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Research Article

Anti-inflammatory potential of alcoholic extract of *Indigofera oblongifolia* Forsk

Nitinkumar Upwar*[1], Roshan Patel[1], Naheed Waseem[2], Naveen Kumar Mahobia[3]

[1]Department of Pharmacognosy, Shree Leuva Patel Trust Pharmacy Mahila College, Amreli, Gujarat, India
[2]Department of Pharmacology, Shree Leuva Patel Trust Pharmacy Mahila College, Amreli, Gujarat, India
[3]Department of Pharmaceutical Chemistry, Shree Leuva Patel Trust Pharmacy Mahila College, Amreli, Gujarat, India

ABSTRACT

Anti-inflammatory activity of the methanolic extracts of the root of *Indigofera oblongifolia* Forsk was studied in Wistar rats using the carrageenan induced left hind paw edema. The methanolic extracts at the dose of 250 mg/kg and 500 mg/kg body weight shows moderate to significant anti-inflammatory activity. The methanolic extracts of *Indigofera oblongifolia* Forsk reduced the edema induced by carrageenan by 32.19 % and 40.97 % respectively on oral administration of 250 mg/kg and 500 mg/kg body weight as compared to the untreated control group. Diclofenac sodium at 10 mg/kg body weight inhibited the edema volume by 39.02 %. The results indicated that the methanolic extract 500 mg/kg body weight shows more significant ($p<0.05$) anti-inflammatory activity when compared with the standard and untreated control.

Keywords: Anti-inflammatory; *Indigofera oblongifolia* Forsk; Carrageenan.

INTRODUCTION

Indigofera oblongifolia Forsk belongs to the family Fabaceae and is widely distributed in Asia and Africa and throughout India. A woody branched undershrub, attaining a height of 4-6 ft. Leaves argenteo-canescent: leaflets 3-7, oblong or oblanceolate (The Wealth of India, 2001). In Ayurvedic formulation root is used as cooling, improve appetite, remove "vata-rakya" and rheumatism. All parts of plant are useful in enlargement of spleen and liver (Kirtikar and Basu, 1984). Its leaves are used in folk medicines in urinary tract infection, urolithiasis, cough and skin infection (Ali et al., 2001). Later these were also reported to possess antimicrobial activity (Dahot, 1999). A methanolic extract of the whole plant showed strong cytotoxicity in brine shrimp lethality test. The plant contains indigin, a novel alkylated xanthene and indigoferic acid, the fatty acid ester of p-hydroxy (E)-cinnamic acid. In addition β-sitosterol and 3-hydroxybenzoic acid. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation (Higgs et al., 1984, Vane, 1971). Arachidonic acid is also converted to leukotrienes via lipoxygenase enzyme. The aim of this present study is to investigate and evaluate the anti-inflammatory effect of *Indigofera oblongifolia* Forsk extracts on carrageenan induced inflammation in rats and provide scientific evidence for development of *Indigofera oblongifolia* Forsk as a potential natural oral anti-inflammatory agent.

* Corresponding Author
Email: nitinupwar@yahoo.com
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MATERIALS AND METHODS

Plant material

Roots of *Indigofera oblongifolia* Forsk were collected from Amreli localities, Amreli district (Gujarat), the plant was identified and authenticated by Dept. of Botany, Smt. U. B. Bhagat Science Mahila college, Amreli, Gujarat. A specimen voucher of the same is deposited in the college museum.

Preparation of extract

Roots of *Indigofera oblongifolia* Forsk (600 g) were extracted with methanol using Soxhlet apparatus. The resulting extract was evaporated in vacuum (Yield = 2.39 % w/w) and stored in desiccators for future use. The crude extract was dissolved 2% Tween 80 prior to the experiment and used.

Phytochemical screening

The Phytochemical examination of methanolic extract of *Indigofera oblongifolia* Forsk root was performed by standard methods (Harbone, 1984).

Animals

Wistar albino rats (150 – 180 g) of either sex were selected for the experiments. Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark sequence; at an ambient temperature of 25±2ºC; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum*. The experiments on animal were conducted in accordance with the international accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional Ethical Committee (Reg. No. 949/a/06/CPCSEA).

Acute toxicity studies

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (5% acacia) while the test groups received graded doses of extracts orally (p. o.) and were observed for mortality till 48 h and the LD_{50} was calculated (Ghosh, 1994).

Carrageenan induced rat paw edema

Methanolic extracts of root was evaluated for anti inflammatory activity by carrageenan induced hind paw edema method (Winter et al., 1962). The rats were divided into four groups of six animals each. First group (control) received 5 ml/kg body weight of normal saline; second group (standard) received 10 mg/kg body weight (i.p) Diclofenac sodium, third group received methanolic extract (250 mg/kg body wt, p.o.) and fourth group received methanolic extract (500 mg/kg body wt, p.o.) of *Indigofera oblongifolia* Forsk, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in solution up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was of UGO Basil Company. The paw volume was measured immediately after injection (0 h) and then every hour till 4 h after injection of carrageenan to each group. The difference between the initial and

Table 1: Effect of methanolic extracts of *Indigofera oblongifolia* Forsk (MEIO) on carrageenan induced rat paw edema

