

Original Article

ANTI-INFLAMMATORY ACTIVITY OF PLANTAGO EROSA WALL EXTRACTS IN RATS

MAXILLINE D. MARAK, A. SUBHALAXMI, R. K. BHARATI, JULIE BIRDIE WAHLANG, DHIRITI KR. BRAHMA

Department of Pharmacology North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Mawdiangdiang, Shillong, Meghalaya, India, Professor of Pharmacology Regional Institute of Medical Sciences, Imphal, Manipur, India
Email: maximarak2611@gmail.com

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ABSTRACT

Objectives: To evaluate *Plantago erosa Wall* (PEW) for anti-inflammatory effects by using aqueous extracts of its leaves in various rat models *in vivo*.

Methods: Fresh leaves of PEW were collected from South Eastern region of Manipur, India during the months of June-July, 2007. Aqueous extracts of fresh leaves of PEW at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg was evaluated for its anti-inflammatory effects on experimental models in rats, e.g. carrageenan induced rat paw oedema, cotton pellet implantation, granuloma pouch and formaldehyde arthritis and compared with the standard drug aspirin.

Results: The extracts at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg showed a significant reduction of acute inflammation in comparison to the control drug aspirin in rat paw oedema model. It also caused highly significant inhibition of granuloma formation and exudate formation in comparison to the control drug. There was also significant reduction of formaldehyde induced arthritis in rats comparable to the control drug aspirin.

Conclusion: The study revealed that the PEW extracts at the doses of 200 mg/kg and 400 mg/kg caused significant anti-inflammatory effects in rats and warrants more studies with more purified form of extracts.

Keywords: *Plantago Erosa Wall*, Anti-Inflammatory.

INTRODUCTION

Traditional medicine is defined as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation transferred from generation to generation [1]. Medicinal herbs constitute the basis of traditional medicinal practice worldwide. According to World Health Organization (WHO), over 80% of population in traditional medicinal system depends on medicinal plants [2].

Northeast India including the state of Manipur is the abode of several native medicinal plants and has a long tradition of using these plants as therapeutic agent. Scientific study and relevant verifications of these plants for their various medicinal properties are the need of the hour. as These traditional claims can be a new source of medicines for future [3].

Plantago erosa Wall, synonym- *Plantago major* Linn. (PEW) is glabrous perennial herb very common in moist, wasteland in the valley of Manipur, India. It is also commonly known as *Yempat* in Manipuri and *Luhuriya* in Hindi. It has been used by the local indigenous people (Maaiba) of Manipur in treating fever, boils, dysentery, diarrhoea, urinary tract complaints, headache, tooth ache, and various inflammatory conditions. The seeds of the plant have been used as abortifacients [4]. The present study was undertaken in the department of Pharmacology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, India to evaluate the pharmacological efficacy of PEW as an anti-inflammatory agent in comparison with a standard non-steroidal anti-inflammatory agent. The experimental animal models selected for the study were the standard classical pharmacological methods used for evaluation of anti-inflammatory agents against acute, sub acute and chronic types of inflammation.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Plantago erosa Wall* (PEW) were collected from South Eastern region of Manipur during the months of June-July, 2007 identified and authenticated by Dr. S.C. Sinha, Professor of Botany (retired) Manipur University.

Preparation of plant extract

The plant leaves were cleaned, dried under shade, powdered by a mechanical grinder and stored in air tight container for future use.

Preparation of aqueous extract was done by the method of Verma SCL and Agarwal SL, (1962) [5] with slight modification. 50 gm of the powdered dry parts of the plant was extracted with distilled water using a soxhlet apparatus. The brownish extract obtained was evaporated, shade-dried, scraped out, weighed and stored in glazed porcelain jar for future use. The yield was 18.2%.

Study methods

Anti-inflammatory effects of PEW were evaluated in the study models of carrageenan induced rat paw oedema model for acute inflammation, cotton pellet implantation & granuloma pouch for sub-acute inflammation and formaldehyde arthritis for chronic inflammation.

Grouping of animals

Healthy albino rats of Wistar strain of either sex weighing between 100 gm to 200 gm obtained from Central Animal House, RIMS, Imphal, India were used throughout the present investigation. Animals were kept for 3 days under laboratory conditions before any experimental work. Animals were fed with standard pellet diet with water ad libitum and maintained at 24-28°C temperature and 12 hours day & night cycle. The rats were divided into five groups with six animals in each group. This arrangement was used throughout the present investigation. Animals divided into 5 (groups) and the drugs were suspended in 1% gum acacia and administered orally at the doses as shown below in the Table - 1.

