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Pharmacological Study

Anti-inflammatory activity of two varieties of *Pippali* (*Piper longum* Linn.)

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Abstract

The present study has beenundertaken to evaluate the anti-inflammatory activity of two varieties of *Pippali* in acute and sub-acute experimental models of inflammation in albino rats. Four different market samples of each variety of *Pippali* were procured from different regions of India. The samples collected from South India which have given more extractive values were selected for screening of anti-inflammatory activity. Randomly selected animals were divided into four groups of six animals each. The test drugs were administered orally at a dose of 200 mg/kg and the activity was compared with standard anti-inflammatory drugs in both models. Among the two different *test* samples studied, it was found that *Chhoti* variety of *Pippali* suppressed inflammation of both acute and sub acute phase, while *Badi* variety of *Pippali* only of acute phase. Thus for the therapeutic utility, *Chhoti* variety of *Pippali* may be considered over the *Badi* variety.

Key words: Anti-inflammatory, carrageenan, *Chhoti Pippali, Pippali,* plethysmograph

Introduction

Pippali (Piper longum Linn.) is one of the prime *Rasayana* (rejuvenator) drugs in Ayurveda and is widely used to treat various diseases especially for the treatment of respiratory disorders.[1] The root of this plant is known as *Pippali Mula* in Ayurveda and its fruits (Spike) are mainly used for *Rasayana* purpose. Several biological activities like immunostimulatory, anti-ulcer, anti-amebic, anti-oxidant, hepatoprotective and anti-inflammatory activities were reported on the fruit of this plant.[2,3] In Ayurvedic formulary of India, *Pippali* is being used in 324 formulations and it is one of the ingredients of the *Trikatu churna.*[4]

However, there are two types of *Pippali* available in the market in the name of *Chhoti Pippali* and *Badi Pippali*. Though both the varieties are used for therapeutic purposes, *Chhoti Pippali* is more preferred by physicians. It is a well-established fact that the *Pippali* is one of the most important drugs in the treatment of *Tamaka Shwasa* (bronchial asthma). The drug which is supposed to be used for the treatment of asthma should have anti-inflammatory activity. Thus, the present pharmacological study has been undertaken to screen the anti-inflammatory activity of two varieties of Pippali in different experimental models to ascertain which variety is having better anti-inflammatory effect.

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Materials and Methods

Procurement, identification and authentication of different samples of *Pippali* fruits

Four different market samples of each variety of *Pippali* were procured from different regions of India viz., East zone (Kolkotta), West zone (Jamnagar), North zone (Jaipur) and South zone (Thiruvananthapuram). Identification and authentication of these materials was done on the basis of organoleptic characters, exomorphology and pharmacognostic study at Pharmacognosy Laboratory. Further, these samples were subjected to preliminary phytochemical tests. Both *Chhoti* and *Badi* varieties of *Pippali* samples collected from South India, which gave higher extractive values (in terms of water soluble, methanolic and dichloromethane extractives), were selected for pharmacological screening.[5] The test drugs were made into fine powder (120 mesh size) and stored in air tight glass jar till the commencement of pharmacological study.

Animals

Wistar strain albino rats of either sex weighing between 180-200 g were selected for the study from the animal house attached to our institute. They were housed at 25 ± 3ºC with constant humidity 50 – 60%, on a 12 h natural day and night cycles. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water *ad libitum*. The experiments were carried out in accordance with the directions of the Institutional Animal Ethics Committee (IAEC/02/2007/MD/04).

Dose selection and schedule

The dose of the test drugs was calculated by extrapolating the human dose to animals (550 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1969).[6] The fine powder of both the varieties of *Pippali* was suspended in distilled water (55 mg/ml) and administered orally at a volume of 1 ml/100 g body weight. The drugs were administered orally with the help of a gastric catheter of suitable size sleeved on to a syringe nozzle. The animals of water control groups received equal volume of distilled water.

Statistical analysis

Students "*t*" test for unpaired data has been used for analyzing the data generated during the study. The values of drug treated groups were compared with water control group. *P* value less than 0.05 is considered as statistically significant.

Study protocol

Carrageenan-induced paw edema

The selected animals were weighed and randomly divided into three groups of six each. First group received distilled water and served as the control group. The second and third groups received test drugs *Badi* variety and *Chhoti* variety, respectively. Fourth group was administered with standard anti-inflammatory drug phenylbutazone in the dose of 100 mg/kg.[7] The vehicles and test drugs were administered to the respective groups for five consecutive days.

