



Research Article

Analgesic and Anti-Inflammatory Activities of Ethyl Acetate Extract of Leaves of *Salvadora persica L.*

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ABSTRACT

The leaves of *Salvadora persica L.* were extracted with petroleum ether, chloroform, and ethyl acetate, ethanol, and water solvents. The ethyl acetate extract was screened for analgesic and anti-inflammatory activities. The analgesic activity was carried out by tail immersion method and anti-inflammatory activity was evaluated by carrageen induced paw edema method. Pretreatment with ethyl acetate extract (500 mg / kg, body weight) showed significant analgesic activity ($p < 0.01$) comparable to the standard drug pentazocine and against carrageen induced paw edema suppressant activity comparable to the standard drug indomethacin.

Key Words: Analgesic activity, anti-inflammatory activity, *Salvadora persica L.*

INTRODUCTION

Salvadora persica L. is an evergreen small tree, belonging to family Salvadoraceae, commonly known as 'Pilu', 'Jal' and 'Tooth brush tree' and is widely distributed in India, Africa, Saudi Arabia, Iran, Israel and Pakistan. It has been claimed in traditional literature to be valuable against wide variety of diseases. Tooth brushes made from roots and small branches have been used for over 1000 years in India, Arabia and Africa. The fruit is aphrodisiac, alexiteric and stomachic, improves appetite and is useful in biliousness. The leaves are used in the treatment of nose trouble, piles, scabies, leucoderma, inflammation, scurvy, gonorrhoea and pain. The bark is useful in the treatment of low fever and amenorrhoea.

The root is useful in the treatment of toothache, chest disease and boils^[1-3]. Miswak is a chewing stick prepared from the roots, twigs, or stems of *Salvadora persica L.* Miswak extract showed high content of sodium chloride and potassium chloride as well as salvadourin and salvadorin, saponins, tannins, vitamin C, silica, and resin in addition to cyanogenic glycoside and benzylisothiocyanate.^[4] Anticonvulsant and sedative effects have been reported from stem extract of *S. persica L.*^[5]

There is no report on the analgesic and anti-inflammatory studies of the ethyl acetate extract of dried leaves of *Salvadora persica L.* so far, though it is used in folk medicine. Thus it was considered worthwhile to take up such an investigation in detail. The present study was, therefore, aimed to explore analgesic and anti-inflammatory effects of ethyl acetate extract of the leaves of *Salvadora persica L.* on laboratory animals.

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MATERIALS AND METHODS

Collection and extraction

Salvadora persica L. leaves were collected from the campus of Central Arid Zone Research Institute, Jodhpur, Rajasthan, India, in the third-fourth week of May. Taxonomic identification of the plant was done by the taxonomist, Botanical Survey of India, Jodhpur (Raj.) as per the herbarium specimen number JNU/PH/2009/S-1 and certificate number A. 199014/SE-1/Estt. Leaves of the *Salvadora persica L.* plant were dried in shade for 10-12 days. After complete drying, leaves were pulverized to a coarse powder of 40 mesh size in a mechanical grinder.

The dried powder (300 gm) of dried leaves of *Salvadora persica L.* was extracted with various solvents having different polarity like petroleum ether (60-80°C), chloroform, ethyl acetate, ethanol (99.5%) and water in succession using Soxhlet extractor. Each extract was concentrated in vacuum to yield a semi solid mass and the extracts were subjected to preliminary qualitative chemical analysis.^[6-9]

Animals used

Albino mice of either sex; weighing between 16-22 g body weight and Wistar albino rats of either sex; weighing between 150-210 g body weight were provided by Animal Housing Facility of Lachoo Memorial College of Sc. And Tech., Pharmacy Wing, Jodhpur, India. Animals housed in standard cages in light- controlled room at $25 \pm 3^\circ$ (12 hour light/ 12 hour dark cycle and 50 ± 5 % RH, were given a standard pellet diet and water ad libitum. All studies were conducted in accordance with protocols, reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The serial number of the approval protocols were IACE/REC/5/2 and /AEC/REC/5/3 for ocular in vivo and ocular safety studies, respectively. Animals free of

any sign of ocular inflammation or gross abnormality, were used. The animals were deprived of food for 24 hr before experiment but allowed free access to drinking water throughout.

Materials used

For analgesic activity; the test sample (ethyl acetate extract of leaves of *Salvadora persica L.*) and reference drug (Pentazocin) were dissolved in sterile distilled water. Normal saline solution was prepared for control vehicle.

For anti-inflammatory activity; the 5% acacia solution was prepared, test sample (ethyl acetate extract of leaves of *Salvadora persica L.*) was dissolved in 5% acacia solution and reference drug (Indomethacin) was dissolved in 5% acacia solution. Carrageen (1%) was prepared in normal saline solution and used when required.

