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Anti-inflammatory activity of *Plumbago capensis*

Charles Dorni A.I*., Vidyalakshmi K.S., Hannah R.Vasanthi., Rajamanickam G.V and Dubey G.P

Centre for Advanced Research in Indian System of Medicine (CARISM)
SASTRA Deemed University, Thanjavur, Tamil Nadu, India
**** For Correspondence:** aicd_charles@yahoo.co.in*

ABSTRACT - The HAEPC (Hydroalcoholic extract of *Plumbago capensis*) showed a maximum of 46.88% inhibitory action on Carrageenan induced paw edema at the dose of 250 mg/kg and inhibited the leukocyte migration in a dose dependent manner. The anti-inflammatory activity observed was comparable to the standard non-steroidal anti-inflammatory drug indomethacin (10mg/kg). *Plumbago capensis*, a lesser known *Plumbago* species has significant anti-inflammatory activity with potential constituents targeting different components of inflammatory process.

KEY WORDS - *Plumbago capensis*, Plumbagin, Peritonitis, HPTLC, Prostaglandin, Carrageenan.

INTRODUCTION

Plants of the genus *Plumbago*, namely *Plumbago capensis (PC)*, *Plumbago rosea (PR)* and *Plumbago zeylanica (PZ)*, grown throughout India, have long been used traditionally in Indian folk medicine to treat inflammatory disorders such as rheumatoid arthritis, laryngitis and skin diseases such as leucoderma, ringworm, scabies, leprosy *etc*., Among the three species *PZ* has been widely assigned for various medicinal properties and is used in formulations of a number of ayurvedic compounds (1). The root of the three species are rich source of 1,4 naphthoquinone, Plumbagin, exhibiting a wide range of pharmacological activities such as anti-bacterial, anti-fungal, anti-coagulant, anti-fertility, anti-cancer activity, anti-helicobacter pylori activity (2,3). PZ and PR have been prescribed in the treatment of cancer in Siddha system of medicine (4).

Plumbago capensis Thunb., a small scandent shrub, indigenous to S.Africa is a lesser known species in ethnopharmacognosy. Previous studies reported the presence of anti fungal protein acting against *Trichosporium vesiculosum* and *Macrophomina phaseolina* in the crude extract of *P.capensis* (5, 6). The present study was focused on the anti-inflammatory effects of the hydroalcoholic extract of *Plumbago capensis* root (HAEPC) in animal models of acute inflammation. The bioactive marker plumbagin has been quantified using HPTLC.

MATERIALS AND METHODS

Chemicals **-** Carrageenan and Indomethacin was obtained from Sigma-Aldrich, Germany. All the solvents used were of analytical grade procured from E.Merck, Mumbai and the standard Plumbagin (RM 2947) was purchased from Hi-Media.

Plant materials **-**

Air dried roots of *Plumbago capensis* were collected from Nagercoil, Tamil Nadu, India and taxonomically identified by the Captain Srinivasa Murti Drug Research Institute (CSMDRI), Chennai and a voucher specimen was deposited in the CARISM herbarium.

Preparation of the Hydroalcoholic Extract **-**

1000 gm of air dried roots was extracted by cold maceration process with 70% Hydroalcohol (Ethanol:Water; 70:30) for 72 hrs. The hydroalcoholic extract (HAEPC) was rotary evaporated at 50°C and concentrated *in vaccuo* to obtain a brown solid mass with a yield of 12.9% w/w. Phytochemical screening of the extract revealed the presence of quinones, flavonoids, terpenoids and amino acids (7).

Acute Oral Toxicity -

Acute oral toxicity of the HAEPC was studied in Swiss albino mice weighing 20-25 gm. HAEPC was administered orally at the dose of 100, 1000, 2000, 3000 or 4000 mg/kg to 6 groups of animals (8 mice in each). Control group received normal saline (5 ml/Kg orally or 0.1 to 0.125 ml for mice weighing 20-25 g). Signs of toxicity and mortality within 24-72 h were noted. CPCSEA Registration No.817/04/AC/CPCSEA.

Estimation of Plumbagin in Hydroalcoholic Extract by HPTLC

To determine the content of plumbagin in the HAEPC, 500 mg of HAEPC is made up to 25 ml with chloroform as naphthoquinones are reported to be easily soluble in chloroform (8, 9). The resulting solution is centrifuged at 3000 rpm for 15 minutes and the supernatant is

analyzed for plumbagin content. 40 µl of the filtered solution is applied on a 10 x 10 cm preactivated HPTLC Silica gel 60F254 plate. HAEPC and standard plumbagin were applied to the plate as 6 mm wide band with an automatic TLC applicator Linomat V with N_2 flow (CAMAG, Switzerland), 8 mm from the bottom. Densitometric scanning was performed on CAMAG scanner III at 265 nm. The plates were pre-washed by methanol and activated at 60° C for 5 min prior to chromatography. The slit dimension was kept at 5 mm x 0.45 mm and 40 mm/s scanning speed was employed. The mobile phase consisted of toluene:ethyl acetate: methanol (8:1:1) and 10 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 10 cm x 10 cm twin glass chamber saturated with the mobile phase. The analysis is repeated for six times and the possibility of interference from other components of HAEPC in the analysis is studied.

