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Original Article

ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF *ARTEMISIA PALLENS* WALL EX.DC

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ABSTRACT

Artemisia pallens was studied for anti-inflammatory action by carrageenin-induced rat paw edema. The analgesic activity was tested by tail flick method and hot plate method in albino rats and mice. The methanolic extract of *Artemisia pallens* in doses of 100, 200 and 500 mg/ml showed 68.85, 74.53 and 81.13% inhibition of paw edema respectively at the end of three hour. In the hot plate and tail flick model, the methanolic extract of *Artemisia pallens* in the above doses increased the pain threshold significantly also administration of *Artemisia pallens* showed dose dependent action in all experimental animal models. The plant had Saponins, flavonoids, Sesquiterpenoids, Oils, Phenols and Tannins. The results of the present study suggest that *Artemisia pallens* has potent analgesic and anti-inflammatory activities.

Keywords: Analgesic, Anti-Inflammatory, Carrageenin, *Artemisia Pallens*

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1.0 INTRODUCTION

Artemisia pallens, family compositea commonly called Davana (Hindi) are a shrub plant. It comprises hardy herbs and shrubs known for their volatile oils. The fern like leaves of many species are covered with white hairs. The stem is thin woody and the plant produced in an annual aromatic south Indian plants. Specially found in Mysore city. *Davana* is mostly cultivated in red soil region in south Indian as Maharashtra, Andhra Pradesh, Karnataka and Tamilnadu[1]. Many Pharmacological activities of *Artemisia pallens* have been reported: perfumenes and as an antifungal and antibacterial agent[2,3]. It is the most important Aromatic plants used in the perfumery and cosmetic industries and India is the major exporters of *Artemisia pallens* oil to the rest of the world. *Davana* is widely used in Iraqi and Indian folk medicine for the treatment of diabetes mellitus. The present study was therefore undertaken to investigate some of the folkloric claims especially the use of the plant as a treatment of diabetes mellitus[4].

2.0 Materials and Methods

2.1 Animals: - Studies were carried out using Sprague-dawley rats weighing 95-100 gm and Swiss albino mice weighing 30-35 gm. The animals were bred and housed in the central animal house of the Faculty of Pharmacy, GRD(PG)IMT, Dehradun, (U.K.). The animals were housed in groups of 6 – 10 under environmentally controlled condition and maintained under standard laboratory conditions (temperature 25 ± 2 ^{0}C) and relative humidity 44 –56 %, with a dark and light cycle of 12 ± 1 h. They were allowed free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided *ad libitum*. The research was conducted in accordance with Ethical Committee, Faculty of Pharmacy, GRD(PG)IMT, Dehradun (U.K.).

2.2 Drugs and Chemicals: - The following drugs and chemicals were used: Carrageenan (Sigma - Aldrich), Acetylsalicylic acid was obtained by (Lupin pharmaceuticals Ltd), Sodium chloride (Sigma chemicals company, U.S.A.), Indomethacin (Jasonpal pharmaceutical Ltd.), Pethidine (Bengal Immunity, Kolkata) and all the other chemicals used were of the analytical and highest purity grade from standard companies. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

2.3 Plant Materials:- The plant (*Artemisia pallens*) used for this study was collected from

South India (Tamilnadu) and identified at Department of Pharmacy, Kumaun University Nainital. A voucher specimen has also been deposited in the herbarium of the institute for future references. The air dried aerial parts of the plant were cleaned and reduced to powder form with the help of mechanical grinder, after which 150 gm of powdered sample was exhaustively extracted with 1.5 lt of methanol (analytical grade), for 3 days (by soxlet apparatus). The plant material was separated by filtration and the methanolic extract was concentrated (by Rotavapour, Büchi, Switzerland) and lyophilized to preserve it. The residue was obtained 3.3gm and dilutions of the extract were made in 2% gum acacia for the various studies. Preliminary phytochemical screening was carried out on the extract using the standard screening method[5].

2.4 Analgesic Activity

Tails flick Method : - The method involves exposure of rat tail to the heated nicrome wire and recording of the reaction time of the animal i.e. Tail flick as originally described[6]. Reaction time is defined as 'interval between exposure to heat and the tail flick'. The Prescreened animals were divided into five groups as shown in Table 2. Acetylsalicylic acid in dose of 300 mg/kg, suspended in NaCl was used as the standard drug and administered by oral route. In this method for every rat, three consecutive readings were taken and the mean of three reading was taken as the individual reading. Thus, the mean reaction time for each group was calculated. The animal showing the reaction time of more than 10 seconds were rejected during the screening procedure. 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 :-

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

Hot Plate Method: - The hot plate latency assay was based on the method[7]. The temperature of the hot plate set at 55 ± 0.5^{0}C. The plant extract, saline, and Acetyl salicylic acid were given to the animals (each group 6 animals) orally after 18 hr. fast. All the animals in each group were placed on a hot plate 30 min. after the administration of extract, standard drug and saline. The average of the two reading was obtained as the initial reaction time (T_b). The reaction time (T_a) was recorded when the animal starts to lick the foot or jumps off the hot plate. The mean of the latency for each group was recorded at 60 and 90 min. after the administration of extract, saline and Acetyl salicylic acid. The analgesic activity was calculated using the following formula :-

$$\text{Percentage analgesic activity} = \frac{Ta - Tb \times 100\%}{Tb}$$

Anti-Inflammatory Activity

Hind Paw edema in rats:- This assay was determined as described[8]. Animals were divided into five groups comprising six animals in each group with 2% gum acacia in normal saline. An injection of 0.1 ml of carrageenin suspension (200µg/paw) was made into the right hind foot of each conscious rats under the plantar aponeurosis (95-100gm). The control, standard and test groups were treated orally with saline, indomethacin and the extracts 1hr. before carrageenin injection. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at '0' and '3' hour after the carrageenin injection. The difference between the two readings was taken as the volume of paw edema and percentage inhibition was calculated by :-

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

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

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.

