

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF TOPICAL  
PREPARATION OF ROOT EXTRACTS OF *ICHNOCARPUS FRUTESCENS* (L.) R.BR

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**ABSTRACT:** The root extracts of *Ichnocarpus frutescens* (L) R.Br is been scientifically proved for its analgesic and anti-inflammatory activity and the present study was under taken to evaluate the topical preparation of methanolic extracts of root of *Ichnocarpus frutescens* for analgesic and anti-inflammatory activities. Four different concentrations of the root extract was made in to a topical preparation i.e IF 1%, IF 2%, IF 4% and IF 6% with the help of a cream base containing cetyl alcohol, white petrolatum ,mineral oil, carbapol ,tween 80, water and Propylene glycol. All the four formulations along with cream base were screened for their analgesic and anti-inflammatory activities using formaline induced paw licking test and carrageenan induce paw edema models respectively. In analgesic activity, the IF 6 % has showed significant analgesic effect by decreasing the no. of paw lickings in formalin induced rat paw licking test; In anti-inflammatory activity the IF 1%, IF 2% has showed slight inhibition and IF 4%, IF 6% have showed significant inhibition of carrageenan induced rat paw edema compared to control group in which only cream base was used. The anti-inflammatory activity of IF 4 % and IF 6% were comparable with 0.5 % piroxicam gel which was used as standard and analgesic activity of the IF 6% was comparable with standard formulation 30% Methyl salicylate ointment.

**Keywords:** *Ichnocarpus frutescens* Topical preparation, Analgesic, Antiinflammatory.

## INTRODUCTION

*Ichnocarpus frutescens* R.Br. (Apocynaceae) is a climbing plant found throughout India. A large, much branched, twining shrub with long, slender, whip-like, finely fulvous to mentose branclilets; leaves simple, opposite, 3.7-7.5cm long, 2-3.8cm broad, ovate oval, rounded at base, acute, glabrous above, slightly hairy and paler beneath (Yoganarasimhan,1996 ; Nadkarni, 1976).

The roots of *Ichnocarpus frutescens* are said to be used to treat gout, rheumatism, cold, fever and catarrhal problems, as well as for relieving flatulence (Joshi, 2003). A tea made from it has also been used externally for skin problems, scrofula, and ringworm infections. It is generally called as a "blood purifier (Ashok Panigrahi & Alaka Sahni, 2000). It is also used for venereal diseases, herpes, skin diseases, arthritis, epilepsy, insanity, chronic nervous diseases, abdominal distention, intestinal gas, debility, impotence, turbid urine, diabetes and stone in gall bladder (Kirtikar &Basu, 1999).

The main constituents of *Ichnocarpus frutescens* (L.) R.Br. are Saponins, alkaloids, flavonoids (Kapoor et al., 1969), sorboside, Kampferol and kampferol -3, glucoside compounds (Gupta, 1988). The methanolic extract of roots of *Ichnocarpus frutescens* was proved for its anti-inflammatory and antioxidant activity (Pandurangan, 2009). The present study was under taken to prepare a suitable topical preparation of methanolic extract of roots of *Ichnocarpus frutescens* and to evaluate the anti-inflammatory and analgesic effect of the topical preparation by using carrageenan-induced rat paw edema and formalin induced paw licking tests.

## MATERIALS AND METHODS

**Raw herbs collection:** The roots of *Ichnocarpus frutescens* (L.) R.Br. were collected from Gandhi Krishi Vignana Kendra (GKVK), University of Agricultural Sciences, Bangalore.

**Authentication:** The plant material collected was identified and authenticated by Dr. K.P. Sreenath, Reader and Taxonomist, Botany Department from Bangalore University, Bangalore. A sample specimen deposited with voucher No.PESCP-IF-61/2007 in our laboratory.

**Extraction:** The roots of the plant were dried in the shade and powdered so that all the material could be passed through a mesh not larger than 0.5mm. Powdered plant material (100g) was macerated and extracted with methanol (700ml) for 18 h. The solvent was removed under vacuum, producing dry extracts. The weight of the extract was found to be 5.3g. Percentage yield of methanolic extract was found to be 5.3% w/w.

**Topical preparation of root extracts of *Ichnocarpus frutescens*:** Cream formulations (o/w) were prepared by the following procedure. Carbopol 941 was hydrated in water for 36 h using methyl paraben and propylene glycol and dispersed with a double bladed mixer (300 rev. /min) for 30 min., then heated to 60°C and oily phase containing different amounts of cetyl alcohol, white petrolatum, liquid paraffin oil and Tween 80 were weighed and melted approximately at 60°C. The aqueous phase was added to the oil phase and mixed with a double bladed mixer (300 rpm) for 30 min. The mixture was neutralized by sodium hydroxide to pH 6.2 and then mixed (300 rpm) for 30 min. *I. frutescens* extract was levigated in propylene glycol and then added to the formulation after neutralizing (Table 1) shows the constituents of investigated preparations).

