



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of anti-inflammatory and analgesic activities of *Diospyros ferrea* leaves

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ABSTRACT

The chloroform and methanolic extracts of the leaves of *Diospyros ferrea* (100-300mg/kg) were tested for its anti-inflammatory and analgesic activities. The extracts showed 37% reduction in inflammation when compared with standard Ibuprofen and also exhibited significant analgesic activity. Carrageenin induced rat paw oedama method for anti-inflammatory activity and Tail flick method for analgesic activity were employed.

Keywords: *Diospyros ferrea*, leaves, Anti-inflammatory, Analgesic



INTRODUCTION

The plant *Diospyros ferrea* (wild) (Syn: *Maba buxifolia* pers. fam: Ebenaceae) is a bushy shrub widely distributed in Orissa and Southern India [1]. It is used to treat infertility and also shows anticancer, antimicrobial, diuretic activities along with termicidal activity [2-4]. In this present study the anti-inflammatory and analgesic activities of *Diospyros ferrea* leaves chloroform and methanolic extracts were have been investigated

MATERIALS AND METHODS

Preparation of extract

The leaves of *Diospyros ferrea* were collected in August 2010 from the Kailas hills of Visakhapatnam, Andhra Pradesh. They were dried, powdered and extracted in soxhlet with chloroform and methanol (2 liters each) and concentrated to a small volume. The concentrated extracts (chloroform extract, yield: 10gm, methanol extract, yield: 12gm) were tested for anti-inflammatory and analgesic activities.

Phytochemical screening

The preliminary phytochemical investigation was carried out for the two different extracts obtained from crude drug. It revealed the presence of naphthoquinones and triterpenoids [5-6].

Animals

Healthy adult male Wister rats weighing (150-200gm) were selected for the studies. Rats were housed in polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (i.e. 12:12hr light and dark sequence; at an ambient temperature of $25 \pm 1^{\circ}\text{C}$). The animals were fed with standard pellet diet and water ad libitum).

Instrument

Plethysmometer, it is a glass tube of 20mm internal diameter and one end fabricated to a glass tube with 0.5mm bore. This tube is fused to a flexible tube and a pump (glass-syringe) and fixed to other end of the tube. This pump is used to adjust the level of mercury in both the flexible tube and graduated up to zero level.

Procedure

Evaluation of Anti-inflammatory activity

The anti-inflammatory activity was assessed by the method suggested by Winter et al [7], using Carrageenin as phlogestic agent on adult Wistar Albino rats of either sex weighing between 150-200g. The selected Albino rats were housed in groups of six. They were fasted overnight and during the experiment but had free access to water. Both the extracts (100,300mg/kg) were suspended in 0.5%w/v sodium carboxy methyl cellulose and administered orally 30min before injection of carrageenin (0.1ml of 1% w/v solution) in normal saline into sub planter region of left hind paw of each rat. The contra lateral paw was injected with an equal volume of saline. The control group received 0.5w/v sodium carboxy methyl cellulose (2ml/kg), standard group of Ibuprofen (10mg/kg) and the tested groups received the chloroform and methanolic extracts of *Diospyros ferrea* (100mg and 300mg/kg) respectively. The paw volumes were measured plethysmographically at each hour, for 4hr after Carrageenin and compared with the standard treated group. Results are recorded in Table No.1

Table no. 1
Anti-inflammatory activity of *Diospyros ferrea* leaves extracts on Carrageenin paw oedema in Albino rats.