Results

In the anti-inflammatory activity, the results show oral treatment of animals with methanolic extract of *Artemisia pallens* (100 – 500 mg/kg b.w.) and Indomethacin (30 mg/kg). The test and standard drugs produced significant inhibition of paw edema in comparison to the control (Table 1). In the analgesic studies, the methanolic extract of *Artemisia pallens* (100 – 500 mg/kg b.w.) show the result by tail flick method significantly in a dose – dependent manner. The increased in reaction times was significant in the dose of 100 mg/kg, 200 mg/kg and 500 mg/kg of methanolic extract when compared with standard drug acetylsalicylic acid. The results were found to be highly significant ($P < 0.001$) in comparison to the control (Table 2). The results from hot plate test show that at 30 min the oral doses of methanolic extract of *Artemisia pallens* and indomethacin increased the reaction time were significantly increased in comparison to the control (Table 3).

Table 1. Effect of the methanolic extract *Artemisia pallens* on carrageenin-induced paw edema in rats.

S.No.	Groups	Dose orally (mg/kg. p.o.)	Initial paw size	Paw edema Inhibition			
				3 hr	4 hr	3 hr	4 hr
1	Control	Normal Saline (0.5 ml)	0.32±0.04	0.79±0.03	0.87±0.05	----	----
2	*Artemisia pallens*	100	0.36±0.02	0.90±0.06	0.93±0.04	68.85	94.89
3	*Artemisia pallens*	200	0.38±0.02	0.81±0.03	0.84±0.03	74.53	82.45
4	*Artemisia pallens*	500	0.35±0.03	0.71±0.05	0.81±.0.04	81.13	94.89
5	Indomethacin	30	0.29±0.01	0.62±0.05	0.68±0.04	21.52	21.84

n = 6 in each group, each values is the mean ± S.E.M.
*P< 0.05 compared to control
**P< 0.001 compared to control

Table 2. Effect of the methanolic extract of *Artemisia pallens* in mice's by tail flick method.

S.No.	Group	Dose (mg/kg, p.o.)	60 min.	90 min.	120 min.	180 min.
1	Control	Normal saline (.2ml)	06.05 ± .84	7.30 ± 0.72	8.41±0.70	8.21±0.67
2	*Artemisia pallens*	100	7.95±0.38	8.42±0.38	8.11±.0.66	8.81±0.24
3	*Artemisia pallens*	200	7.61±0.57	7.52 ±0.78	7.52±0.78	7.84±0.67
4	*Artemisia pallens*	500	7.87±0.73	8.41±0.61	8.10±0.48	7.95±0.99
5	Acetylsalicylic acid	300	7.02 ± 0.58	7.64 ± .41	7.41±0.27	6.44±0.39

n= 6 in each group, each value is the mean ± S.E.M.
*P< 0.05 compared to control
**P< 0.001 compared to control

Table 3. Effect of the methanolic extract of *Artemisia pallens* on hot plate test method in rats.

S.No.	Groups	Dose (mg/kg, p.o.)	Reaction time[a] (s)		
			30	60	90
1	Control	Normal saline (0 .2ml)	10.05 ± .82	9.25 ± .91	8.86±.01
2	*Artemisia pallens*	100	12.67 ± 1.4	14.36 ± 1.61	18.05±.72
3	*Artemisia pallens*	200	16.34 ± .618	18.78 ± .63	21.44±.09
4	*Artemisia pallens*	500	18.54 ± .43	21.87 ± .78	27.37±.84
5	Acetylsalicylic acid	300	27.10 ± .63	28.87 ± 3.02	31.50±0.66

n= 6 in each group, each value is the mean ± S.E.M.
*P< 0.05 compared to control
**P< 0.001 compared to control

Discussion

Two different analgesic laboratory models were employed in the current investigation with the objective of identifying possible increase in latency and central effect of the test substances, using both tail flick method and hot plate thermal stimulation. It was observed that the methanolic extracts of *Artemisia pallens* possessed analgesic effects against both models. This observation indicates that *Artemisia pallens* has both latency and central (thermal reaction time prolongation) effects. The analgesic activity was expressed as "mean increase in latency after drug administration ± SEM" relative to controls by different doses of the extracts of aerial parts of *Artemisia pallens.* In hot plate method, the latency of the animals were highly increased compared to the control, likewise the licking time was significantly reduced by administration of the extract showing analgesic activities. This observation can provide useful information if a choice is desired to be made regarding the species of *Artemisia pallens* as a source of analgesic drugs.

The anti-inflammatory effects of the extract on acute inflammatory process such as carrageenin – induced edema in rats paw was dose dependent[9]. At 200 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals and the efficacy of indomethacin (30

mg/kg) was comparable to 500 mg/kg of the extract.

Preliminary 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]. Further studies may reveal the mechanisms of action responsible for the analgesic and anti-inflammatory activities of *Artemisia pallens*.

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