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Research Article

PRELIMINARY *IN VIVO* EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES OF VARIOUS SOLVENT EXTRACTS OF *CADABA INDICA* LAM ON CARRAGEENAN-INDUCED PAW EDEMA IN SWISS ALBINO RATS

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ABSTRACT

Objective: Among the invention of novel anti-inflammatory agents from modern research and drug development, the natural sources exist as evergreen to produce potential secondary metabolites that possess multiple efficacy against inflammatory mediators with no adverse reactions.

Methods: Accordingly, *Cadaba indica* lam (Capparidaceae) produced the positive results for phenol, flavonoids, steroid, and saponins in preliminary phytochemical screening and exhibited the potent anti-inflammatory activity (100 mg/kg, 200 mg/kg) by methanolic leaf extract against carrageenan-induced paw edema using rats in dose-dependent manner stayed closer to reference standard indomethacin (25 mg/kg) compared to petroleum ether and aqueous extract.

Results: Thus, the plant *C. indica* lam might be considered to posses potential secondary metabolites against inflammatory agents and act as lead to isolation of novel therapeutic compounds.

Conclusion: The phytochemical test indicates the presence of phenol, flavonoids, steroid, and saponins in leaf extract of *C. indica* may be known to possess anti-inflammatory property. The result of anti-inflammatory activity produced by the methanolic extract was threshold of isolation of bio molecules from the natural sources in diverse drug development in the near future being responsible for the pharmaceutical industries.

Keywords: Preliminary phytochemical, *Cadaba indica,* Anti-inflammatory.

INTRODUCTION

Inflammation is protective and defense mechanism of the body and thus, during inflammation various pathological changes take place [1]. It is characterized by elaboration of inflammatory mediators [2] and movement of fluid and leukocytes from the blood into extra vascular tissues. This response localizes and eliminates altered cells, foreign particles, microorganisms, and antigens and paves the way for the return to normal structure and function. Although a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain, or intensify numerous diseases [3]. Medicinal plants are considered to be an important source of new chemical substances with potential therapeutic effects [4]. In traditional medicine systems, different parts of the plant have been described to be useful against a variety of diseases. *Cadaba indica* belonging to the family Capparidaceae is an unarmed straggling much branched shrub up to 3 m height. Leaves simple or trifoliate, 8-12 cm length, entire, elliptic, oblong or ovate, mucronate dull green in few flowered terminal corymbs, fruit berry, and cylindrical torulose [5]. The leaves of *C. indica* lam plant are rich in lactones, steroids, flavonoids, phenol [6], reducing sugars, and tannins [7,8]. The plant *C. indica* was medicinally used for treating skin diseases, uterine obstruction, anthelmentic, purgative, deobstruent, emmenagogue and antisyphillictic, eczema, swelling, constipation and boils; its leaf juice is used as eye drops [9]. The leaf and flower liquid extract mixed with castor oil and turmeric is taken as a remedy for menorrhagia, syphilis, and as a purgative [9]. However, the activity of the ethanol extract of the leaves was found to be most effective against bacteria and fungi [10], antioxidant activity (Umesh BT), and antidiabetic activity [11]. This work was aimed to evaluate the anti-inflammatory potential of the leaf part of *C. indica*.

MATERIALS AND METHODS

Collection of the plant materials

The *C. indica* lam plants were collected in and around of Achirupakkam area in Kancheepuram District, in Tamil Nadu, India, and identified by the taxonomist, Department of Botany, Annamalai University, Chidambaram, India. Immediately after collection, the leaf sample was cut into small pieces and spread thinly on a flat, clean tray to prevent spoilage by moisture condensation and allowed to dry at room temperature for 3 days.

Preparation of extracts

To about 300 g of the plant sample was used for extraction process using in Soxhlet apparatus by hot percolation method. The extract was obtained by successive solvent extraction using petroleum ether, methanol, and aqueous using Soxhlet apparatus. The mixture was boiled for 45 minutes, and allowed to cool. Each extract obtained following successive extraction was filtered using Whatman No 1 filter paper, dried to a semisolid mass using a water bath and stored in a refrigerator at 4°C till further use [12].

Preliminary phytochemical screening

The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. The phytochemical test was carried out adopting standards procedure [12-15].

Animals

About 30 Wister Rats of either sex, weighing 300-400 g were procured from Animal House, GRD IMT, Dehradun (U.K.). The animals were housed in cages under standard laboratory conditions (12:12 hrs light/dark

cycle at 25±2°C). They had free access to standard commercial diet and water. The animals were divided into 6 groups of 5 each. The 1st group served as the control group, the 2nd, 3rd, 4th, and 5th groups were used as test groups, and the 6th group was the standard group [16].

Acute toxicity test

The acute oral toxicity study [17] was conducted as per the guideline set by the Organization for Economic Cooperation and Development guidelines 425 received from the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was evaluated in Sprague-Dawley males rats in 6 groups of 6 animals per group for each dose according to the carrageenan-induced paw edema described by Dimo *et al*. [18]. The aqueous extract at doses of 100 and 200 mg/kg body weight were given to groups (3 and 4), respectively, the methanolic extract at doses of 100 and 200 mg/kg body weight were given to groups (5 and 6) petroleum ether extract at doses of 100 and 200 mg/kg body weight were given to groups (7 and 8), respectively, and were administered orally an hour before the subcutaneous injection of 0.1 ml of sterile normal saline solution of carrageenan 1% (w/v) into the sub planter region of the right hind paw. The control group, (2) received distilled water whiles the reference drug indomethacin 25.0 mg/kg was also given to group (1) before induction of edema (baseline). Paw volumes were measured using plethysmometer 30 minutes before administration of carrageenan and thereafter, readings were taken hourly until the 4th hr last plant extracts administration.

