EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *DIOSPYROS CORDIFOLIA* EXTRACT

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Abstract

In this study we evaluated the analgesic and anti- inflammatory activities of the methanol extract of stem bark of *Diospyros cordifolia* (MEDC) Roxb. The analgesic effects of the stem bark of the plant was assessed in mice using the tail-flick method while carrageenan, histamine and dextran induced paw oedema was used to study the antiinflammatory effects in rats. The MEDC exhibited significant ($p<0.01$) analgesic effects comparable to the reference drug diclofenac sodium. MEDC also was evaluated for its anti-inflammatory potential against carrageenan, histamine and dextran induced rat paw edema. The methanol extract (25 and 50 mg / kg body weight) exhibited significant ($p<0.01$) activity against all phlogistic agents used in a dose dependent manner. All these effects were compared with reference drug phenylbutazone (100 mg/kg body weight).

Keywords: Diospyros cordifolia, analgesic, anti-inflammatory.

Introduction

Inflammation is a process, accompanied by local liberation of chemical mediators that include histamine, 5-HT, bradykinin and eicosanoids (Lawrence and Bennet, 1992). Modern research in the field of anti-inflammatory compounds with improved tolerability and reduction in major side effects viz. gastric discomfort, dizziness, blurring of vision, rashes, itching etc. Drugs from plant origin are used in India for treatment of many diseases in traditional system of medicine. Diospyros cordifolia Roxb. belonging to family Ebenaceae is found in natural forest in West Bengal, India. The plant holds very important position in Indian folk medicine where it is mostly used as the treatment of liver disorder, whooping cough, leprosy, dysentery, abdominal pain, wounds, gonorrhoea, fever, inflammation, as emetic and anthelmintic (Nadakarni et al, 1954; Chopra et al, 1956). Chemical constituents that include ursolic acid, α-amyrin, β-amyrin, lupeol, taraxerol, nentriacontane, hentriacontanol and β-sitosterol (Suresh et al, 1989) were isolated from leaves of Diospyros cordifolia Roxb. Present study was undertaken to evaluate the analgesic and anti-inflammatory effect of stem bark extract of Diospyros cordifolia Roxb. in different animal models.

Material and Methods

Plant material

The plant Diospyros cordifolia Roxb. was collected in the month of November 2008 from the forest region of West Bengal, India. The taxonomical identification of the plant was done by Botanical Survey of India, Shibpur, India and the voucher specimen (PMU-5/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Preparation of Extract

The stem bark of the Diospyros cordifolia was dried under shade and then powered with mechanical grinder. The powder plant material was extracted with 80% methanol using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (yield 14.5 w/w). The extracts were stored in a vacuum dessicator for further use.

Animal used

Wistar albino rats (150-180 gm) and Swiss albino mice (20-25 gm) of either sex were maintained in identical laboratory conditions (temperature 25±2°C and 12/12 h light/dark cycle) and fed with commercial pellet diet (Hindustan Lever,

Kolkata, India) and water ad libitum. All procedures described were reviewed and approved by Jadavpur University animal ethical committee (ref no. 367001/C/CPCACA).

Analgesic activity

Analgesic activity was assayed by Tail-flick method (Seth U.K and Kamt U.G, 1972)

Tail-Flick method

Swiss albino mice (25-30 gm) of either sex (reaction time: 3-4 sec) were divided into four groups of 6 each. Diclofenac sodium (50 mg/kg) was used as a standard. The tail-flick latency was assayed by the analgesiometer (Techno, India). The magnitude of the current which was passing through the nichrome wire was kept constant at 6 ampere. The radiant heat (55^0C) in the tail was applied and maintained at 2.5 cm measured from the root of the tail. In order to avoid tissue damage, the cut of reaction time was kept at 10 sec. The mean scores in control, reference (Diclofenac sodium) and test groups were recorded and tabulated in table 1.

Anti-inflammatory activity

Anti-inflammatory activity was assayed by carrageenan, histamine and dextrane induced rat paw edema model.

Table 1. Analgesic activity of Diospyros cordifolia extract by tail-flick method in mice (n=6).

Reaction time in sec.(MEAN ± SEM)				
Treatment	Dose (mg/kg)	Tail-flick at 30 mint	Tail-flick at 60 mint	Tail-flick at 90 mint
Control	5 ml	3.16±0.06	3.52±0.04	3.66±0.03
Extract	25	7.11±0.08	8.44±0.04	9.78±0.04
Extract	50	8.45±0.03	10.21±0.07	11.36±o.05
Diclofenac Sodium	50	9.05±0.06	10.56±0.02	11.58±0.07

* P< 0.01 when compared with control group; statistically analysis was evaluated by Dunnett's vs. control.

Table 2: Effect of Diospyros cordifolia extract and phynylbuyazone on carrageenan induced paw edema in rats (n=6).

