washed by placing them under running tap water and then passed through a series of following solvents as per schedule for dehydration, clearing and paraffin infiltration.

Alcohol 70% - 20 minutes , Alcohol 80% - 20 minutes , Alcohol 90% - 20 minutes , Alcohol 95% (2 changes) - 20 minutes , Isopropyl alcohol - 20 minutes, Acetone (2 changes) - 20 minutes, Chloroform (3 changes) - 20 minutes , Melted paraffin wax (60°C) (2 changes) - 30 minutes. Next the tissues should be embedded in paraffin wax to prepare tissue blocks. Tissue blocks were fixed to metal object holder after trimming them to suitable size.

Section cutting: The tissue sections of the 5-6 μ m thickness were cut with the help of Spencer type rotating microtome and floated in a water bath at 50-55°C for 30 minutes. Then they were mounted on clear glass slides with a drop of Mayer's egg albumin dried on hot plate at about 50°C for 30 minutes.

Staining: After fixing the section on slide, the sections were stained by serially placing them in the following reagents- Xylol (2 changes) - 3 min, Acetone - 3 min, Alcohol 95% - 3 min, Running water - 3 min, Haematoxylin stain - 20 min, Running water wash - 20 min, Eosin working solution - 2 min, Alcohol 95% (3 changes) - 3 min, Acetone (2 changes) - 3 min, Xylol (2 changes) - 3 min. After passing through all the above reagents and stains, the slides would be covered with D.P.X. (Diphenylphthalein Xylene) and cover slip were placed. Care was taken to avoid the air bubble formation during mounting the slide. The slides are viewed under binocular research Carl-Zeiss's microscope at various magnifications to note down the changes in the microscopic features of the tissues studied.

Blood samples were collected before sacrificing the rat for the biochemical investigation for Serum C- reactive protein (CRP).

Preparation and stability of working reagent

CRP Calibrator: Reconstitute the calibrator with 1mL of distilled water.

Table 1: Assay procedure of serum CRP

	Calibrator	Sample / control
CRP R1	900 μ L	900 μ L
Calibrator	5 μ L	-
Sample	-	5 μ L
CRP R2	100 μ L	100 μ L
Mix and read the absorbance immediately (A1) and after 2 minutes (A2) of sample addition.		

Calculation

CRP con. in mg/ L = $\frac{(A2 - A1) \text{ sample} \times \text{Calibrator concentration}}{(A2 - A1) \text{ calibrator}}$

Statistical analysis

The experimental data were expressed as mean $\pm$ SEM. The data obtained was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test for determining the level of significance of the observed effects. A 'p' value of less

than 0.05 was considered statistically significant. Graph Pad In Stat-3 was used for statistical analysis of the generated data.

RESULTS**Acute inflammation / Carrageenan induced paw edema****Table 2: Effect of *Karanja* on Carrageenan induced paw oedema in time interval**

Group	Basal	1 st h	3 rd h	6 th h	24 th h
Control	0.93 $\pm$ 0.02	1.42 $\pm$ 0.05**	1.51 $\pm$ 0.06**	1.80 $\pm$ 0.11**	1.04 $\pm$ 0.04
Standard	0.90 $\pm$ 0.04	1.03 $\pm$ 0.03	1.10 $\pm$ 0.07**	0.93 $\pm$ 0.10	1 $\pm$ 0.04
<i>Karanja</i> leaf	1.05 $\pm$ 0.05	1.26 $\pm$ 0.05**	1.35 $\pm$ 0.03**	1.2 $\pm$ 0.05*	1.2 $\pm$ 0.01*

Data : MEAN (ml) $\pm$ SEM, *P < 0.05, ** P < 0.01

The effect of *Karanja* leaf swarasa on Carrageenan induced paw edema is depicted in Table 3.

The data shows there was increase in paw volume in control group of 1st, 3rd and 6th hour when compared to the basal paw volume of control group, the observed increase was found to be statistically very significant. And the increase observed at 24th hour was found to be non-significant.

In reference standard group the increase in paw volume was much less in comparison to the control group. Only the increase observed at 3rd hour was found to be statistically significant in comparison to the basal values. In *Karanja* leaf swarasa treated group also significant increase in paw volume was observed at different time intervals. However, it was moderate and less in comparison to the oedema formation observed in control group.

