

ANTI-INFLAMMATORY ACTIVITY OF SEED EXTRACTS OF *PONGAMIA PINNATA* IN RAT

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Abstract : *Pongamia pinnata* is a marsh growing Indian tree. Its seeds are used in febrile and inflammatory diseases in Indian system of medicine. Previous preliminary studies with ethanolic seed extract of this plant had exhibited an anti-inflammatory effect in rat. Different solvent fractionated extracts were evaluated in the present study for anti-inflammatory effect in chemically induced paw inflammation in rats. Anti-inflammatory effects of *P. pinnata* were best seen against bradykinin and PGE₁-induced inflammation. In contrast minimal effects were seen against histamine and 5-HT-induced inflammation. The predominant action of extracts of *Pongamia pinnata* appears to be a modulation of eicosanoid-events in inflammation.

Key words : *Pongamia pinnata* inflammation eicosanoids

INTRODUCTION

Pongamia pinnata (L) Pierre (Family - Leguminosae (Papilionoidae); Hindi - Karanj grows in humid environment all over India (1). Its seeds are useful in fever, abdominal colic, inflammation (externally) and gout (2, 3). Preliminary studies showed anti-inflammatory effect of seed extracts against carrageenin induced rat hind paw oedema (11). Chemical mediators like histamine, 5-HT, bradykinin and PGE₁ are involved in carrageenin-induced acute inflammation (5, 6). The present study aimed at evaluation of anti-inflammatory effect of fractional extracts of *P. pinnata* seeds following individual chemical insults. Relative efficacy of fractionated constituents and their possible mode of inhibitory action in inflammation is thus appraised.

METHODS

315 g of dried of Karanj seed (*P. pinnata*) powder was extracted with 95% ethanol. The

extract was concentrated under steam bath to a final yield of 91 g. Further, 325 g seed kernel was extracted and sequential fractions were obtained with petroleum ether, chloroform, acetone and ethanol. The extracts were concentrated under steam bath with final yields of 90 g, 0.6 g, 2 g and 10 g respectively.

All fractions of *P. pinnata* (seed) i.e. direct ethanolic (DE), petroleum ether (PE), chloroform (CE), acetone (AE) and ethanol (EE) extracts were positive for the presence of glycosides. The extracts were suspended in 1% gum acacia in double distilled water.

The chemicals used were; carrageenin (Marine Colloids FMC Corporation, Springfield, NJ, USA), histamine dihydrochloride (Sigma, USA), serotonin creatinine sulphate (E. Merck, Germany), bradykinin (Sigma, USA), PGE₁ (Sigma, USA), phenylbutazone (SG Pharmaceuticals, India).

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Albino rats (CF Strain) of either sex, weighing 100-120 g (6 per group) were used. Right hind-paw oedema was induced by subplantar injection of 0.1 ml of carrageenin (1%); histamine (1 mg/ml); 5-HT (1 mg/ml); bradykinin (20 µg/ml) and PGE₁ (1 µg/ml). The paw volume, upto the ankle joint, was measured before and at 3 h after insult by the above inflammatory agents. The test extracts were administered ip, 30 min prior to insult. Phenylbutazone (PBZ) 100 mg/kg, ip, 30 min before, was used as a standard anti-inflammatory drug.

None of the extracts in suspension given ip caused signs of irritation. Thus ip route was

selected for the study to avoid uncertainties of the absorption in comparative study as this. In preliminary experiments, 50 mg/kg, ip of direct ethanolic extract was found to be the lowest dose producing significant inhibition of carrageenin induced oedema. The comparative experiments therefore employed the same dose level of the different extracts.

Statistical analysis was done using Student's 't' test for unpaired data.

RESULTS AND DISCUSSION

Data emanated from various experiments are presented in Tables I and II.

TABLE I : Anti-inflammatory effect of *P. pinnata* extracts against various inflammogens in albino rats (n=6 in each group).

