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Anti-Inflammatory and Anti-Arthritic Activities of the Mixture of Flavonoids Isolated from *Pluchea lanceolata*



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ABSTRACT

A mixture of four flavonoids isolated from whole plant of *Pluchea lanceolata* has been shown to possess significant anti-inflammatory and anti-arthritic effects against acute pedal inflammation induced by carrageenin and formaline and subacute inflammation induced by croton oil and cotton pellet implantation together with pleurisy produced by turpentine. The results were compared with betamethasone and found to be qualitatively similar, though less effective, but with advantage of producing no gastric lesions. The present findings suggested that the flavonoids of *Pluchea lanceolata* has major role of anti-inflammatory and anti-arthritic activities of the plant.



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INTRODUCTION

Pluchea lanceolata (Oliver and Hiern) (Family: *Asteraceae*) is a small shrub found in hotter parts of India including Punjab, Rajasthan, Upper West Bengal and Uttar Pradesh. It is locally known as Rasna. In Indigenous system of medicine its use has been described as an antipyretic, analgesic, bitter, laxative, nervine tonic and recommended for dyspepsia, rheumatoid arthritis and bronchitis [1-3]. The plant is greatly used in neurological diseases, sciatica, cough, psoriasis and piles [4]. The Ayurvedic practitioners of the region use this drug for treating pain and swelling of the body joints [5]. For the treatment of rheumatism, it is given as Rasnaguggulam or Rasnapanchak orally and as Mahanarayan oil or Mahamesa oil externally [6]. The plant contains a number of different secondary metabolites viz. quercetin, quercitrin, isorhamnetin, daidzein, triterpenes, sitosterols, taraxasterol, neolupinol, pluchine [4, 7-13]. Anti-inflammatory and anti-arthritis activities of the crude methanolic extracts of the plant has been reported [4, 6, 14, 15]. The triterpene, neolupinol isolated from the plant possess anti-inflammatory activity [9]. Flavonoids of the plant are reported as scavengers of free radicals [16]. In the present study we have investigated the anti-inflammatory and anti-arthritis activities of the mixture of four flavonoids isolated from *Pluchea lanceolata*.

MATERIALS AND METHODS

Isolation of flavonoids

The whole plant *Pluchea lanceolata* was collected during the month of June from Varanasi District and adjacent areas. It was identified by Dr. N. K. Dube, Department of Botany, Banaras Hindu University and a voucher specimen is kept in the Department. The plant was air dried at room temperature under shade. The powdered plant material (2 kg) was extracted successively with petroleum ether (60-80°), benzene, ethyl acetate and methanol. The solvents of individual extracts were distilled at lower temperature under reduced pressure. They were finally dried on water bath to give pet ether extract (10 g) as colourless gummy mass, benzene extract (15.5 g) as light brown mass, ethyl acetate extract (24.5 g) as light yellow semi-solid and methanol extract (30g) as brown gummy mass. All these extracts were tested for the presence of flavonoids. A little amount of ethyl acetate extract was dissolved in MeOH and added Zn pieces which on heating gave reddish yellow color indicated in presence of flavonoids in ethyl acetate extract. The ethyl acetate extract was further treated

with few drops of NaOH solution which gave intense yellow colour; becomes colourless on addition of few drops of dilute acid, further supports the presence of flavonoids in ethyl acetate extract. The pet. ether and benzene extracts did not show the test of flavonoids. The MeOH extract exhibited only mild test for flavonoids. The ethyl acetate extract was directly crystallized from MeOH which on filtration furnished a good amount of yellow solid (1.5 g) designated as FPL. A little amount of FPL was dissolved in few drops of MeOH and chromatographed on thin layer chromatographic plate using solvent system $\text{CHCl}_3 - \text{MeOH}$ (8: 2). The spots on plate were visualized by developing with UV and NH_3 vapour which showed yellow/yellowish green coloured spots of four flavonoids having R_f values 0.23, 0.38, 0.45 and 0.65. FPL is thus a mixture of major four flavonoids. FPL was subjected for screening of anti-inflammatory and anti-arthritic activities.

Pharmacological studies

The animals used in this study were adult albino rats of either sex, weighing between 100-150 g. They were divided into groups of ten animals each. In every experimental parameter, one group received only the vehicle (Arachis oil I.P.) to serve as control whereas another group was treated with betamethasone in arachis oil, for comparison. The following tests were used:

1. Carrageenin induced acute pedal inflammation [17]-The animals were pretreated with I.P. injections of test drug one hour before injecting 0.05 ml of 1% freshly prepared suspension of carrageenin in 0.9% saline into the plantar side of hind paw. The volume of the paw before and three hours after carrageenin treatment was measured by the method of Buttle *et al.*[18].