The volume of the medicament was kept constant at 1ml/100 gm of body weight of animals. Both control and treated groups in a particular series received, the same phlogestic agent.

Determination of percentage of inhibition of edema

The percentage of anti-inflammatory effect was calculated according to the method described by Diniz et al, (1978) [6] by the

formula: Percentage of inhibition = $V_c - V_t / V_c \times 100$, where V_c = mean increase in paw volume in control group and V_t = mean increase in paw volume in drug treated group.

Acute Inflammation on carrageen induced rat paw oedema model

The animals in 30 numbers kept fasting overnight and during the experiment, but water was allowed ad libitum. Treated groups of rats were pre-treated with test drugs and aspirin orally and control group received gum acacia suspension one hour before carrageenan

injection. Freshly prepared carrageenan (1%) in 0.9% sodium chloride solution was injected in a volume of 0.1ml into sub-plantar region of the right hind paw of the rat.

The foot volume was measured in normal rats without an anaesthetic by a plethysmometer immediately after and again three hours after carrageenan injection and the 'volume of oedema' was recorded as the difference between two readings. Paw volume was measured by a modified plethysmographic method described by Singh H and Ghosh MN, (1968) [7].

Table 1: Grouping of animals in the study

Group	Drugs
A (Control)	Vehicle - gum acacia 1%
B (Test)	PEW (100 mg/kg)
C (Test)	PEW (200 mg/kg)
D (Test)	PEW (400 mg/kg)
E (Standard)	Aspirin (100 mg/kg)

Sub-acute Inflammation on cotton pellet implantation model

The study methods used was the method described by Ghosh MN and Singh H (1974) [8]. Cotton pellets cut out from dental rolls to size approx 0.5 x 0.5 cm and weighing 20 ± 1 mg were sterilized at 120°C for 2 hours. They were then soaked in 0.2 ml distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg) and then inserted in both axillas an ether anaesthetized rat. While rats from the treated groups were treated with the test drugs; the standard and control group received 1% gum acacia suspension orally for 6 days respectively. On the seventh day, after ether anaesthesia the cotton pellets together, with the granuloma were dissected out carefully and dried in incubator at 37°C for 24 hours. Any increment in the dry weight was taken as a measure of granuloma formation. Then the percentage inhibition of granuloma formation was calculated for various test drugs and compared with the control group receiving aspirin.

Sub-acute Inflammation on granuloma Pouch model

Study method used was the method described by Selye H, (1953) [9] with slight modifications. Rats were anaesthetized with ether and a subcutaneous dorsal pouch was prepared in between the shoulder blades by injecting 20 ml of air. Then 0.5 ml of turpentine oil was injected into the pouch. Treated groups of rats received the test drugs aspirin and the control group received 1% gum acacia suspension, orally for six days beginning from the day of pouch formation. On the seventh day, the pouch was opened under ether anaesthesia and the exudate were sucked out and the amount measured.

The percentage inhibition was then calculated for the different group of drugs as compared to the control group.

Chronic Inflammation on modified Formaldehyde arthritis

Study used was the method described by Selye H, (1949) [10] with slight modifications on Formaldehyde arthritis. A subcutaneous injection of 0.1 ml of 2% formalin was given under the plantar aponeurosis of the right hind foot of the rats. The paw volume was measured plethysmometrically for 13 days to assess the degree of inflammation. Groups of animal, were treated with test drugs, gum acacia and aspirin orally daily for 13 days.

RESULTS AND DISCUSSION

Test on acute inflammation model

The results are tabulated in **Table 2**. While the mean increase in paw volume in the control group was 0.63 ± 0.06 ; the groups B, C, D and E showed 0.41 ± 0.08 ($p < 0.001$), 0.33 ± 0.06 ($p < 0.001$), 0.26 ± 0.06 ($p < 0.001$) and 0.24 ± 0.05 ($p < 0.001$) respectively.

The test drug PEW in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg produced 34.92%, 47.62% and 58.73% inhibition of paw oedema compared to 61.90% inhibition produced by 100 mg/kg of the standard drug, aspirin. Increasing the concentration of the test drug produced increase percentage of inhibition of paw oedema. Both the test and standard drug produced highly significant inhibition of paw oedema in comparison to the control ($p < 0.001$).