Drugs and dose mg/kg, ip	Oedema volume in ml (mean±SE) and % inhibition over respective control		
	Carrageenin	Bradykinin	PGE ₁
Control (1% gum acacia)	0.57 ± 0.02	0.20 ± 0.01	0.18 ± 0.01
PBZ 100	0.07 ± 0.01 ^d (87.7)	0.10 ± 0.01 ^d (50.0)	0.04 ± 0.01 ^d (77.8)
DE 50	0.48 ± 0.01 ^c (15.8)	0.11 ± 0.01 ^c (45.0)	0.10 ± 0.02 ^c (44.4)
PE 50	0.45 ± 0.01 ^c (21.1)	0.13 ± 0.01 ^d (35.0)	0.14 ± 0.01 ^b (22.2)
CE 50	0.35 ± 0.03 ^d (38.6)	0.16 ± 0.01 (20.0)	0.08 ± 0.01 ^d (55.6)
AE 50	0.45 ± 0.03 ^c (21.1)	0.14 ± 0.02 ^a (30.0)	0.10 ± 0.02 ^c (44.4)
EE 50	0.50 ± 0.01 ^b (12.3)	0.17 ± 0.01 (15.0)	0.11 ± 0.01 ^d (38.9)

P values : ^a < 0.05, ^b < 0.02, ^c < 0.01, ^d < 0.001

TABLE II : Anti-inflammatory effect of *P. pinnata* extracts against various inflammogens in albino rats (n=6 in each group).

Drugs and dose mg/kg, ip	Oedema volume in ml (mean±SE) and % inhibition over respective control	
	Histamine	5-HT
Control (1% gum acacia)	0.26 ± 0.01	0.55 ± 0.01
PBZ 100	0.12 ± 0.01 ^d (53.9)	0.17 ± 0.02 ^d (69.1)
DE 50	0.18 ± 0.03 ^c (30.8)	0.45 ± 0.02 ^c (18.2)
PE 50	0.14 ± 0.01 ^d (46.2)	0.54 ± 0.01 (0.0)
CE 50	0.17 ± 0.01 ^d (34.6)	0.43 ± 0.01 ^d (21.8)
AE 50	0.23 ± 0.01 (11.5)	0.39 ± 0.02 ^d (29.1)
EE 50	0.18 ± 0.01 ^d (30.8)	0.55 ± 0.01 (0.0)

P values : ^b < 0.02, ^c < 0.01, ^d < 0.001

P. pinnata seed is used for febrile and inflammatory illness in Indian system of medicine (2, 13). Confirmatory evidence for anti-inflammatory activity of the extract was indicated by preliminary study (11). Present study was conducted to examine the relative potency of the constituents in fractional extracts and their possible mode of anti-inflammatory action.

Maximum anti-inflammatory effect was seen in bradykinin-induced oedema, where the effect of 50 mg/kg dose of *P. pinnata* direct ethanolic seed extract fraction nearly matched with that of 100 mg/kg of PBZ. The chloroform extract was similarly effective in PGE₁-induced model of inflammation.

All the extract fractions of *P. pinnata* seeds exhibited an anti-inflammatory effect in various models. Generalizing the outcome, the polar constituents in the seeds appear to have the anti-inflammatory principles and modulate late phases involving eicosanoid mechanisms which may be the prominent basis of anti-inflammatory action.

It is interesting further, that relative anti-inflammatory effect is different against different inflamogens used. Thus chloroform extract is most potent against carrageenin and PGE₁-induced paw oedema, while the direct ethanolic extracts exhibited most prominent anti-inflammatory effect against bradykinin-induced paw oedema. Since these two extract fractions represent more polar constituents, their site of anti-inflammatory action should be located superficially at membranes of inflammatory cells, i.e. leucocytes or the target cells of mediators. Broadly therefore, the polar constituents appear to inhibit eicosanoid mechanisms in inflammation, as they inhibit the sustained

late phases in inflammation (7). Chloroform extract was most effective against carrageenin and PGE₁ but least effective against bradykinin. This further suggest an effect on prostaglandin system.