Carrageenan induced Paw Edema.

Male wistar rats weighing 120-150 gm were used. The animals were put on standard diet and water was provided *ad libitum*. The animals were fasted overnight before the experimentation. The rats were divided into five groups (n=6). The anti-inflammatory activity of HAEPC was assessed by the method described by Winter *et al,* (10). Rats in Group I were given normal saline and were treated as control. Rats in Group II were administered Indomethacin in normal saline at the dose of 10 mg/kg b.w. orally and were kept as standard (11).

Rats in Group III to Group V were administered orally with the HAEPC in normal saline at the doses of 150, 250 & 350 mg/kg b.w. respectively. Since the LD 50 has not been determined during the acute toxicity study, the doses for this study were selected by trial and error method. The standard and HAEPC were given orally to the animals one hour prior to Carrageenan injection. Acute paw edema was induced by injecting 0.1ml of 1 % (w/v) Carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. The perimeter of paw was measured by using vernier caliper. Measurements were taken at 0, 1, 2, 3 & 4 hours after the administration of the Carrageenan.

% Inhibition of Edema = {[C-T}/C}*100

Where, C – Control Paw Edema ; T – Test Paw Edema

Carrageenan Induced Peritonitis

Inflammation was induced by the modified method of Griswold *et al.*, 1987 (12). Male Swiss albino mice weighing 20-25 gm were divided into five groups (n=4). Group I served as control. Group II served as standard and was dosed with Indomethacin (10 mg/kg, p.o) and Groups III – V were dosed with HAEPC at the doses of 150, 250, 350 mg/kg, p.o. Since the LD 50 has not been determined during the acute toxicity study, the doses for this study were selected by trial and error method. The standard drug and HAEPC were administered orally one hour prior to the induction of peritonitis. After one hour, Carrageenan (0.25 ml, 0.75 % w/v in saline) was injected intraperitoneally. Four hours later, the animals were sacrificed by cervical dislocation and 2 ml of Ca^{2+} and Mg^{2+} free phosphate-buffered saline (PBS) was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined (13,14). The percentage of the leukocyte inhibition was calculated using the following formula: Leukocyte Inhibition (L I %) = (1- T/C) X 100

where 'T 'represents the treated groups' leukocyte count and 'C' represents the control group leukocyte count.

Inhibition of Neutrophil migration was calculated by the following equation: Inhibition of Neutrophils Migration = 100 – {[NT/NC] X 100}.

Where, NT = Neutrophil counts of treated groups; NC = Neutrophil counts of Control group.

Statistical analysis

Results are expressed as mean ±S.D. and difference in means are determined by one-way ANOVA followed by post-hoc analysis with Dunnet's t-test ; values $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Plumbago species is one of the most important medicinal plants grown throughout India. The whole plant and its root have been used as a folk medicine in India as well as in countries like Taiwan, Vietnam etc., for the treatment of rheumatic pain, menostasis, carbuncle, and injury by bumping.(15). Despite the traditional use of the species in rheumatic diseases, the anti-inflammatory activity of the *Plumbago* species has not been studied in detail in the past.

Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenan induced inflammation is a biphasic event, the first phase occurs within an hour of injection is attributed to the release of histamine, 5-HT and kinins, while the second phase which can be measured around 3-4 hours time is related to the release of

prostaglandins (16). The presence of prostaglandin E_2 in inflammatory exudates from the injected foot can be demonstrated at three hours time period and thereafter. (17) Indomethacin is used as standard reference drug as it is reported to inhibit inflammation by its effect upon plasma exudation associated with carrageenan mediated inflammation. (18). HAEPC showed a maximum of 46.88% edema inhibition at 3 hrs at the dose of 250 mg/kg and the effect lasted for 3 hrs for *P.capensis*. The inhibitory activity decreased after 3 hrs and above 250 mg/kg dose the anti-inflammatory effect recedes.

Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induce accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility (19) Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility. In the present study the standard NSAID, Indomethacin (10mg/kg) has produced 63.36% of leukocyte inhibition and 42.16% inhibition of neutrophil migration. HAEPC exhibited a maximum of 48.85% leukocyte inhibition at 350 mg/kg dose and the effect increases dose dependently. The results suggest that the HAEPC have potential constituents interacting with the different cellular processes of inflammation.