### Pharmacological Activity:

In present study the topical preparation of root extracts of *Ichnocarpus frutescens* (L.) R.br was examined for anti-inflammatory and analgesic properties by Carrageenan induced rat paw edema in rats and Formalin induced paw licking test in rats respectively.

**Antiinflammatory activity - Carrageenan-induced rat paw edema:** Animals were allowed to free access to feed and water before the experiment. Approximately 50 µl of a 1% suspension of carrageenan in saline was prepared 1h before each experiment and was injected into the plantar surface of right hind paw of rat. 0.2g of cream containing 1-6% of *Ichnocarpus frutescens* methanolic extract were applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control groups received only the cream base and 0.5% Piroxicam gel (Cipla, Batch No.V136, Ahmedabad, India) applied in the same way as a reference standard. 1h after the application of the cream base, topical preparation of *Ichnocarpus frutescens* methanolic extract and 0.5% Piroxicam gel; 50 µl of a 1% suspension of carrageenan in saline was administered in to plantar surface of right hind paw of rat. Paw volume was measured immediately after carrageenan injection and at 1h, 2h, 3h and 4h after the administration of the noxious agent by using a plethysmometer (model 7159, Ugo Basile arese, Italy) (Niemegeer et al., 1964). The paw volume was recorded at different time points . The percentage inhibition in paw volume is calculated by using the formula.

$$\% \text{ Inhibition} = \frac{\text{Control paw volume} - \text{Test paw volume}}{\text{Control paw volume}} \times 100$$

**Analgesic activity - Formalin induced paw licking test in rats :** The formalin test possesses two distinctive phases, which possibly reflecting different types of pain. 0.2g of cream containing 1-6% of *Ichnocarpus frutescens* methanolic extract, cream base (vehicle control) and Methyl salicylate ointment 30 % (standard) were applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger. Fifteen minutes later, the antinociceptive activity was determined using formalin test described by Dubuisson and Dennis (Dubuisson & Dennis, 1977). Fifty micro liters of 2.5% formalin was injected into the dorsal surface of the left hind paw. The rat was observed for 60 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is known as the early phase and the period between 15 and 60 min as the late phase.

The percentage inhibition in paw volume is calculated by using the formula

$$\% \text{ Inhibition} = \frac{\text{Control paw volume} - \text{Test paw volume}}{\text{Control paw volume}} \times 100$$

**Statistical analysis:** The results of various studies were expressed as mean  $\pm$  SEM. Data analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability values of 0.05 ( $p < 0.05$ ) or less were considered statistically significant.

## RESULTS AND DISCUSSION

### Anti-inflammatory Activity:

The results of anti-inflammatory activity after topical administration of *Ichnocarpus frutescens* methanolic extract are given in Table 2. Statistical analysis showed that the topical preparations containing *Ichnocarpus frutescens* methanolic extract has showed significant inhibition of carrageenan induced rat paw edema when compare control group at all the tested concentrations and the activity is dose-dependent. The results showed that the anti-inflammatory effect of the formulation containing 6% of the *Ichnocarpus frutescens* methanolic extract was similar to the effect of 0.5 % Piroxicam gel (Standard).

**Table 1. Compositions of formulations of Topical preparation of root extracts of *Ichnocarpus frutescens***

Formulation	Oily phase				Aqueous phase			<i>I. frutescens</i> extract (g)
	Cetyl Alcohol(g)	White Petrolatum (g)	Mineral oil (g)	Tween 80 (g)	Carbopol (g)	Water (g)	Propylene Glycol (g)	
Cream Base	3	6	5	1	0.5	75	5	-
IF 1%	3	6	5	1	0.5	70	5	1
IF 2%	3	6	5	1	0.5	70	5	2
IF 4%	3	6	5	1	0.5	65	5	4
IF 6%	3	6	5	1	0.5	65	5	6

Methyl paraben (0.18% w/w) and propyl paraben (0.02% w/w) were used as preservative agents.

All of formulations were neutralized by sodium hydroxide solution (18%) in 0.2g per 100g of creams.