Table 1: Preliminary phytochemical screening

Phytoconstituents	Aqueous extract	Methanol extract	Petroleum ether extract
Phenol	+	+	-
Flavonoid	+	+	+
Saponin	+	+	-
Steroid	-	+	+
Alkaloid	-	-	-
Tannin	-	-	-
Protein	-	+	-
Glycosides	-	-	-
Carbohydrates	+	+	-
Volatile oil	-	-	-

+: Indicates presence, -: Indicates absence

Table 2: Acute toxicity test for the extracts of *C. indica* Lam

Parameters	Aqueous extract	Methanol extract	Petroleum ether extract
Species and strain			
Number of animals	12	12	12
Sex	Male	Male	Male
Number of groups	3 (n=4)	3 (n=4)	3 (n=4)
Route of administration	Oral	Oral	Oral
Formulation	Freeze dried	Freeze dried	Freeze dried
Dose administered (mg/kg)	1250, 2500, 5000	1250, 2500, 5000	1250, 2500, 5000
Period of observation	48 hrs	48 hrs	48 hrs
Number of deaths	Nil	Nil	Nil
Approximate LD_{50}	>5000 mg/kg	>5000 mg/kg	>5000 mg/kg
Signs of toxicity	Nil	Nil	Nil

C. indica: Cadaba indica, LD_{50}: Lethal dose

RESULTS AND DISCUSSION

The lethal dose is >5000 mg/kg and may be classified as practically nontoxic and within the acceptable margin of safety (Hodge and Sterner scale) at the recommended dose. Thus, 1/50th and 1/25th (i.e., 100 mg/kg and 200 mg/kg) were selected for the study.

Mean values of paw edema inhibition (%) after treatment with standard and solvent extracts. Values are significantly different from reference drug of indomethacin ($p<0.05$).

The preliminary phytochemical investigation of the extracts showed (Table 1 and Fig. 1) that phenol, flavonoids, saponins, steroids, etc., the major phytoconstituents were present in methanolic leaf extract of *C. indica* compared to petroleum ether and aqueous extract. The secondary metabolites such as saponins, steroids, and flavonoids were considered to be responsible for anti-inflammatory agents of natural products as reported by [19-21]. The LD_{50} value observed at the dose of above 5000mg/kg for all the three extracts as shown in the results (Table 2). The anti-inflammatory activity was demonstrated by three extracts may be attributed to the presence of phytochemicals. The extracts showed modest anti-inflammatory activity in a dose-dependent manner as summarized in the results (Table 3). The results showed that the methanolic leaf extract of *C. indica* exhibited the highest percentage inhibition against carrageenan-induced paw edema in a dose-dependent manner, whereas petroleum ether leaf extract showed moderate result than the aqueous leaf extract.

CONCLUSION

The phytochemical test indicates the presence of phenol, flavonoids, steroid, and saponins in leaf extract of *C. indica* may be known to possess anti-inflammatory property. The result of anti-inflammatory activity produced by the methanolic extract was threshold of isolation of bio molecules from the natural sources in diverse drug development in the near future being responsible for the pharmaceutical industries.

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Table 3: Anti-inflammatory activity of leaf extracts of *Cadaba indica*. (paw edema (%) inhibition)

Time in hrs	Control drug 25.0 mg/kg	Aqueous extract (mg/kg)		Methanol extract (mg/kg)		Petroleum ether (mg/kg)	
	indomethacin	100	200	100	200	100	200
1	60.15	10.23	18.23	25.17	36.52	22.36	31.98
2	64.85	17.02	25.74	33.28	41.05	30.15	37.58
3	69.24	24.91	34.18	41.98	52.01	36.97	41.26
4	73.65	31.47	40.32	48.63	57.09	42.01	48.23
5	76.21	38.14	46.25	51.38	63.52	48.91	55.97

C. indica: Cadaba indica, mean values of paw edema inhibition (%) after treatment with standard and solvent extracts. Values are significantly different from reference drug of indomethacin ($p<0.05$)

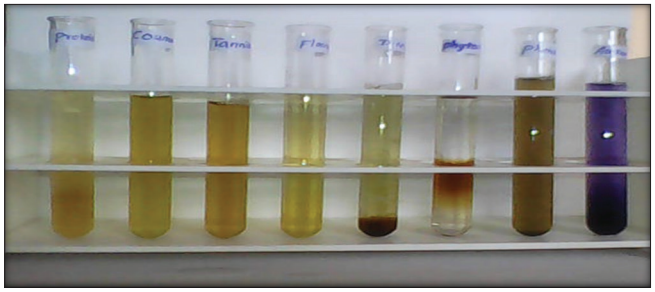

Fig. 1: Preliminary phytochemical screening

Research Centre, Thanjavur, Transdisciplinary University, Institute of Ayurveda and Integrated Medicine, Bengaluru and Dr. K. Pugazhendy, Assistant Professor, Department of Zoology, Annamalai University, Tamil Nadu, India.

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