PHYTOCHEMICAL AND PHARMACOLOGICAL POTENTIAL OF *ARTEMISIA INDICA* IN EXPERIMENTAL ANIMAL MODELS

Manoj Kumar Sagar[1*], Praveen Kumar Ashok[1], Himanshu Chopra[1] Kumud Upadhyaya[2]

1- Faculty of Pharmacy, GRD (PG) IMT, Dehradun(U.K.)

2- Department of pharmacy, Kumaun University Bhimtal, Nanital (U.K.)

Summary

Artemisia indica was evaluated for anti-inflammatory action by carrageenin-induced rat paw edema. The analgesic activity was tested by acetic acid-induced writhing response in albino mice and tail flick method in albino rats. The methanol extract of *Artemisia indica* in doses of 100, 200 and 500 mg/ml showed 52.4, 54.2 and 55.8 % inhibition of paw edema respectively at the end of three hour and the percentage of protection from writhing was 48.5, 52.3 and 67.9 respectively. In the tail flick model, the methanol extract of *Artemisia indica* in the above doses increased the pain threshold significantly after 30 min., 1, 2, and 4 hr. of administration. *Artemisia indica* showed dose-dependent action in all experimental animal models.

Keywords*: Artemisia indica;* Anti-inflammatory; Analgesic

* Corresponding author. Tel.: +91-9458411789.
E-mail address: manojsagar@live.com.
1 Present address: College of Pharmacy, GRD (PG) IMT, 214, Rajpur Road Dehradun.

Introduction

Artemisia indica (Astereaceae) is a perennial herb found in different hilly areas of India. These have been employed by local people successfully to alleviate chronic fever, dyspepsia and hepatobilary ailments[1]. Phytopharmacological evaluation of this genus shows the presence of antimalarial[2]. The alleged antimalarial activity of *Artemisia indica* reported in the guidelines of Thai medicinal plants used in primary health care[3]. The prevalence of malaria in many regions of the world, together with the lack of vaccine and the emergence of strains resistant to antimalarial drugs in use, makes it necessary to continue to search for new synthetic and naturally occurring antimalarials and anti-leishmanial. Artemisia *indica* has been used for general malaise and fevers of unknown origin whereas artemisinins, the sesquiterpine lactones isolated from *Artemisia indica* have been used to treat multidrug-resistant malaria, analogs of which have been reported to exhibit both antimalarial and anti-leishmanial activity[4]. The leaves and flowering stems are anthelmintic, antiseptic, and antispasmodic, emmenagogue, expectorant and stomachic.

Material and methods

Plant Material - fresh aerial parts of *Artemisia indica* were collected from their natural habitats in and around Dehradun. The plants were authenticated by comparison with the herbarium and voucher specimen was lodged in the departmental herbarium of Botanical Research survey of India Dehradun. Aerial parts of *Artemisia indica* (500gm) were air dried at room temperature and powdered coarsely. The powder obtained (125g). Hundred gram of the pulverized plant was extracted with methanol using a soxlet apparatus. The extract was filtered, pooled and concentrated on rotavapour. The yield was 14.2% in powder extract. The extract of *Artemisia indica* was administered as a suspension in 2% Gum acacia to the animals. Preliminary phytochemical screening method was carried out on the standard screening method[5].

Animals - Male Wistar rats (150–250 g) and either male or female Swiss albino mice (20–25 g) were used. These animals were obtained from colonies maintained at the Department of Pharmacy, GRD (PG) IMT, Dehradun, U.K. (India). The animals were housed in groups of 6–10 under environmentally controlled conditions with free access to water and standard food. Food was with held overnight prior to experiments while water was still provided ad libitum. The handling and use of animals were in accordance to the Guidelines of Institute Animal Ethics Committee, while using live animals. All the animals were acclimatized to the laboratory environment for 5 days before the experiment. Six animals (rats or mice) per group comprising of three males and three females, were used in each experiment, unless otherwise specified. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water.

Chemicals – Carrageenin, Sodium chloride were purchased from Sigma Chemicals Company, U.S.A, Pethidine was obtained from Bengal Immunity, Kolkata, acetylsalicylic acid and Kaolin was purchased from Hi-Media Laboratories, Mumbai, India. Standard orogastric cannula distilled water was used for oral drug administration.

Anti-inflammatory study

Hind Paw edema in rats - In present study anti- inflammatory activity was determined in albino rats of either sex according to the method[6]. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gam acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile) at ‘0’ and ‘3’ hours after the carrageenan injection. Aspirin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug.

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

Where C_t = paw circumference at time t, C_0 = paw circumference before carrageenin injection

Acetic acid-induced writhing test - The prescreened animals were divided into groups as shown in Table 1. Aspirin in doses suspended in 2% gam acacia was used as the standard drug. The drugs were autoclaved at $121^{O}C$ for 30 min and administered subcutaneously. Writhing was induced 30 min later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water[7]. The number of writhes was counted for 30 min immediately after the acetic acid injection.