Groups	Dose (mg/kg)	Left hind paw volume (mean±S.D) (ml)					% inhibition in paw edema at 4th hour
		0 hr	1 hr	2 hr	3 hr	4 hr	
Control	--	1.70	1.75	1.85	1.91	2.05	--
MEIO	250	1.69±0.05*	1.55±0.06*	1.50±0.10*	1.44±0.09*	1.39±0.15*	32.19
MEIO	500	1.67±0.08*	1.52±0.07*	1.45±0.08*	1.32±0.11**	1.21±0.09*	40.97
Diclofenac Sodium	10	1.59±0.11*	1.54±0.03*	1.47±0.12*	1.33±0.06**	1.25±0.04*	39.02

*Values are expressed as mean ± S.D. (n=6).*p value compared to control (p < 0.001) i.e. significant; ** p value compared to control (p <0.05).*

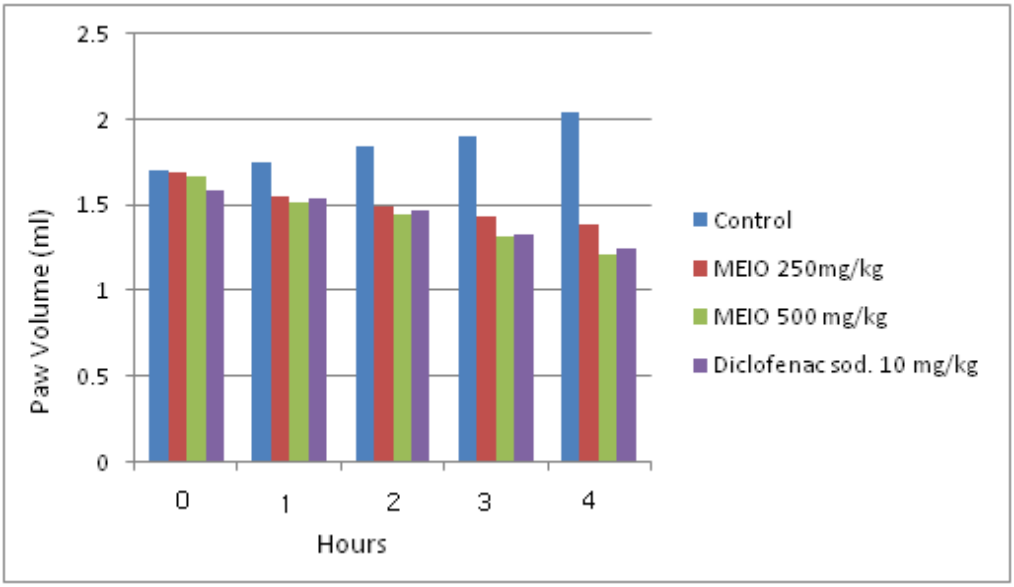

Figure 1: Graph of Anti-inflammatory activity of *Indigofera oblongifolia* Forsk

subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula,

$$\% \text{ inhibition} = 100 (1-Vt/Vc)$$

Where 'Vc' represents edema volume in control and

'Vt' edema volume in group treated with test extracts.

Statistical analysis

The experimental data were calculated as mean ± SEM., evaluated by unpaired one way ANOVA test. Values of $p< 0.001$ were considered statistically significant.

RESULTS

The average percentage yield of the methanol extracts of *Indigofera oblongifolia* Forsk root was found to be 2.39 % w/w. Preliminary phytochemical screening of the root of *Indigofera oblongifolia* Forsk revealed the presence of alkaloids, saponins, flavonoids and tannins. The LD_{50} was found to be 5000 mg/kg for methanolic extract of *Indigofera oblongifolia* Forsk. So the 1/10 of LD_{50} dose was considered as an effective dose. The effect of methanolic extract of *Indigofera oblongifolia* Forsk on carrageenan induced edema in rats is shown in Table 1. The results obtained indicate that the methanolic extract at a 500 mg/kg body weight had more significant anti-inflammatory activity in rats, The methanolic extracts of *Indigofera oblongifolia* Forsk reduced the edema induced by carrageenan by 32.19 % and 40.97 % respectively on oral administration of 250 mg/kg and 500 mg/kg body weight as compared to the untreated control group. Diclofenac sodium at 10 mg/kg body wt inhibited the edema volume by 39.02 %. The results indicated that the methanolic extract 500 mg/kg body weight shows more significant ($p<0.05$) anti-inflammatory activity when compared with the standard and untreated control.

DISCUSSION

Carrageenan induced paw edema was taken as a prototype of exudative phase of inflammation where development of edema being described as biphasic. The initial phase is attributed to release of histamine, serotonin and kinins after injection of carrageenan. A more prolonged second phase is related to the release of prostaglandins like substance (Vogel, 2002). The present study has shown that the methanolic extracts of *Indigofera oblongifolia* Forsk At dose 500 mg/kg exhibited significant anti-inflammatory activity being reported for first time. Preliminary phytochemical screening showed that the *Indigofera oblongifolia* Forsk revealed the presence of alkaloids, saponins, flavonoids and tannins. The flavonoids are known to possess anti-inflammatory activity by inhibiting the cyclooxygenase responsible for synthesis of inflammatory prostaglandins. Thus the anti-inflammatory activity of many plants have been attributed to their flavonoids, it is assumed that the effect could be due to the constituents such as flavonoids supporting the results for the present study (Zakaria et al., 2001, Reddy et al., 2007). It can be concluded that methanolic extract of *Indigofera oblongifolia* Forsk is endowed with centrally acting anti-inflammatory activity on acute inflammatory processes.

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