Table 2: Effect of *Plantago erosa* Wall on acute Inflammation model:

Group	Drug dose	Mean increase in paw volume (in ml) after 3 hrs.	% inhibition of paw oedema
A (control)	2% Gum acacia in D/W, 10ml/kg	0.63 ± 0.06	-
B (test)	PEW, 100mg/kg	$0.41 \pm 0.08^*$	34.92%
C (test)	PEW, 200mg/kg	$0.33 \pm 0.06^*$	47.62%
D (test)	PEW, 400mg/kg	$0.26 \pm 0.06^*$	58.73%
E (standard)	Aspirin, 100mg/kg	$0.24 \pm 0.05^*$	61.90%

Table 3: Effects of aqueous extract of *Plantago erosa* Wall on sub-acute inflammation as tested on cotton pellet implantation:

Group	Dose (mg/kg), p.o.	Mean dry weight granuloma (mean \pm SD) in mg	%inhibition of granuloma formation
A Control	10ml/kg	81.83 ± 13.58	-
B Test	100	$67.50 \pm 12.05^*$	17.51%
C Test	200	$57.33 \pm 10.13^{**}$	29.94%
D Test	400	$45.17 \pm 6.40^{**}$	44.80%
E (Standard)	100	$48.33 \pm 6.59^{**}$	40.94%

One day F = 13.0067, ANOVA $d_f = 4, 25$; $P < 0.01$, All values are mean \pm SD, $n = 6$ in each group. * $p < 0.05$ and ** $p < 0.001$ when compared to control.

Table 4: Effect on the sub-acute inflammation of aqueous extract of *Plantago erosa* Wall on granuloma pouch

Group	Dose (mg/kg), p.o.	Mean volume of exudate (Mean ± SD) in ml	% inhibition of exudate formation.
A (Control)	10ml/kg	3.42 ± 0.89	-
B (Test)	100	2.02 ± 0.62*	40.93
C (Test)	200	1.75 ± 0.46*	48.83
D (Test)	400	1.45 ± 0.43*	57.6
E (Standard)	100	1.48 ± 0.39*	56.72

One way ANOVA 11.4231, df4, 25. P < 0.01, Values are mean ± SD, n=6 in each group; *p < 0.01 when compared to control.

Test on sub-acute inflammation model (cotton pellet)

Results are tabulated in **Table - 3**. While the mean dry weight in the control group was 81.83±13.58; the groups B, C, D and E had 67.50±12.05 (p<0.05), 57.33±10.13 (p<0.001), 45.17±6.40 (p<0.001) and 48.33±6.59 (p<0.001) respectively. The test drug in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg produced 17.51%, 29.94%, 44.80% inhibition of granuloma formation. Both the test and standard drug produced highly significant, inhibition of granuloma formation in comparison to control.

Test on sub-acute inflammation model (granuloma pouch)

Results are tabulated in **Table 4**. The volume of exudate in the control group was 3.42 ± 0.89. The said volume for the groups B, C, D and E was 2.02 ± 0.62 (p < 0.01), 1.75 ± 0.46 (p < 0.01), 1.45 ± 0.43 (p < 0.01) and 1.48 ± 0.39 (p < 0.01) respectively.. The test drug in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg produced 40.93%, 48.83%, 57.6% inhibition of exudate formation compared to 56.72% produced by 100 mg/kg of the standard drug, aspirin. Increasing concentration of the test drug produced increased inhibition of exudate formation. Both, the test and standard drug produced significant inhibition of exudate formation compared to the control.

Test on chronic inflammation in formaldehyde arthritis

The mean increase in paw volumes in the control group were 0.50±0.038, 0.57±0.053, 0.63±0.059, 0.65±0.045 and 0.62±0.045 respectively on the 3rd, 5th, 9th, 11th and 13th day. On pre treatment with aqueous extract of PEW the mean increase in paw volumes for group B were 0.40±0.37 (p<0.001), 0.45±0.047 (p<0.001), 0.45±0.041 (p<0.001), 0.39±0.32 (p<0.001) and 0.38±0.065 (p<0.001) respectively on the mentioned days. While the Group C showed an increase of 0.39±0.025 (p<0.001), 0.42±0.043 (p<0.001), 0.44±0.052 (p<0.001) 0.37±0.065 (p<0.001) and 0.35±0.052 (p<0.001) respectively; the group D animals showed 0.37±0.052 (p<0.001), 0.40±0.032 (p<0.001), 0.39±0.039 (p<0.001), 0.34±0.064 (p<0.001) and 0.33 ± 0.052 (p<0.001) increase in paw volume respectively on the mentioned days. Group E animals, with the standard drug aspirin, showed the mean increase in paw volume of 0.34±0.043 (p<0.001), 0.36±0.047 (p<0.001), 0.35±0.029 (p<0.001), 0.32±0.058 (p<0.001) and 0.31±0.056 (p<0.001) respectively on the mentioned days.

