In vitro and *in vivo* Antiinflammatory Activity of *Clerodendrum paniculatum* Linn. Leaves

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Preliminary phytochemical screening showed the presence of terpenes, flavonoids, tannins, alkaloids, phenolic acid, sterols, and glycosides. This study was intended to evaluate the antiinflammatory activity of various extracts of fresh leaves of *Clerodendrum paniculatum* Linn experimentally by *in vitro* (human red blood cell membrane stabilization method) and *in vivo* methods (0.1 ml of 1% w/v carrageenan-induced rat paw oedema model). Petroleum ether, chloroform, ethyl acetate, alcohol, and aqueous extracts were screened for *in vitro* antiinflammatory activity. Petroleum ether and chloroform extracts which showed, best *in vitro* antiinflammatory activity was screened for *in vivo* antiinflammatory activity at the dose level of 200 and 400 mg/kg. Indomethacin at the dose level of 10 mg/kg was used as reference standard drug. Both the extracts showed a dose dependent significant (P<0.001) reduction in paw edema when compared to the control, at all the time intervals and comparable to indomethacin (reference standard) treated group. The results of the present study demonstrate that petroleum ether and chloroform extracts possess significant (P<0.001) antiinflammatory potential which provide scientific basis for the traditional claims of *Clerodendrum paniculatum* Linn leaves as an antiinflammatory drug.

Key words: Antiinflammatory activity, carrageenan, *Clerodendrum paniculatum* Linn, human red blood cells membrane, verbenaceae

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Clerodendrum paniculatum Linn. (Family Verbenacea) commonly known as 'Red Pagoda plant' is a semi woody shrub of 1-2 m height growing naturally in shady places throughout India. It is used traditionally in India, China and Japan, in the treatment of rheumatism, neuralgia, ulcer, inflammation, and for healing wounds[1-3]. Preliminary phytochemical screening showed the presence of terpenes, flavonoids, tannins, alkaloids, phenolic acid, sterols, glycosides, phenolic acid, sterols, and glycosides.

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair, which are aimed at host, defense and usually activated in most disease condition[4]. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration[5]. Currently much interest have been paid in the search of medicinal plants with antiinflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response amplifies the disease process.

Bibliographical survey showed that there is no report on the antiinflammatory activity of *Clerodendrum paniculatum*. Linn. This study was intended to evaluate the antiinflammatory activity of various extracts of fresh leaves of *Clerodendrum paniculatum* Linn experimentally by *in vitro* (human red blood cell membrane stabilization method) and *in vivo* methods (0.1 ml of 1% w/v carrageenan-induced rat paw edema model).

Clerodendrum paniculatum Linn. leaves were collected in November 2008 from Ettumanoor in Kottayam distt., Kerala. The leaves were identified and authentified by the botanist from Department of Environmental sciences, M.G. University and voucher specimen (No: CPS 12/09) was deposited in the Herbarium of same department.

Fresh *Clerodendrum paniculatum* L. leaves were extracted with petroleum ether AR (Nice Chemical Pvt. Ltd., New Delhi) by simple maceration (yield: 1.16%). Residue was extracted with chloroform LR, ethyl acetate LR, ethanol LR (Nice Chemical Pvt. Ltd, New Delhi) by continuous hot percolation in a Soxhlet apparatus (yield: 2.17, 1.05, 0.89%, respectively). Aqueous extract was prepared by refluxing the residue in water in a reflux condenser (yield: 0.5%).

Male Wistar rats (150-200 g) were used for the *in vivo* antiinflammatory activity. The animals were housed in standard environmental conditions. Food and water were available *ad libitum*. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of University College of Pharmaceutical science, Cheruvandoor (Animal ethical Protocol number: IAEC/MGU/CHE/-M. Pharm/001/2009).

