

Basic Science In Medicine

PHARMACOLOGICAL EVALUATION OF MEDICINAL PLANTS FOR THEIR ANALGESIC ACTIVITY IN MICE

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ABSTRACT

The selected parts of four medicinal plants, *Achillea millefolium*, *Hibiscus rosasinensis*, *Linum usitatissimum* and *Pluchea lanceolata* were extracted in absolute methanol to determine their analgesic activity. Analgesic activity was assessed on intact mice by tail flick latency via the tail immersion method. The analgesic activities of these plant extracts were compared with acetylsalicylic acid (300 mg/kg) which was used as the standard drug. Extracts were given orally in doses of 300, 500 and 1000 mg/kg. 0.9% saline was administered to the control group of animals. Results showed that *Linum usitatissimum* and *Pluchea lanceolata* possessed highly significant analgesic activity, while *Achillea millefolium* and *Hibiscus rosasinensis* did not show any significant effects.
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INTRODUCTION

Drugs presently used for the management of pain and inflammatory conditions are either narcotics (e.g., opioids), non-narcotics (e.g., salicylates) or corticosteroids (e.g., hydrocortisone). All of these drugs cause well known side- and toxic effects. Moreover, synthetic drugs are very expensive to develop, as the successful introduction of a new product calls for approximately 3000-4000 compounds to be synthesized, tested and screened, the cost of development of which ranges from 0.5 to 5 million dollars. On the other hand, many medicines of herbal origin have been used since long ago without any adverse effects. It is therefore essential that efforts be made to develop cheaper drugs.¹ Medicinal plants and herbal medicine are one of the current areas of investigation that possess all the hallmarks of modern biomedical science.² This necessitates efforts in order to identify plants that have potential for medical cure.

The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study, in which *Achillea millefolium*, *Hibiscus rosasinensis*, *Linum usitatissimum* and *Pluchea lanceolata* have been selected for their reported biological activities in the indigenous system of medicine.³⁻⁶

MATERIALS AND METHODS

Plant material

Achillea millefolium (Compositae), *Hibiscus rosasinensis* (Malvaceae), *Linum usitatissimum* (Linaceae) and *Pluchea lanceolata* (Compositae) were obtained from various parts of Karachi during July and August 1991 and identified with the help of herbarium specimens.

The flowers of *A. millefolium* and *H. rosasinensis*, seeds of *L. usitatissimum* and whole plant of *P. lanceolata* were

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Table I. Analgesic effect of medicinal plants (methanolic extract) in mouse tail immersion method.

Treatment	Oral dose (mg/kg)	Analgesia TFLD or mean increase in latency after drug administration \pm S.E.M. (s)					
		+ 60	+ 90	+ 120	+ 150	+ 180	+ 210 (min.)
Saline	0.2 ml	0.06 \pm 0.17	0.38 \pm 0.31	0.33 \pm 0.29	0.54 \pm 4.65**	0.36 \pm 0.27	0.46 \pm 0.32
<i>Achillea millefolium</i>	300 mg	0.17 \pm 0.33	0.45 \pm 0.29	0.38 \pm 0.18	0.11 \pm 0.33	0.28 \pm 0.38	0.28 \pm 0.50
<i>Achillea millefolium</i>	500 mg	0.44 \pm 0.28	0.43 \pm 0.16	0.53 \pm 0.24	-0.11 \pm 0.36	0.93 \pm 0.32	0.53 \pm 0.42
<i>Achillea millefolium</i>	1000 mg	0.24 \pm 0.15	0.35 \pm 0.42	0.62 \pm 0.52	0.41 \pm 0.48	0.69 \pm 0.27	0.52 \pm 0.28
<i>Hibiscus rosasinensis</i>	300 mg	-0.29 \pm 0.17	0.16 \pm 0.50	-0.63 \pm 0.33	-0.33 \pm 0.26	-0.04 \pm 0.27	-0.13 \pm 0.22
<i>Hibiscus rosasinensis</i>	500 mg	0.01 \pm 0.14	0.20 \pm 0.29	0.43 \pm 0.29	0.57 \pm 0.20	0.40 \pm 0.17	0.37 \pm 0.27
<i>Hibiscus rosasinensis</i>	1000 mg	0.24 \pm 0.16	0.10 \pm 0.30	0.42 \pm 0.49	-0.14 \pm 0.13	-0.01 \pm 0.18	0.66 \pm 0.26
<i>Linum usitatissimum</i>	300mg	0.56 \pm 0.20*	1.16 \pm 0.34	1.82 \pm 0.27**	2.06 \pm 0.22**	2.59 \pm 0.32**	2.49 \pm 0.23**
<i>Linum usitatissimum</i>	500 mg	1.33 \pm 0.30**	1.83 \pm 0.14**	2.43 \pm 0.32**	2.46 \pm 0.30**	2.36 \pm 0.32**	3.40 \pm 0.36**
<i>Linum usitatissimum</i>	1000 mg	4.17 \pm 0.93**	15.66 \pm 7.60*	26.90 \pm 10.70*	38.40 \pm 12.50**	28.00 \pm 10.10*	25.50 \pm 13.00*
<i>Pluchea lanceolata</i>	300 mg	-0.06 \pm 0.39	0.58 \pm 0.18	1.13 \pm 0.30*	1.26 \pm 0.18*	1.43 \pm 0.22**	1.06 \pm 0.32
<i>Pluchea lanceolata</i>	500 mg	0.30 \pm 0.20	0.96 \pm 0.09	1.20 \pm 0.26*	1.26 \pm 0.16*	1.86 \pm 0.21**	2.06 \pm 0.15**
<i>Pluchea lanceolata</i>	1000 mg	1.86 \pm 0.37**	4.66 \pm 0.19**	4.60 \pm 0.54**	11.60 \pm 4.90*	23.00 \pm 8.50*	18.70 \pm 7.00**
Acetylsalicylic acid	300 mg	0.90 \pm 0.15**	2.22 \pm 0.19**	2.31 \pm 0.14**	3.48 \pm 0.14**	3.48 \pm 0.20**	4.14 \pm 0.23**

n/group = 5

*P <0.05

**P <0.01

dried in shade at 21-30°C for 15 to 30 days. These parts were then chopped and ground.