**Table 2: Effect of topical administration of *Ichnocarpus frutescens* methanolic extract on carrageenan-induced paw edema in rats**

Treatment <sup>a</sup>	n	Paw volume (ml) <sup>b</sup> at time after carrageenan injection				
		Initial	1 h	2 h	3 h	4 h
Cream Base	6	0.09±0.01	0.15±0.01	0.22±0.01	0.35±0.01	0.38±0.01
IF-1%	6	0.06±0.03	0.14±0.01 (3.30)	0.19±0.01 (13.63)	0.20±0.01* (41.42)	0.23±0.01* (39.61)
IF-2%	6	0.10±0.01	0.13±0.01 (10.00)	0.16±0.01* (27.27)	0.20±0.01* (42.14)	0.23±0.01* (38.96)
IF-4%	6	0.05±0.02	0.13±0.01 (13.33)	0.14±0.01* (34.09)	0.18±0.01** (46.42)	0.20±0.01** (46.75)
IF-6%	6	0.08±0.02	0.13±0.01 (12.66)	0.14±0.01** (36.36)	0.11±0.01** (68.57)	0.13±0.01** (65.58)
Piroxicam gel 0.5 %	6	0.09±0.0	0.12±0.01 (16.66)	0.13±0.01** (40.90)	0.09±0.01** (74.28)	0.11±0.01** (71.42)

n: Number of animals.

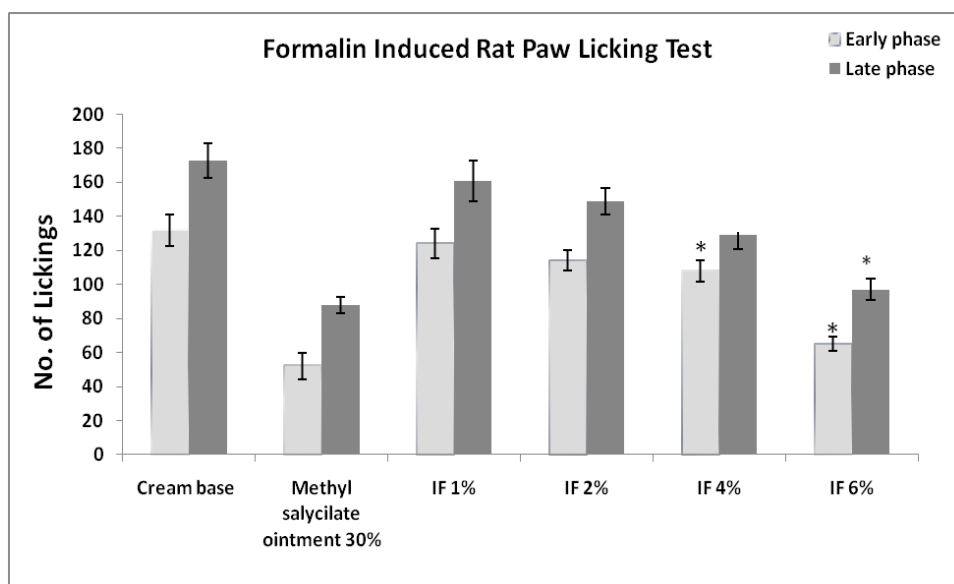
<sup>a</sup> 0.2g of Preparation were applied to the plantar surface of the right hind paw by gently rubbing 50 times with the index finger.

<sup>b</sup> Values are mean±S.E.M. (percent reduction). \*  $P < 0.05$ , \*\*  $P < 0.01$

### Analgesic activity

The effects of *Ichnocarpus frutescens* cream on formalin induced paw licking test are shown in **Figure. 1**. The groups that received topical preparation containing 6% of the *Ichnocarpus frutescens* methanolic extract and the group receiving 30% Methyl salicylate ointment have showed statistically significantly inhibition of formalin induced paw licking in both early phase and late phase when compare to control group

**Figure.1. Analgesic effect of topical preparation of methanolic extract of roots of *Ichnocarpus frutescens* on formalin-induced paw licking test in rats.**



Values are Mean  $\pm$  S.E.M. (n=6); Significance vs. Control group: \*P<0.05.

## CONCLUSION:

From these overall results, we can conclude that the topical preparation containing 6% w/w of methanolic extract of roots of *Ichnocarpus frutescens* possesses significant anti-inflammatory and analgesic effect, which can be useful for the treatment of acute pain and local inflammation. Preliminary phytochemical investigation of this plant showed the presence of alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids which might be in part responsible for analgesic and anti-inflammatory effects.

**Acknowledgement:** The authors are thankful to Prof. Dr. S. Mohan, Principal and management members of P.E.S.College of Pharmacy for providing all necessary facilities to carry out the research work.

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