Both the drugs, standard and test, produced highly significant inhibition of paw edema when compared to control. There was no significant reduction in the mean paw volume of the animals of control group A, even on the 13th day. The aqueous extract of PEW showed significant reduction in the paw volume. The test drug at a dose of 400 mg/kg had similar efficacy to aspirin (100 mg/kg).

Our study reveals that the mean increase in paw volume after 3 Hrs of the carrageenan injection in the control group was 0.63±0.06 ml which corresponds to the findings 0.55±0.01 ml of Janu et al (1999) [11] and 0.57±0.14 ml of Chakraborty et al (2004) [12]. The test drugs in concentrations of 100 mg, 200 mg and 400 mg/kg produced 34.92%, 47.62% and 58.73% inhibition of paw oedema respectively and were significant when compared to that of the control (p<0.001). The standard drug, aspirin (100 mg/kg) produced 61.90% inhibition of paw oedema. Higher doses of the test drug (200 mg and 400 mg/kg) showed comparable efficacy in reducing the oedema volume with that of the standard drug, aspirin (100 mg/kg). The mean increase in paw volume for the group of animals

with standard drug was 0.24±0.05 which corresponds to the finding 0.24±0.01 ml of Jana U et al, 1999 [11]. Thus the study of the aqueous extract of *Plantago erosa* Wall on the carrageenan induced paw oedema model in albino rats showed significant anti-inflammatory property.

In our cotton pellet implantation study, the mean dry weight of granuloma of the control group was 81.83±13.58 (mg±SD) which corresponds to the previous findings by Ghosh MN [13] (77.24±4.82), Singh H, 1974 [14] (79±10) and Islam CN et al, 1995 [15] (70.0±0.12). While the test drugs in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg produced 17.51%, (p<0.05), 29.94% (p<0.05), 44.80% (p<0.001) inhibition of granuloma formation; the standard drug aspirin produced 40.94% inhibition of granuloma formation. The effect of test drug (400 mg/kg) was comparable to that of standard drug aspirin (100 mg/kg). The mean dry weight of granuloma of the standard group was 48.33±6.59 (mg ± SD) and was comparable to that of Thangam C and Dhananjayan R, 2003 (41.66±2.81) [16].

In the granuloma pouch experiment, the mean value of exudate in the control group was 3.42 ± 0.89 (Mean ± SD) corresponding to the findings of Islam CN et al, (1995) [15] 2.50 ± 0.05. The test drug in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg produced 40.93%, 48.83% and 57.6% inhibition of exudate formation respectively. The standard drug aspirin produced 56.72% inhibition of exudate formation. The test drug 400 mg/kg is found to be more effective than the standard drug aspirin (100 mg/kg). In the study of chronic inflammation, the mean increase of paw volume in the control group was found to be 0.50±0.038 (ml±SD) on the 3rd day and 0.63±0.059 on the 9th day. The test drug in concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg orally produced mean increase of 0.40±0.037, 0.39±0.025 and 0.37±0.052 on the 3rd day and 0.45±0.041, 0.44±0.052 and 0.39±0.039 respectively on the 9th day. The mean increase of paw volume of the standard drug aspirin (100 mg/kg) was 0.34±0.043 on 3rd day and 0.35±0.029 on the 9th day. Thus, it is evident that the test drug at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg significantly inhibited rat paw oedema induced by formaldehyde. The test drug in dose of 400 mg/kg was found to be similar in efficacy to the standard drug aspirin (100 mg/kg).

CONCLUSION

The present study reveals the significant anti-inflammatory actions of aqueous extracts of the whole plant of *Plantago erosa* Wall. With out any significant adverse effects in the used dose range. Thus, the traditional age old cure used by local Indigenous people in this part of India has proved its pharmacological efficacy as an anti-inflammatory agent. Further studies with more purified form of extracts may open the gateway for potential drug development for future.

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