Histamine and 5-HT involvement is said to occur in earlier stages in mounting of vascular reactions in chemically-induced inflammation (7). Lipophilic constituents in petroleum ether and acetone extracts of *P. pinnata* seeds most prominently inhibited histamine and 5-HT induced inflammation respectively. Since these mediators bring about their effects through specific receptor types, the lipophilic plant constituents most likely intervene at the level of cell membranes in vascular capillaries. Their interaction with membrane lipids may alter receptor functions. AE was most effective against 5-HT but was ineffective against histamine. Similarly, PE was most effective against histamine while it was ineffective against 5-HT.

Glycosides were the most prominent pharmacological constituents of *P. pinnata* seeds (11) and presence of variety of flavonoids and sterols in smaller quantities is reported (12). The flavonoids are known to prominently inhibit histamine and 5-HT induced vascular responses. They also inhibit prostaglandin synthesis (8, 9, 10). Glycosides as major determinants of anti-inflammatory and anti-allergic action of *P. kurroa* has been established in earlier work of this research unit (14, 15, 16).

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REFERENCES

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, CSIR, New Delhi, 1956; pp. 201.
2. Bhavaprakash Nighantu of Guduchyadi Varga. Hindi commentary by KC Chunekar and GS Pandey, Chaukhambha, Varanasi, 1969; pp. 350-353.
3. Parmer BS, Sahrawal KL, Mukherjee SK. *Pongamia glabra* : Constituents and uses. *J Scient Ind Res* 1976; 35; 608-611.
4. Winter CA, Risely EA, Nuss GW. Carrageenin-induced oedema in hind paw of rat as an assay of anti-inflammatory drugs. *Proc Soc Exp Biol Med* 1962; 111: 544-547.
5. Ghosh MN. Some common evaluation technics chapter 25. Fundamentals of Experimental Pharmacology, Second edition, Scientific Book Agency, Calcutta, 1984; pp.144-152.
6. Parmar NS, Ghosh MN. Anti-inflammatory activity of gossypin isolated from HV Linn. *Indian J Pharmacol* 1978; 10: 277-293.
7. Willoughby DA. Mediation of increased vascular permeability of inflammation. In : Zweifach BW, Grant L, McCluskey RT (Eds). *The inflammatory Process* 2nd Ed. New York-London Academic Press, 1973; II : 303-331.
8. Banerjee RS. Auto-Immunopharmacological studies with some bioflavonoids. *Ph. D. Thesis, BHU, 1989.*
9. Pathak VP, Saini TR, Khanna RN. Isopongachromene a chromenoflavone from *Pongamia glabra* seeds. *Phytochemistry* 1983; 22: 308-309.
10. Pathak VP, Saini TR, Khanna RN. A new furanoflavone from seeds of *Pongamia glabra*. *Planta Med* 1983; 49: 61-65.
11. Singh RK, Joshi VK, Goel RK, Gambhir SS, Acharya SB. Pharmacological actions on *Pongamia pinnata* seeds - A Preliminary Study. *Indian J Exp Biol* 1996 (in press).
12. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, CDRI & PID, New Delhi, 1990; I : pp153-154.
13. Agharkar SP. Medicinal Plants of Bombay Presidency. Scientific Publishers, Jodhpur, India, 1991; pp174.
14. Pandey BL, Das PK. Immunopharmacological studies on *Picrorhiza kurroa* Royle-ex Benth Part II. Antiallergic activity. *Indian J Allergy Appl Immunol* 1988; 2: 21-34.
15. Pandey BL, Das PK. Immunopharmacological studies on *Picrorhiza kurroa* Royle-ex Benth Part III. Adrenergic mechanisms of anti-inflammatory action. *Indian J Physiol Pharmacol* 1988; 32 : 120-125.
16. Pandey BL, Das PK. Immunopharmacological studies on *Picrorhiza kurroa* Royle-ex Benth Part IV. Cellular mechanisms of anti-inflammatory action. *Indian J Physiol Pharmacol* 1989; 33 : 28-30.