Initially left hind paw volumes up to the tibio-tarsal articulation were recorded prior to Carrageenan injection by using plethysmograph.[8] The plethysmograph employed consisted of a 10 ml glass vessel (25 × 65mm) fixed to a 2 ml glass syringe through pressure tubing. About 4 ml of mercury was filled in the syringe and the mercury level was adjusted to zero mark on the micropipette. The space between the zero mark and the fixed mark on the glass vessel was filled with water and few drops of teepol. The initial level of fluid was adjusted and set at zero. The paw was immersed in water exactly up to the tibio-tarsal articulation. The increased level of water in the glass vessel was adjusted to the prefixed mark by releasing the pressure of the connected syringe. The level where water and mercury interface in the micropipette was recorded as paw volume.

On fifth day 1 h after drug administration, edema was produced by injecting 0.1 ml of freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered tap water in the dose of 2 ml per 100 g body weight to ensure uniform hydration and hence to minimize variations in edema formation. Paw volume was recorded 3 h after carrageenan injection. Results were expressed as an increase in paw volume in comparison to the initial paw volumes and also in comparison with the control group.

Formaldehyde-induced paw edema

In this model, test conditions and groupings were similar to carrageenan-induced paw edema except the standard anti-inflammatory drug used i.e, diclofenac sodium (5 mg/kg) was used as the standard anti-inflammatory drug. Pedal inflammation was induced by injecting 0.1 ml of 3% formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 24 and 48 h after formaldehyde injection. Results were expressed as an increase in paw volume in comparison to the initial paw volumes and also in comparison with the control group.[9]

Results

Data pertaining to effect of *Badi* variety and *Chhoti* variety on carrageenan-nduced hind paw edema in rats are given in Table 1. Both the test drugs significantly inhibited the paw edema in comparison to water control group. Among the two varieties, *Chhoti* variety shows better suppression of paw edema in comparison to *Badi* variety.

Table 2 shows data related to the effect of *Badi* variety and *Chhoti* variety on formaldehyde-induced hind paw edema in rats. Apparent and statistically non-significant edema suppression was observed in *Badi* variety at both 24 and 48 h of formalin injection. In *Chhoti* variety administered rats, statistically highly significant suppression of paw edema was observed at both time intervals in comparison to water control group. As expected, in diclofenac sodium treated group, the edema suppression was observed at both 24 and 48 h of formalin injection. The observed suppression of edema in *Chhoti* variety treated group at both 24 and 48 h is comparable to standard anti-inflammatory drug, especially at 48 h it shows better suppression of paw edema than that of standard drug.

Discussion

Carrageenan-induced edema is commonly used in animal models for acute inflammatory agent(s) and is believed to be a biphasic event. The initial phase is attributable to the release of various biochemicals, viz. histamine, 5-HT, various kinins in the first hour injection of carrageenan. A more pronounced second

Table 1: Effect on carrageenan-induced paw edema in albino rats

Treatment	% increase in paw volume (3 h)	Percentage inhibition
Control	70.64±03.19	--
Badi Pippali (200 mg/kg, po)	57.33±04.85*	18.84 ↓
Chhoti Pippali (200 mg/kg, po)	50.66±06.36**	28.28 ↓
Phenyl butazone (100 mg/kg, po)	17.68±02.07***	74.98 ↓

The test formulations were administered by the oral route to groups of rats (n=6) for five days. The percentage increase in paw volume was measured at 3 h. The data are expressed as mean±SEM; significant differences in each group *vs.* the control are; * P< 0.05, ** P< 0.01, *** P< 0.001, ↓ - Decrease

Table 2: Effect on formalin-induced paw edema in albino rats

Treatment	Percentage increase in paw edema			
	24 h	Percentage inhibition	48 h	Percentage inhibition
Control	52.62±06.32	---	44.44±06.30	---
Badi Pippali (200 mg/kg, po)	44.04±03.89	16.30 ↓	38.29±05.41	13.83 ↓
Chhoti Pippali (200 mg/kg, po)	28.33±02.83**	46.16 ↓	17.74±04.00**	60.00 ↓
Diclofenac sodium (5mg/kg, po)	26.10±01.30***	50.40 ↓	27.16±02.18**	38.88 ↓