Extracts and the standard drugs were administered in the form of suspension in water with 1% Sodium carboxymethyl cellulose (SCMC) as suspending agent. Acute oral toxicity study was performed as per OECD-423 guidelines. Petroleum ether and chloroform extracts were safe up to a dose of 2000 mg/kg. Hence, 200 and 400 mg/kg were used as suitable doses for the evaluation antiinflammatory activity.

The human red blood cells (HRBC) membrane stabilization had been used as a method to study the antiinflammatory activity. Petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous extracts were used for the *in vitro* antiinflammatory activity[6-8]. Briefly blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.9% w/v NaCl) and a 10% suspension was made with isosaline. Various concentrations of the extracts were prepared (250, 500 and 1000 µg/ml) using distilled water and to each concentration 1 ml phosphate buffer, 2 ml hyposaline, and 0.5 ml HRBC suspension were added. These were incubated at 37º for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm. Indomethacin (100 µg/ml) was used as the reference

standard and a control was prepared omitting the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced by the control group as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula, percent protection=100−((OD of drug treated sample/OD of control)×100).

Carrageenan-induced rat paw edema model was used to study the *in vivo* antiinflammatory activity of petroleum ether and chloroform extracts. The extracts were suspended in distilled water using 1% SCMC as suspending agent. Male Wistar rats were divided into six groups each composed of six animals. Group I: Control animals received (1% SCMC, 10 ml/kg, p.o.) Group II: Animals received petroleum ether extract at the dose of 200 mg/kg p.o. Group III: Animals received petroleum ether extract at the dose of 400 mg/kg p.o. Group IV: Animals received chloroform extract at the dose of 200 mg/kg p. o. Group V: Animals received chloroform extract at the dose of 400 mg/kg p. o. Group VI: Animals received standard indomethacin (10 mg/kg, p.o.).

Paw edema was induced by injecting 0.1 ml of 1% carrageenan in physiological saline into subplantar tissues of hind paw of each rat. Petroleum ether and chloroform extract at the dose of 200 and 400 mg/kg were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180, and 240 min by the mercury displacement method using a plethysmograph. Percent inhibition (%IE) of edema was calculated using the equation, $\%IE=(V_c-V_t)/V_c\times100$, where V_c is the inflammatory increase in paw volume in control group of animals and V_t is the inflammatory increase in paw volume in drug treated animals. Inhibition of paw volume in drug-treated group was compared with the carrageenan control group (Group 1), whereas indomethacin (10 mg/kg p.o.) was used as reference drug.

The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The NSAIDs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC is similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis is taken as a measure of antiinflammatory activity of the drugs[9]. As shown in Table 1 the petroleum ether, chloroform, ethyl acetate, alcohol, and aqueous extracts of the leaves of *Clerodendrum paniculatum* Linn. were used to study the *in vitro* antiinflammatory activity at 250, 500, and 1000 µg/ml. Among all the extracts, petroleum ether and chloroform extract showed significant *in vitro* antiinflammatory activity in a concentration-dependent manner. Petroleum ether extract at a concentration of 1000 µg/ml showed 57.15% protection and chloroform extract at a concentration of 1000 µg/ml showed 48.98% protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 71.43% protection.

Petroleum ether extract and chloroform extract of *Clerodendrum paniculatum* Linn. at 2000 mg/kg body weight in two groups of mice did not produce any mortality for 14 days. There was no significant change in the body weight compared to the control (Table 2) and food consumption of animals of both groups. It indicated the safety of the extracts of *Clerodendrum paniculatum* Linn. in the experimental species.

The time course of edema development in carrageenan-induced rat paw edema model in rats is generally represented by a biphasic curve[10].