Figure 1: Paw edema**Chronic inflammation****Cotton pellet implanted granuloma****Table 3: Effect of *Karanja* on cotton pellet induced granuloma test**

Group	Cotton pellet granuloma formation mg/100g body weight	% change
Control	151.92 $\pm$ 15.61	
Standard	130.07 $\pm$ 20.18	14.38↓
<i>Karanja</i> leaf	156.73 $\pm$ 10.98	3.16↑

Data : MEAN $\pm$ SEM

The effect of *Karanja* leaf on cotton pellet induced granuloma test (body weight index of cotton) has been depicted in Table 4.

From the data presented above it can be observed that administration of reference standard lead to moderate but

statistically non-significant decrease in cotton pellet granuloma formation. In *Karanja* leaf swarasa given group no suppression of granuloma formation could be observed in comparison to the control vehicle group.

Table 4: Effect of *Karanja* on Weight of Spleen, Adrenal gland and Lymph nodes in cotton pellet implanted rats

Group	Weight of spleen (g)	% change	Weight of adrenal gland (g)	% change	Weight of lymph node (g)	% change
Control	0.94 $\pm$ 0.05	--	0.07 $\pm$ 0.00		0.30 $\pm$ 0.05	
Standard	1.21 $\pm$ 0.09	28.72↑	0.082 $\pm$ 0.01	17.14↑	0.22 $\pm$ 0.06	26.66↓
<i>Karanja</i> leaf	1.04 $\pm$ 0.06	10.63↑	0.07 $\pm$ 0.00	0	0.18 $\pm$ 0.01	40↓

Data : MEAN $\pm$ SEM

The data in the depicted table shows that there was increase in weight of spleen in standard group and in *Karanja* leaf group when compared to the control group. So the observed increase was found to be statistically non significant. Also there was no change in weight of adrenal gland in *Karanja* leaf group when

compared to the control group but there was decrease in the weight of lymph node in standard group and *Karanja* leaf group when compared to the control group. Hence the observed decrease was found to be statistically non significant.

Biochemical investigation

Table 5: Result of Serum C- Reactive Protein level in Cotton pellet implanted rats

Group	CRP	% change
Control	0.78 ± 0.16	-
Standard	1.28 ± 0.15	64.10 ↑
Karanja leaf	0.89 ± 0.15	14.10 ↑

Data : MEAN $\pm$ SEM

The effect of Karanja leaf swarasa on Serum CRP has been depicted in the Table 6.

From the data it is found that the standard administered group has shown slight increase in Serum CRP level than normal control group and found to be statistically non significant. The test drug administered group has also shown increase in the CRP level when compared to that of control group and was found to be statistically non significant.

Histological examination

Adrenal gland: Microscopic examination of the adrenal gland from control group exhibited well developed and differentiated cortex and medullary regions. Sections of adrenal gland from Karanja leaf swarasa administered group and reference standard group also exhibited normal cytoarchitecture. [Plate 1]

Spleen: microscopic examination of the spleen sections from control group showed normal cytoarchitecture. Sections from standard group also showed normal cytoarchitecture with slight decrease in cellularity in some sections. The spleen sections from Karanja leaf swarasa administered group showed normal cytoarchitecture. [Plate 2]

Lymph node: microscopic examination of the lymph node sections from control group showed normal cytoarchitecture with normal and good cellularity. The sections from standard group showed decrease in cellularity in majority of the sections examined. The lymph node sections from Karanja leaf swarasa administered showed normal cytoarchitecture in majority of sections except for decrease in cellularity in sections from one rat. [Plate 3]

Control Group

Standard Group

Test Group

PLATE 1: Showing micrographs of Adrenal gland of control, standard and Test groups

Control Group

Standard Group

Test Group

PLATE 2: Showing micrographs of lymph nodes of control, standard and Test groups

Control Group

Standard Group

Test Group

PLATE 3: Showing micrographs of Spleen of control, standard and Test groups

DISCUSSION

The leaves of Karanja is having indication in Shotha according to Bhavaprakasha Nighantu and Kaiyadeva Nighantu. Karanja possess Ushna Veerya which helps in treating lakshana like davathu, sira ayama and siratanutva due to vata dosha. The Katu and Tikta rasa, Laghu and Teekshna guna and Ushna veerya are responsible to subside the signs and symptoms like *Gaurava* and *Utsedha* caused due to Kapha dosha. The Tikta rasa relieves Ushma and Angavivarnata which is due to Pitta dosha. Therefore, the properties of this drug bring about Shothahara karma.