The test formulations were administered by the oral route to groups of rats (n=6) for five days. The percentage increase in paw volume was measured at 24 and 48 h. The data are expressed as mean±SEM; a significant difference in each group *vs.* the control are; * P< 0.05, ** P< 0.01, *** P< 0.001, ↓ - Decrease

phase is related to the release of prostaglandin-like substances in 2 to 3 h.[10,11]

Both the varieties of *Pippali* produced a considerable suppression of edema formation against carrageenan-induced paw edema in rats. The observed effect may be due to inhibition of one or more of the phlogistic mediators, antagonizing their interaction with their respective receptors, inhibition of phlogistic mediators or it may be due to general mechanism like increasing the membrane stability in the cell.

Inhibition of formaldehyde-induced pedal edema in rats is one of the most suitable tests to evaluate the anti-proliferative activity of inflammation in which inflammation occurs through proliferation and migration of fibroblast which are mainly concerned with the formation of connective tissue.[12] In this model, the *Badi* variety suppressed edema at both 24 h and 48 h time intervals in a non significant manner and *Chhoti* variety produced highly significant suppression of pedal edema which is almost equal to the effect of standard drug at 24 h and more effective than standard drug at 48 h of formaldehyde injection. The mechanism involved here may be attributed to suppression of this phase of inflammation by active constituents present in *Chhoti* variety of test drug. The suppression of edema by standard reference drug, diclofenac sodium; which is a non-selective COX inhibitor, was also found to be significant. The most remarkable point of this study was that the *Chhoti* variety at 200 mg/kg produced more inhibition of edema than the standard anti-inflammatory drug, diclofenac sodium.

Pippali (*Piper longum* Linn.) fruit contains a number of constituents, including volatile oil, alkaloids, isobutylamides, lignans and esters. Piperine, which is the prime constituent of fruit, is reported to be having significant anti-inflammatory activity.[13,14] In this study also, Piperine may be responsible for observed anti-inflammatory activity.

Conclusion

This study shows that *Chhoti* variety of *Pippali* suppressed inflammation of both acute and sub acute phase while *Badi* variety of *Pippali* only of acute phase. Thus for the therapeutic utility, *Chhoti* variety of *Pippali* may be considered over the *Badi* variety.

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हिन्दी सारांश

पिप्पली की शोथहर क्रिया का अध्ययन

ममता कुमारी, अशोक बी. के., रविशंकर बी., तरुलता एन. पण्ड्या, रबिनारायण आचार्य

इस अध्ययन में भारत के विभिन्न क्षेत्रों से एकत्रित दो प्रकार की पिप्पली के चार अलग अलग बाजार के नमूनों को लिया गया । इन चार प्रकार के नमूनों मे से दक्षिण भारत से लिये गये नमूने में सार की मात्रा अधिक होने के कारण इसको शोथहर प्रभाव के अध्ययन हेतु चयन किया गया । यह अध्ययन दोनों प्रकार की पिप्पली के शोथ (एक्यूट एवम् सब्एक्यूट) की स्थिति में पिप्पली के प्रभाव को प्रायोगिक माडल एल्बिनो चूहों में देखने हेतु किया गया । इस अध्ययन में चार वर्ग में छः – छः एल्बिनो चूहों को रखा गया । ग्रुप अ एवं ब में दोनों प्रायोगिक पिप्पली को क्रमशः २०० मि.ग्रा. प्रति किलोग्राम चूहे की मात्रा से प्रत्येक चूहे को मुख द्वारा दिया गया । ग्रुप स को बिना प्रायोगिक औषधि के एवं ग्रुप द को स्टैंडर्ड शोथहर औषधि पर रखा गया । पश्चात् शोथहर क्रिया का तुलनात्मक अध्ययन किया गया । दोनों प्रायोगिक नमूनों मे से छोटी पिप्पली को शोथ की एक्यूट एवम् सब्एक्यूट दोनों अवस्था में प्रभावी पाया गया, जबकि बड़ी पिप्पली को केवल एक्यूट अवस्था में ही प्रभावी पाया गया । अतः चिकित्सात्मक उपयोगिता के लिये बड़ी पिप्पली की अपेक्षा छोटी पिप्पली का प्रयोग करना श्रेयकर है । तमक श्वास में श्वसन नलिका में अत्यंत शोथ को कम करने में भी यह प्रायोगिक औषधि प्रभावी है ।