2. Formaline induced acute pedal inflammation [19] – The animals were pretreated with I.P. injections of the test drug one hour before injecting 0.05 ml of 4% (v/v) formaldehyde solution into the plantar side of hind paw. The volume of the paw, before and four hours after the injection of the phlogistic agent was measured.

3. Croton oil granuloma pouch [20] – Croton oil was injected S.C. between the shoulder blades into sac produced by injecting 10 ml air. The animals were sacrificed on the sixth day and the volume of inflammatory exudates in the sac was measured. Drug treatment was started one day prior to injection of the phlogistic agent and given daily till the day of sacrifice.

4. Cotton pellet implantation [21] – Pellets of surgical cotton weighing 9.0 ± 1 mg sterilized in hot air oven, were implanted into both axillae and groins under ether anaesthesia. The test drug was given daily I.P. for six days starting one day prior to the implantation. On the sixth day, the pellets dissected out under light ether anaesthesia, dried for two hours at 120° and weighed after cooling.

5. Pleural exudation method [22] – Experimental pleurisy was produced by injecting 0.1 ml of turpentine into the right pleural space under light ether anaesthesia. Test drug was given orally two hours before turpentine injection. Rats were decapitated and pleural exudate was collected half an hour after turpentine injection.

The animals used in the cotton pellet implantation and croton oil granuloma pouch methods were kept throughout the experimental period in metabolic cages and a record of urinary and faecal output was kept. The stomach was examined in each rat after scarifying them at the end of these two experimental procedures, for signs of gastric erosion, haemorrhage or ulceration.

RESULTS AND DISCUSSION

A mixture of four major flavanoids FPL isolated from *Pluchea lanceolata* was screened for their anti-inflammatory and anti-arthritic activities in albino rats using five established experimental methods of clinical arthritis and rheumatoid arthritis. The flavonoid mixture FPL was found to have significant anti-inflammatory and anti-arthritic activities. Results are summarized in Table-1. *Pluchea lanceolata* flavonoids FPL did not have any significant effect on faecal and urinary output of albino rats during the period of treatment in the croton oil granuloma pouch and cotton implantation groups. Gastric lesions were entirely absent as against 50% incidence of gastric erosion/haemorrhage ulceration in the betamethasone groups. The drug was less effective than betamethasone in all parameters tested, but had the advantages of producing no adverse gastric lesion, as did betamethasone. The present findings substantiated the anti-inflammatory and anti-arthritic uses of the plant.

CONCLUSION

The results of the above study revealed that the mixture of flavonoids of the plant *Pluchea lanceolata* showed significant anti-inflammatory and anti-arthritic activities. The future prospective demands the isolation and characterization of individual flavonoids of the plant

which might help in the findings of new flavonoids in the field of anti-inflammatory and anti-arthritic drug research. There is a sufficient scope to develop the use of *Pluchea lanceolata* in Indian Medicine and as a base for the development of potent anti-inflammatory and anti-arthritic drugs.

Table 1: Screening of Anti-inflammatory and Anti-arthritic activities of mixture of flavonoids of *Pluchea lanceolata*.

Tests	Control (No. of animals 10)	<i>Pluchea lanceolata</i> flavonoids FPL (10 mg/100 g)* (No. of animals -10)	Betamethasone (20 µg/100g)* (No. of animals 10)
1. Carrageenin induced pedal oedema (Mean increase in paw volume ±SE) **	12.8±0.95	5.3±0.82 P < 0.001	3.5 ± 0.28 P < 0.001
2. Formaline induced pedal oedema (Mean increase in paw volume ±SE)**	12.2 ± 0.80	6.4 ± 1.00 P < 0.05	5.8 ± 0.85 P < 0.05
3. Cotton pellet method (Mean weight of granulation tissue in mg ± SE)	0.72 ± 0.1	0.03 ± 0.08 P < 0.001	0.13 ± 0.08 P < 0.001
4. Croton oil induced granulation pouch (Mean volume of exudate± SE) in ml	185 ± 6.4	90 ± 10.3 P < 0.05	53 ± 8.5 P < 0.005
5. Pleural exudation method (Mean volume of exudate in ml ± SE)	2.4 ± 0.24	0.85 ± 0.02 P < 0.01	0.6 ± 0.05 P < 0.001

* Route of administration I.P. except pleural exudation method where it is P.O.

** Increase in paw volume expressed in terms of mm rise of mercury level in the plethysmograph.

CONFLICT OF INTEREST

No conflict of interest for the above work.

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