As, plumbagin is reported to have cytotoxic activity (20) its quantity in the HAEPC is of greatest importance in terms of toxicity and biological activity. Hence, we quantified the amount of plumbagin in the hydroalcoholic extract and studied the acute toxicity in albino mice. The spot at Rf = 0.78 corresponding to plumbagin was observed in the chromatogram of the HAEPC along with other components. There is no interference from other components present in the chromatogram. The HAEPC was found to contain 0.43 % (w/w) of plumbagin in dried extract.

The mice administered with the HAEPC showed no toxic signs or mortality up to a dose of 4000 mg/kg showing the safety of the extract. Plumbagin as a pure molecule also exhibited concentration dependent immunomodulatory activity on macrophages in BALB/c mice (21). Plumbagin which is also found as active ingredient in the common species of *Plumbago*, *P.zeylanica*, reported to have significant antioxidant activity. In an antioxidant and pulse radiolysis study (22), the PZ extracts (aqueous/alcoholic) and plumbagin showed significant antioxidant abilities in FRAP, ABTS and DPPH assays. The observed anti-inflammatory activity of HAEPC can also be attributed to the anti-oxidant potential of the naphthoquinone, plumbagin in the extract, as inflammation involves oxidative damage.

Table.1 - Effect of Plumbago capensis on carrageenan-induced paw edema in rats (n=6)

Groups	Paw volume (cm, Mean ± SD)				Edema value(cm, Mean ± SD)			
	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	0.62 ±0.02	0.67 ±0.03	0.74 ±0.02	0.69 ±0.02	0.21 ±0.01	0.25 ±0.02	0.32 ±0.02	0.27 ±0.01
Indomethacin 10 mg/kg	0.56* ±0.02	0.57* ±0.02	0.55* ±0.01	0.53* ±0.02	0.16* ±0.03 (23.81)	0.17* ±0.02 (32.0)	0.15* ±0.02 (53.13)	0.13* ±0.01 (51.85)
PC 150 mg/kg	0.59 ±0.01	0.62* ±0.002	0.66* ±0.02	0.63* ±0.02	0.18 ±0.02 (14.29)	0.21* ±0.03 (16.0)	0.25* ±0.03 (21.88)	0.22 ±0.03 (18.52)
PC 250 mg/kg	0.56* ±0.04	0.58* ±0.02	0.56* ±0.02	0.58* ±0.03	0.17* ±0.03 (19.05)	0.19* ±0.02 (24.0)	0.17* ±0.02 (46.88)	0.19* ±0.04 (29.63)
PC 350 mg/kg	0.56* ±0.02	0.58* ±0.02	0.61* ±0.01	0.60* ±0.02	0.16* ±0.02 (23.81)	0.18* ±0.01 (28.0)	0.21* ±0.01 (34.38)	0.20* ±0.01 (25.93)

*Values represent the mean ± S.D. of 6 animals for each group. Values in parenthesis indicate the percentage inhibition rate. * Experimental groups compared with control ($p<0.05$)*

Table.2 Effect of *Plumbago capensis* on Carrageenan induced Leukocyte Aggregation (n=4)

Groups	Total Leukocyte Count (10^6 Cells/cmm)	% Leukocyte Inhibition	Neutrophils ($10^5 mL^{-1}$)	% Inhibition of Neutrophil Migration
Control	16.13± 2.37	-	0.67±0.02	
Indomethacin 10 mg/kg	5.91± 2.05*	63.36	0.39±0.01*	42.16
PC 150 mg/kg	12.83± 2.20	23.46	0.53±0.02*	22.64
PC 250 mg/kg	10.19± 0.66*	36.83	0.50±0.03*	25.37
PC 350 mg/kg	8.25± 2.30*	48.85	0.40±0.29*	40.30

*Values are mean±SD; * Experimental groups were compared with control ($p<0.05$)*

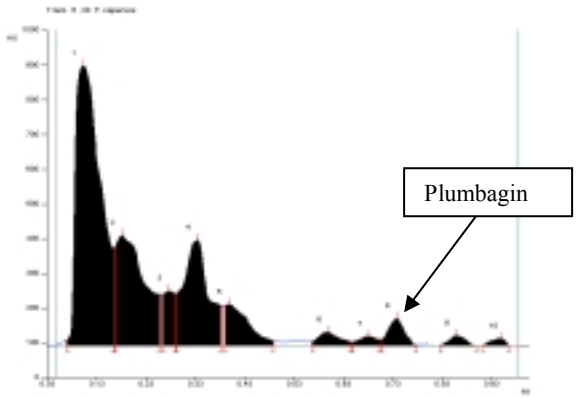

Fig.1 HPTLC chromatogram of Plumbago capensis

Thus the hydroacloholic extract obtained from the root of *Plumbago capensis* is found to have a significant anti-inflammatory activity due to the synergistic effect of multiple ingredients of the extract including the known bioactive marker plumbagin.

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