Preparation of extract

Approximately 1 kg of ground plant material was soaked in 500 ml absolute methanol for about six weeks. The alcoholic extracts were then evaporated under reduced pressure in a rotary evaporator (Eyela) and the syrupy residue so obtained was dissolved in a small quantity of water and subjected to freeze-drying. Freeze-dried extracts were collected in small glass bottles and kept at -30°C for further evaluation. In terms of dry starting material, the yields of the methanol extracts of *A. millefolium* and *H. rosasinensis* flowers were 0.88% and 1.65%, respectively, while the yield

of *L. usitatissimum* seeds was 0.98% and the yield of the extract of the whole plant *P. lanceolata* was 0.86%.

Preparation of samples for bioassay

Acetylsalicylic acid in a quantity of 300 mg and extracts of plants in the quantities of 300, 500 and 1000 mg were homogenized in 1.5% aqueous gum tragacanth. The suspensions were then administered orally (10 ml/kg) to the test animals on the basis of body weight.

Animals

Albino mice of either sex bred at the animal house of Welcome Pakistan Ltd. were used in the present study. Weights of the mice ranged from 20-25 g. All animals were

maintained in groups of five at $22\pm 1^\circ\text{C}$ with a light/dark cycle of 12:12 hours. They were starved overnight but allowed fresh water before administration of the plant extracts.

Tail immersion method

In the present study analgesia was assessed according to the method of Di Stasi et al.⁷ Mice were divided into groups of five each and held in position in a suitable restrainer with the whole tail extending out. The tail area (2-3 cm) was marked and immersed in a water bath which was thermostatically maintained at 51°C . The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail-flick latency. The maximum cutoff time for immersion was 180 seconds in order to avoid injury of the tail tissues. 0.2ml of 0.9% NaCl solution was administered to control animals, while plant extracts in doses of 300, 500 and 1000 mg/kg were given orally by intubation to the study group. The initial reading was taken immediately before administration of test and standard drugs and then 60, 90, 120, 150, 180 and 210 minutes following administration.

The criterion for analgesia was postdrug latency which was greater than two times the predrug average latency.⁸ Tail-flick latency difference (TFLD) or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs. Analgesia TFLD was calculated as follows:

Analgesia TFLD = postdrug tail flick latency minus predrug tail flick latency

Statistical analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration \pm SEM" in terms of seconds. The significance of difference between means was determined by Student's t-test. Values of $P < 0.05$ were considered significant and $P < 0.01$ as highly significant.

All statistical procedures were performed according to the method of Alcaraz and Jimenez.⁹

RESULTS AND DISCUSSION

Acetylsalicylic acid was used as a standard or reference drug in this study since it is known to have both analgesic and anti-inflammatory action and it is the prototype used for comparison against other analgesic, anti-inflammatory and anti-pyretic drugs.^{10,11} Acetylsalicylic acid continues to be widely regarded as the drug of choice among the available non-steroidal analgesic anti-inflammatory drugs.¹²

In this study, the methanolic extracts of *Achillea millefolium* and *Hibiscus rosasinensis* exhibited no analgesic activity at any dose level, on intact mice.

Linum usitatissimum is known to have analgesic and anti-inflammatory activity in Unani Tibb.¹³ In this study,

the methanolic extract of this plant showed significant analgesic activity at doses of 300 and 500 mg/kg, but it was less potent than that of acetylsalicylic acid at 300 mg/kg. The most pronounced effect was produced by *Linum usitatissimum* at a dose of 1000 mg/kg in the tail immersion test. Despite this pronounced effect, the results were not significantly different from the controls utilizing Student's t-test because of the large variation of responses in the test group. As all the values in the tail immersion test of *L. usitatissimum* with a dose of 1000 mg/kg were higher than the highest value in the corresponding control group (this would give a highly significant value utilizing a non-parametric statistical method), we think that the results with this extract must be considered as very promising.

In this study *Pluchea lanceolata*, at a dose of 300 and 500 mg/kg showed slight analgesic activity, but with a dose of 1000 mg/kg of the plant extract, showed highly potent analgesic activity which peaked at +180 minutes where it showed an analgesia TFLD of 23.0 ± 8.5 seconds (and after which the effect began to decrease). At this dose the analgesic effect of the plant was much better than acetylsalicylic acid (300 mg/kg), in terms of duration as well as intensity of analgesia. The plant extract also showed a rapid onset of analgesic action. In another study, a new quarternary base chloride called pluchine was isolated from *P. lanceolata*. This substance harbors smooth muscle relaxing, spasmolytic and anti-inflammatory effects. *P. lanceolata* reduced the foot volume when it was screened for anti-inflammatory activity against formalin-induced arthritis.⁴ The results of this study need to be verified in other experimental models, and pharmacodynamic studies should also be carried out in order to establish the modes of action of these plant extracts.

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