TABLE 1: *IN VITRO* ANTIINFLAMMATORY ACTIVITY BY HRBC MEMBRANE STABILIZATION METHOD

Treatment	Conc. (mcg/ml)	Absorbance (560 nm)	% inhibition
Control		0.49±0.02	
Petroleum ether extract	250	0.26±0.001	51.03
	500	0.23±0.001	53.07
	1000	0.21±0.001	57.15
Chloroform extract	250	0.27±0.001	44.90
	500	0.26±0.09	46.94
	1000	0.25±0.001	48.98
Ethyl acetate extract	250	0.31±0.001	36.74
	500	0.30±0.08	38.78
	1000	0.28±0.07	42.86
Alcohol extract	250	0.37±0.001	24.49
	500	0.36±0.001	26.54
	1000	0.35±0.003	28.58
Aqueous extract	250	0.36±0.001	26.50
	500	0.33±0.001	32.66
	1000	0.32±0.001	34.70
Indomethacin	100	0.14±0.001	71.43

Values are expressed as mean±SEM, $n=3$, $P<0.05$ compared to control group

TABLE: 2 CHANGE IN BODY WEIGHT OF MICE (G)

Treatment	Day 0	Day 4	Day 8	Day 12	Day 14
Control	20.55±12	20.58±43	20.65±60	20.90±32	21.05±53
Petroleum ether extract	20.43±0.72	20.64±0.51	20.92±0.62	21.11±0.33	21.32±0.82
Chloroform extract	20.86±0.55	20.98±1.00	21.22±0.77	21.30±0.63	21.46±0.88

TABLE 3: PAW EDEMA VOLUME

Group	60 min	120 min	180 min	240 min
I	0.35±0.002	0.54±0.001	0.72±0.001	0.77±0.002
II (%)	0.27±0.001* (22.8)	0.38±0.002* (29.6)	0.33±0.004* (54.2)	0.29±0.002* (62.3)
III (%)	0.24±0.003* (31.5)	0.37±0.002* (30.6)	0.32±0.003* (55.6)	0.23±0.001* (69.5)
IV (%)	0.27±0.002* (21.4)	0.44±0.001* (18.5)	0.44±0.004* (38.9)	0.39±0.002* (49.4)
V (%)	0.25±0.003* (28.6)	0.40±0.002* (26)	0.40±0.002* (44.5)	0.32±0.002* (58.5)
VI (%)	0.20±0.001* (42.9)	0.32±0.002* (40.8)	0.24±0.003* (66.7)	0.14±0.001* (81.8)

Values are expressed in milliliter (ml) and mean±SEM of six animals in each group. Comparisons were made between Group I *vs*, II, III, IV, V, and VI, *P* values* *P*<0.05, Percentage protection values are given in parenthesis, Difference between the control and the treatment was tested for significance using ANOVA followed by Dunnet's *t*-test

As shown in Table 3 there was no significant inhibition of paw edema in the early hours of study by the petroleum ether and chloroform extracts of *Clerodendrum paniculatum* Linn leaves at the dose level of 200 and 400 mg/kg. Hence, it can be concluded that there is no inhibition of histamine and seritonin. Carrageenan-induced rat paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of nonsteroidal antiinflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis[11]. It plays a major role in the development of second phase of inflammatory reaction, which is measured at the third hour[12]. As shown in Table 3 the petroleum ether extract at the dose of 400 mg/kg showed high significant activity (P<0.05) at 4 h, where it caused 69.5% inhibition, as compared to that of 10 mg/kg of indomethacin which exhibited up to 81.8% inhibition. Therefore, it can be inferred that the inhibitory effect of petroleum ether and chloroform extracts on carrageenan-induced inflammation may be due to inhibition of the cyclooxygenase leading to inhibition of prostaglandin synthesis.

The results of the present study have provided scientific basis for the traditional uses of *Clerodendrum paniculatum* Linn. leaves in inflammation, possessing significant antiinflammatory activity. This may be due to the presence of terpenoids and flavonoids which deserve further studies to isolate the active constituent responsible for the activity and to find out its mechanism of action.

ACKNOWLEDGMENTS

Authors wish to thanks Mr. Joby Paul, Botanist from Department of Environmental Sciences, M.G. University for plant authentication.

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Accepted 30 April 2013
Revised 29 April 2013
Received 07 October 2011
Indian J Pharm Sci 2013;75(3):376-379