Analgesic Activity

Tail flick method - The prescreened animals (reaction time: 3-4 sec) were divided into groups as shown in Table 2. Pethidine 5 mg/kg acted as the standard drug. The drugs were administered intraperitoneally. The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage[8]. Acetylsalicylic acid is a well-known peripheral analgesic drug and was used as a positive control in the present investigation. The analgesic activity was calculated using the following formula:-

$$\% \text{ potential} = \frac{\text{Drug latancy (Test) - Base line lantency (Control)}}{\text{Base line latency (Control)}} \times 100$$

Statistical analysis - Results are expressed as mean ± S.E.M. statistical evaluations were made using ANOVA followed by t-test (Prism 3.0) and P values less than 0.05 were considered significant. Data are represented as mean ± S.E.M.

Discussion

The methanolic extract of *Artemisia indica* (100, 200 and 500 mg/kg, s.c.) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner (=0.99). The results were found to be highly significant (P<0.001) in comparison to the control. In the tail flick model, there was no significant difference in the mean predrug reaction time between the different groups. The results of the present study suggest that the methanolic extract of *Artemisia indica* in doses of 100, 200 and 400 mg/kg significantly suppressed carrageenan-induced paw edema in rats and demonstrated significant analgesic activity in acetic acid-induced writhing and tail flick models. The anti-inflammatory effects of the extract on acute inflammatory process such as carrageenan-induced edema in rats paw was dose dependen[9]. At 200 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals was comparable to 500 mg/kg of the extract. Phytochemical screening of the methanolic extract shows the presence of flavonoids and saponins. Flavonoids act as an anti-inflammatory response in the same way as the non – steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins[10].

Conclusion

The present study indicates the *Artemisia indica* has highly significant anti-inflammatory and analgesic properties.

Table: 1- Effects of the aqueous extract of *Artemisia indica* on carrageenan-induced rat paw edema and acetic acid-induced writhing response in mice

Carrageenan-induced rat paw edema				Acetic acid-induced writhing response in mice		
Group	**Dose (mg/kg, p.o.)**	**Increase in paw volume**	**% inhibition of paw edema**	**Dose (mg/kg, s.c.)**	**No. of writhing movements**	**% of protection**
N/saline	10 ml/kg	0.42 ± 0.21	–	9 ml/kg	82.23 ± 0.45	–
AI	100	0.22 ± 0.06*	52.4	100	44.82± 3.16**	48.5
AI	200	0.20 ± 0.03*	54.2	200	41.22± 4.21**	52.3
AI	500	0.18 ± 0.03*	55.8	500	29.82 ± 2.86*	67.9
Aspirin	100	0.16 ± 0.01**	62.8	100	18.72± 5.42**	82.5

n= 6 in each group, each value is the mean ± S.E.M.; *P< 0.01 compared to control; **P< 0.001 compared to control

Table: 2 - Analgesic activity of the methanolic extract of Artemisia *indica* on tail flick response in rats

Group	**Drug dose mg/kg, p.o.**	**Predrug (mean ± sem) reaction time (in sec)**	**Reaction time in sec (mean ± sem)**			
			30 min.	**1hr.**	**2hr.**	**4hr.**
D.water	1ml/kg	3.6 ± 0.11	4.42 ± 0.16	4.22 ± 0.4	4.51 ± 0.3	4.18 ± 0.8
A.I.	100	3.8 ± 0.09	6.21 ± 0.2*	8.22 ± 0.5*	7.42 ± 0.2*	7.90 ± 0.9*
A.I.	200	3.9 ± 0.16	8.62 ± 0.6*	7.75 ± 0.3*	7.61 ± 0.5*	9.12 ± 0.6*
A.I.	500	3.8 ± 0.12	8.21 ± 0.5*	10.52 ± 0.4*	9.45 ± 0.2*	9.22 ± 0.5*
Pethidine	5	4.2 ± 0.08	9.42 ± 0.2*	9.72 ± 0.3*	9.65 ± 0.7*	7.92 ± 0.6*

n= 6 in each group, each value is the mean ± S.E.M.; *P< 0.01 compared to control; **P< 0.001 compared to control

References

1. Said HM. The disease of liver: Greco-Arab Concepts. Hamdard Foundation Press Karachi, 1984; 45-48.
2. Zafar MM, Hamdard ME, Hameed A. Screening of Artemisia absinthium for antimalarial effects of Plasmodium berghei in mice, a premilinary report. J. Ethnopharmacol. 1990; 30:223-226.
3. Bunyapraphatsara, N. Thai Medicinal Plants Used in Primary Health Care; 1986; 2: 32.
4. S. Ganguly, S. Bandyopadhyay, A. Bera, M. Chatterjee Antipromastigote activity of an ethanolic extract of leaves of Artemisia indica, Indian J Pharmacol, Vol 38, Issue 1 2006:64-5
5. Trease, G.E., Evans, W.C.: Pharmacognosy. Balliere Tindall Press, London; 1983; 309- 706.
6. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med 1962; 111:544-7.
7. Ghosh MN., Evaluation of analgesic agents, Fundamentals of experimental pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984.
8. Sheth UK, Dadkar NK, Kamt UG. Drugs acting on CNS, Selected topics in experimental pharmacology. 1st ed. Bombay: Mohanlal B. Kothari Book De- pot; 1972.
9. DiRosa M., Biological properties of carrageenan. Journal of Pharmacy and Pharmacology. 1972; 24:89–102.
10. Berknow R. The Merck Manual of Diagnosis and Therapy, 16th ed. Merck Research Laboratories Rathway, New Jersey, 1992; 1407–1420.