GROUP	TREATMENT	DOSE	VOLUME OF MERCURY DISPLACED (ml)				PERCENTAGE INHIBITION OF PAW OEDEMA AT 4hr
			0 hr	1 hr	2 hr	4 hr	
I	Control (0.5% sodium CMC)	2 ml/kg	0.967 ± 0.015	1.277 ± 0.026	1.36 ± 0.018	1.457 ± 0.019	–
II	Standard (Ibuprofen)	10 mg/kg	0.986 ± 0.192	1.27 ± 0.014	1.327± 0.018	1.28 ± 0.017**	41.105
III	Chloroform extract of <i>D.ferrea</i>	100 mg/kg	0.99 ± 0.014	1.323 ± 0.021	1.35 ± 0.015	1.36 ± 0.009*	26.178
IV	Chloroform extract of <i>D.ferrea</i>	300 mg/kg	0.99 ± 0.017	1.29 ± 0.015	1.33 ± 0.015	1.35 ± 0.015*	28.173
V	Methanol extract of <i>D.ferrea</i>	100 mg/kg	0.993 ± 0.001	1.293 ± 0.009	1.333 ± 0.011	1.347 ± 0.018*	29.583
VI	Methanol extract of <i>D.ferrea</i>	300 mg/kg	1.013 ± 0.01	1.293 ± 0.009	1.327± 0.01	1.333 ± 0.013**	37.604

Results expressed as mean ± SEM from six observations * p<0.01, ** p<0.001.

Evaluation of Analgesic activity

The analgesic activity was tested using analgesiometer [8].Albino rats (125-150gm) were randomly distributed in control and test groups of four animals each. The chloroform and

methanolic extracts (100 and 300mg/kg) were administered to each test group orally after 12hrs fast and standard drug pentazocin (30mg/kg) was administered to the control group orally. The basal reaction time was noted at 15min, 30min, 45min and 60min. After administration the tip of the rat was placed in the radiant heat of analgesiometer at $55^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The actual tail flick response of rats was calculated and compared with control group. The results are recorded in Table No.2

Table No 2. Analgesic activity of *Diospyros ferrea* leaves extracts (Tail flick method)

GROUP	DOSE	DOSE	BASAL REACTION TIME (sec)	REACTION TIME (sec)			
				15 min	30 min	45 min	60 min
I	Control (0.5% sodium CMC)	2 ml/kg	2.17 \pm 0.281	2.5 \pm 0.204	2.67 \pm 0.192	2.67 \pm 0.192	2.67 \pm 0.192
II	Standard (Pentazocine)	30mg/kg	2.33 \pm 0.192	3.0 \pm 0.408	4.67 \pm 0.385	6.33 \pm 0.304**	8.67 \pm 0.451**
III	Chloroform extract of <i>D.ferrea</i>	100mg/kg	2.17 \pm 0.281	3.17 \pm 0.28	3.5 \pm 0.204	4.5 \pm 0.391*	5.5 \pm 0.312**
IV	Chloroform extract of <i>D.ferrea</i>	300mg/kg	2.67 \pm 0.192	3.33 \pm 0.192	4.83 \pm 0.436*	5.83 \pm 0.549**	6.83 \pm 0.683**
V	Methanol extract of <i>D.ferrea</i>	100mg/kg	1.83 \pm 0.28	2.33 \pm 0.192	4.0 \pm 0.33*	5.0 \pm 0.236**	6.67 \pm 0.304**
VI	Methanol extract of <i>D.ferrea</i>	300mg/kg	2.0 \pm 0.236	3.17 \pm 0.436	4.5 \pm 0.391*	5.83 \pm 0.436**	7.33 \pm 0.304**

Results expressed as mean \pm SEM from six observations * p<0.01, ** p<0.001.

RESULTS AND DISCUSSION

The chloroform and methanolic extracts of leaves of *Diospyros ferrea* showed significant anti-inflammatory and analgesic activity at both the dose levels (100 mg/kg and 300 mg/kg). The percentage inhibition of paw oedema by the methanolic extract is found to be higher than the chloroform extract. The degree of analgesia observed with methanol extract revealed a higher degree of analgesic activity than the chloroform extract.

ACKNOWLEDGEMENT

Authors are thankful to the management and Principal of A.S.N Pharmacy college, Tenali, Andhra Pradesh, for providing necessary support for Research Programme.

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