Treatment	Dose (mg/Kg)	Paw volume (ml)	Percentage of inhibition	*P value
Carrageenan control		0.9052±0.032	-----	------
Extract	25	0.4326±0.024	51.29%	< 0.01
Extract	50	0.2624±0.052	66.46%	< 0.01
Phenylbutazone	100	0.2120±0.017	76.42%	< 0.01

*P<0.01 when compared with control group; statistically analysis was evaluated by Dunnett's vs. control.

Carrageenan-induced rat paw edema

The rats were divided into four groups (n=6). Acute inflammation was produced by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hind paw of the rats. The paw volume was measured at 0-h and 3-h after carrageenan injection by using plethysmometer (Winter et al, 1962; Kavimani S, 1996; Oyemitan et al ,2008) . The animals of group I received normal saline (3 ml/kg) and served as saline control. The groups II, III and IV received methanol extract of Diospyros cordifolia (25 and 50 mg/kg b.w., intraperitoneal i.p) and reference drug phenylbutazone (100 mg/kg b.w., i.p) respectively. The animals of all groups were treated with the extract and reference drug 1 hour before the administration of carrageenan.

Table 3: Effect of Diospyros cordifolia extract and phynylbuyazone on histamine induced paw edema in rats (n=6).

Treatment	Dose (mg/Kg)	Paw volume (ml)	Percentage of inhibition	*P value
Histamine control		1.1326±0.0365	-----	----
Extract	25	0.5827±0.0327	47.37%	< 0.01
Extract	50	0.4612±0.0221	58.67%	< 0.01
Phenylbutazone	100	0.3026±0.0472	70.08%	< 0.01

*P<0.01 when compared with control group; statistically analysis was evaluated by Dunnett's vs. control.

Table 4: Effect of Diospyros cordifolia extract and phynylbuyazone on dextran induced paw edema in rats (n=6).

Treatment	Dose (mg/Kg)	Paw volume (ml)	Percentage of inhibition	*P value
Dextran control		1.2076±0.0462	-----	-----
Extract	25	0.7152±0.0224	41.18%	< 0.01
Extract	50	0.4982±0.0473	56.50%	< 0.01
Phenylbutazone	100	0.3831±0.0264	67.33%	< 0.01

*P<0.01 when compared with control group; statistically analysis was evaluated by Dunnett's vs. control.

Mediator-induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (viz. Histamine, Dextran) which act as mediator of inflammation. The paw edema was introduced in rats by sub plantar injection of freshly prepared histamine (1 mg/ml) and dextran (1 mg/ml) solutions respectively (Mazumder et al., 2003) and paw edema was measured as mentioned earlier.

Statistical analysis

All results are expressed as the mean ± SEM. The results were analyzed for statistical significance (P<0.01) by one-way (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software, U.S.A.

Results

Phytochemical tests showed the presence of flavonoids, tannin, terpenoids and saponins.

The methanol extract of the stem bark of *Diospyros cordifolia* showed significant dose-dependent analgesic activity in the tail-flick method (Table 1). Analgesic activity of the extract increases with time. From the results it was showed that the extract possessed significant (p<0.01) analgesic activity in dose dependent manner.

The anti-inflammatory activity of *Diospyros cordifolia* against carrageenan induced paw edema has been shown in Table 2 and the results were comparable to that of phenylbutazone, a protype of non-steroidal anti-inflammatory agent. The methanol extract showed maximum inhibition of 66.46% at the dose 50 mg/kg body wt. after 3 hrs of the extract treatment in carrageenan induced paw edema (Table 2) whereas the reference drug (phenylbutazone) produced 76.42% of inhibition. In case of histamine and dextran induced paw edema, the methanol extract produced 58.67% and 56.50% of inhibition (Tables 3 and 4) at the dose 50 mg/kg body wt. whereas the reference drug produced 70.08% and 67.33% of inhibition respectively.

Discussion

The tail-flick method is very effective for evaluating drugs possessing analgesic property, which act centrally (Vale et al., 1999; Haque et al., 2001; Silva et al., 2003; Al-Naggar et al., 2003). The extract increased the reaction time significantly at the dose levels used compared to control group. It is evident that carrageenan induced edema is commonly used as an experimental model for inflammation and is believed to be biphasic; the first phase is attributed to the release of histamine, serotonin and kinin and the second phase is elated to the release of prostaglandin and bradykinins(Castro et al ,1968; Vane and Booting, 1987). So the effect of the extract against inflammation produced by these individual mediators was studied. The extract effectively suppressed the inflammation produced by histamine and dextran. The methanol extract was found to possess flavonoids, tannin, terpenoids and saponins. So the anti-inflammatory activity of this plant may be due to the presence of these chemical constituents. Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce analgesic and anti-inflammatory effect (Alcatra and Jimenex, 1998; Della Loggia, 1986).

Thus, it is concluded that the methanol extract of stem bark of *Diospyros cordifolia* produced significant analgesic and anti-inflammatory activity against the tested models. The results support the traditional use of this plant in some painful and inflammatory conditions and suggest the presence of biologically active components which may worth further investigation and elucidation.

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