In the present study, coumarins, steroids and terpenoids were the chemical constituents found in the drug Karanja which are said to modify the activity of inflammatory mediators. In addition to these constituents, the drug was also found to have alkaloids, phenols, tannins, and Quinone. The general mechanism of these chemical constituents in inhibiting the pathways of inflammation can be explained by: immunoprotective or immunomodulatory property, inhibition of NFB, NO, COX and ROS generation, inhibition of enzymes-tyrosine and preventing the entry of microorganism. The Swarasa of Karanja leaf had been evaluated against Carrageenan induced paw edema in rats this model represents acute inflammation. Similarly it was evaluated against granuloma formation against cotton pellet implantation in rats as representative of chronic inflammation. Carrageenan induced hind paw edema test is based on the principle of release of various inflammatory mediators by carrageenan¹⁶. Edema formation due to carrageenan in the rat paw is biphasic, where in the first phase begins immediately after injection of carrageenan with the release of histamine, serotonin and kinins from local tissue damage and act on the vascular permeability lasting up to two hours. The second phase begins at the end of first phase and is due to the release of prostaglandins, protease and lysosomes by tissue macrophages after 3rd hour of tissue damage and lasts for a duration of three to six hours. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation, along with neutrophil extravasation, due to the metabolism of arachidonic acid. In order to assess its efficacy against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed.

In the present study Swarasa of Karanja leaves was found to produce significant effect on carrageenan induced paw odema at all the time intervals studied except 24th hour reading. This provides clear evidence for the presence of anti-inflammatory activity against acute inflammation. The initial phase is reported to be due to formation and release of inflammatory mediators like serotonin, bradykinins and the more pronounced later phase is mediated through release of prostaglandins. In Diclofenac and Karanja leaf swarasa groups inhibition of the release or activity or both of the inflammatory mediators seems to be involved in the expression of anti-inflammatory activity. The effect was quite pronounced and compares quite well with that of Diclofenac. Cotton pellet implanted granuloma model is based on the foreign body granuloma that can provoke by subcutaneous implantation of pellets of compressed cotton in rats. The cotton pellets implanted in the intrascapular region induces a chronic inflammation process in which development of proliferative cells, monocyte migration, liquid accumulation, apoptosis, damage and so on will occur in the surrounding tissue of the pellets and these accumulations will produce a granulation tissue that covers the pellets¹⁷. During the repair process of inflammation, there is proliferation of macrophages, neutrophils,

fibroblasts and multiplication of small blood vessels, which are the basic sources of forming a highly vascularised reddish mass, termed granulation tissue.¹⁸

Analysis of the results indicates mild non significant increase in the weight of spleen and moderate increase in the weight of Lymph nodes. But there was no significant increase or decrease in the weight of Adrenal gland in the Karanja group. So the observed result is non-significant. If the activity occurs as a result of adrenal involvement decrease in the weight of adrenal is expected since it is not so it can be inferred that adrenal stimulation is not involved in the activity observed. Weight changes in spleen and lymph node may be indicative of immunomodulation- since no significant change could be observed it may be indicative of no significant effect on immunomodulation mechanism. It could be observed from the data given above that the reference standard produced moderate and statistically non-significant effect against chronic inflammation in cotton pellet granuloma model in rats. The test formulation – *Karanja* leaf *Swarasa* had no effect on the formation of granulation tissue formation indicating that it has no effect on chronic inflammation and its anti-inflammatory activity is limited to acute inflammation only. In the present study *Karanja* leaf *Swarasa* failed to suppress chronic inflammation in the form of granulation tissue formation in the cotton pellet granuloma.

CONCLUSION

The drug Karanja (*Pongamia pinnata* Linn. Pierre) has been used since Vedic period for different diseases. Nighantus have mentioned the use of Karanja patra in *Shotha*. In Carrageenan induced paw edema for acute inflammation, the drug Karanja (*Pongamia pinnata* Linn. Pierre) has shown significant suppression in edema and has shown weak anti inflammatory effect in Cotton pellet implanted granuloma model for chronic inflammation. Hence, Karanja (*Pongamia pinnata* Linn. Pierre) leaves in 4.32 ml /kg rat dose is said to have Shothahara (Anti inflammatory) activity especially in acute inflammation is justified.

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