



ANTI-INFLAMMATORY POTENTIAL OF METHANOLIC EXTRACT OF *INDIGOFERA ENNEAPHYLLA* LINN.

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ABSTRACT

Anti-inflammatory activity of the methanolic extract of *Indigofera enneaphylla* Linn. was studied in Wistar rats using the carrageenan induced left hind paw edema. The methanolic extract at the dose of 200 mg/kg and 400 mg/kg body weight shows moderate to significant anti-inflammatory activity. The methanolic extract of *Indigofera enneaphylla* Linn reduced the edema induced by carrageenan by 23.10 % and 31.55 % respectively on oral administration of 200mg/kg and 400 mg/kg body weight as compared to the untreated control group. Aspirin at 20 mg/kg body weight inhibited the edema volume by 30.75 %. The results indicated that the methanolic extract 400 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 enneaphylla* Linn, Carrageenan.

INTRODUCTION

Indigofera enneaphylla Linn. belongs to the family Leguminosae and is widely distributed in Asia and Africa and throughout India. A non woody branched under shrub, attaining a height of 4-6 ft. Leaves pinnately Compound: leaflets 3-7, oblong or oblanceolate (The Wealth of India, 2001). Leaves, flowers and tender shoots are employed in decoction as a cooling and demulcent drink and in elephantiasis, leprosy and cancer, and as an alternative in secondary syphilis. Root is chewed as a remedy for toothache. The whole plant rubbed with butter is applied to reduce oedematous tumours. A preparation made from the ashes of the burnt plant is used to cure dandruff from the hair. Inflammation is considered as primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. 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 anti-microbial activity (Dahot, 1999). A methanolic extract of the whole plant showed strong cytotoxicity in brine shrimp lethality test. 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 enneaphylla* Linn. extract on carrageenan induced inflammation in rats and provide scientific evidence for development of *Indigofera enneaphylla* Linn as a potential natural oral anti-inflammatory agent.

MATERIALS AND METHODS

Plant material

The fresh plants of *Indigofera enneaphylla* Linn. were collected from Tiruchirappalli, Tamilnadu. Taxonomically identified and authenticated by Dr.V. Chelladurai, Research officer- Botany, CCRAS, (Govt. of India) Survey of Medicinal Plants Unit, Govt. Siddha Medical College Campus, Palayamkottai, Tamil Nadu.

Preparation of extract

Indigofera enneaphylla Linn. whole plants were dried under shade and pulverized separately by a mechanical grinder and passed through sieve No 40. The powdered plant materials were extracted with methanol by soxhlation for 24 hrs (Harborne JB, 1984). After filtration through Whatman filter paper No. 40, the filtrates were evaporated to dryness in vacuum at 35°C– 40°C. The percentage yield of the extract was calculated with reference to air dried powder.

The dried methanolic extract *I. enneaphylla* (MEIE) were stored separately in screw cap vial at 4°C in a refrigerator until further use. The dried extract was subjected to preliminary phytochemical screening and pharmacological studies. The crude extract was dissolved 2% Tween 80 prior to the experiment and used.

Phytochemical screening

The Phytochemical examination of methanolic extract of *Indigofera enneaphylla* Linn 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±2°C; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai) and water *ad libitum*.

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 LD50 was calculated (Ghosh, 1994).

Carrageenan induced rat paw edema

Anti inflammatory activity was evaluated by Carrageenan induced hind paw edema model method by using male albino rats (120-150gm). All experimental animals were divided into four groups of six animals each. First group (control) received 1 ml/kg body weight of propylene glycol; second group (standard) received 20 mg/kg body weight (i.p) Aspirin, third group received

methanolic extract (200 mg/kg body wt, p.o.) and fourth group received methanolic extract (400 mg/kg body wt, p.o.) of *Indigofera enneaphylla* Linn, 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 subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula,

$$\% \text{ inhibition} = 100 (1 - V_t/V_c)$$

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.

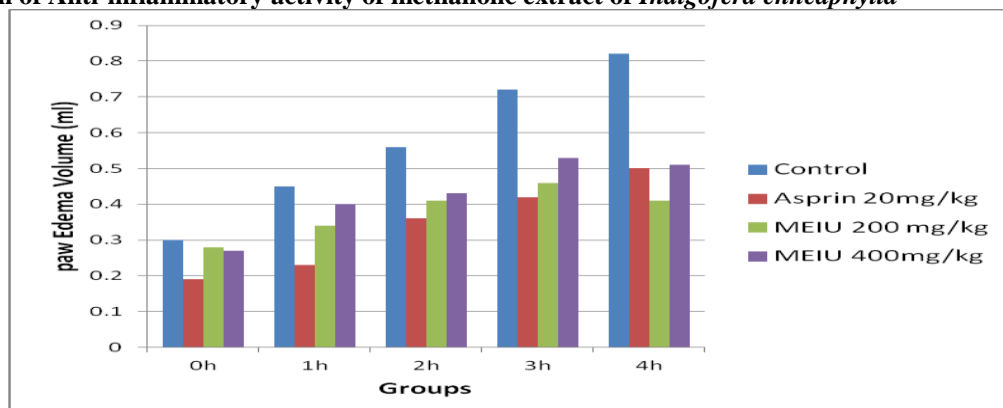
RESULTS

The average percentage yield of the methanol extract of *Indigofera enneaphylla* Linn was found to be 1.8% w/w. Preliminary phytochemical screening of the *Indigofera enneaphylla* Linn revealed the presence of alkaloids, saponins, flavonoids and tannins. The LD50 was found to be 2000 mg/kg for methanolic extract of *Indigofera enneaphylla* Linn. So the 1/10 of LD50 dose was considered as an effective dose. The effect of methanolic extract of *Indigofera enneaphylla* Linn on carrageenan induced edema in rats is shown in Table 1. The results obtained indicate that the methanolic extract at a 400 mg/kg body weight had more significant anti-inflammatory activity in rats, The methanolic extract of *Indigofera enneaphylla* Linn reduced the edema induced by carrageenan by 23.10 % and 31.55 % respectively on oral administration of 200 mg/kg and 400 mg/kg body weight as compared to the untreated control group. Aspirin at 20 mg/kg body wt inhibited the edema volume by 30.75 %. The results indicated that the methanolic extract 400 mg/kg body weight shows more significant (p<0.05) anti-inflammatory activity when compared with the standard and untreated control.

Table 1. Effect of MEIE on carrageenan induced Rat Paw Edema

Treatment	Dose mg/kg	Mean increase in paw Edema Volume (ml)					% Inhibition at 4 th hour
		0h	1h	2h	3h	4h	
Control	--	0.30±0.006	0.45±0.008	0.56±0.014	0.72±0.023	0.82 ±0.04	-
Aspirin	20	0.19±0.007**	0.23±0.004**	0.36±0.005**	0.42±0.005**	0.50±0.05**	30.75
MEIE	200	0.28±0.004*	0.34±0.007	0.41±0.005**	0.46±0.014**	0.41±0.022**	23.10
	400	0.27±0.005**	0.40±0.005**	0.43±0.008**	0.53±0.006**	0.51±0.024**	31.55

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Figure 1. Graph of Anti-inflammatory activity of methanolic extract of *Indigofera enneaphylla*

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 extract of *Indigofera enneaphylla* Linn at dose 400 mg/kg exhibited significant anti-inflammatory activity being reported for first time. Preliminary phytochemical screening showed that the

Indigofera enneaphylla Linn 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 enneaphylla* Linn is endowed with centrally acting anti-inflammatory activity on acute inflammatory processes.

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