Analgesic activity

This method is based on tail immersion method.^[10-12] Prior to the analgesic experiment, the animals were screened for sensitivity test by immersing tip of the tail gently in hot water maintained at 55-55.5 °C. The animals which lifted the tail from hot water within 5 seconds were selected for the study.

The albino mice (16-22 g) were divided into three groups, each group consisting of six animals. The lower 5 cm portion of the tail of each animal was marked. Group (I), serving as control, was administered normal saline solution (5 ml/kg, body weight, i.p.), Group (II), serving as standard, was administered Pentazocine (10 mg/kg, body weight, i.p.) and Group (III), serving as test, was administered ethyl acetate extract of leaves of *Salvadora persica L.* (500 mg/kg, body weight, i.p.). The mice were screened by immersing their distal 5 cm. of the tail in hot

water maintained at $55 \pm 1^\circ\text{C}$. The reaction time was measured at 0, 15, 30, 45 and 60 minutes respectively. The cut off time of the immersion was fixed at 15 seconds to prevent damage to the tail. The results are summarized in the **Table 1**.

Anti-inflammatory activity

The anti-inflammatory activity was assessed by Carrageen Induced Paw edema Method.^[10, 12] The Wistar albino rats (150-210 g) were divided into three groups, each group consisting of six animals. A mark was made on left hind paw of each rat just beyond tibio-tarsal junction. The initial paw volume of each rat was noted by mercury displacement method. The Group-I, serving as control, was administered 5% acacia solution in a volume of 1 ml/100g, body weight, orally. Group-II, serving as standard, was administered Indomethacin (10 mg/kg, body weight, orally). Group-III, serving as test was administered ethyl acetate extract of leaves of *Salvadora persica L.* in the dose of 500 mg/kg body

weight, orally. One hour after the oral administration of control, standard and test drug, 0.1 ml of 1% Carrageen in normal saline solution was injected into the plantar aponeurosis of the left hind paw of each rat. The paw volume was measured by a plethysmometer in 1, 2, 3 and 4 hour after Carrageen suspension injection. The difference between the initial and subsequent readings gives actual edema volume.

The percent inhibition was calculated by following formula-

$$\% \text{ Inhibition} = (1 - V_t/V_c) * 100$$

Where, V_t and V_c are the mean change in paw volume of treated and control rats respectively. The results are summarized in the **Table 2**.

Statistical analysis

The data were calculated per group as mean \pm SEM. The significance was calculated using student's t-test. The minimum level of significance was set at $P < 0.01$.

Table-1 Effect of Ethyl Acetate Extract of Leaves of *Salvadora persica L.* in Tail Immersion Test.

Treatment	Dose	Analgesic Response					
		Mean time (sec) \pm SEM					
		0 min	15 min	30 min	45 min	60 min	120 min
Control (Normal Saline)	5 ml/kg i.p.	3.16	3.36	3.46	4.09	4.09	3.54
		\pm 0.047	\pm 0.168	\pm 0.217	\pm 0.210	\pm 0.304	\pm 0.198
Standard (Pentazocine)	10 mg/kg i.p.	3.49	4.64	6.52	8.31	8.82	7.08
		\pm 0.165	\pm 0.179	\pm 0.207*	\pm 0.319*	\pm 0.173*	\pm 0.375*
		3.18	3.57	4.37	6.54	7.89	5.46
		\pm 0.047	\pm 0.065	\pm 0.230	\pm 0.267*	\pm 0.231*	\pm 0.302*

Each value represents the Mean \pm SEM (n = 6).* ($P < 0.01$) control vs. treated group EAESP- Ethyl acetate extract of *Salvadora persica L.*

Table -2Effect of Ethyl Acetate Extract of Leaves of *Salvadora persica L.* on Carrageen Induced Paw Edema

Treatment	Dose	Mean time ± SEM (Paw Volume)				
		0 hr	1 hr	2 hr	3 hr	4 hr
		0.38 ± 0.034	0.68 ± 0.052	0.93 ± 0.073	0.72 ± 0.044	0.63 ± 0.036
Standard (Indomethacin)	10 mg/kg body weight	0.35 ± 0.025 (7.89)	0.52 ± 0.034 (23.53)	0.45 ± 0.024* (51.61)	0.40 ± 0.039* (44.44)	0.42 ± 0.034* (33.33)
Test (EAESP)	500 mg / kg body weight	0.37 ± 0.023 (2.63)	0.59 ± 0.034 (13.24)	0.51 ± 0.052* (45.16)	0.48 ± 0.034* (33.32)	0.55 ± 0.035* (12.69)

Each value represents the Mean ± SEM (n = 6).* (P < 0.01) control vs. treated group EAESP- Ethyl acetate extract of *Salvadora persica L.*

RESULTS AND DISCUSSION

The preliminary phytochemical investigation revealed the presence of fats and tannins in petroleum ether extract; phytosterols in chloroform extract; phytosterols, alkaloids and tannins in ethyl acetate extract; phytosterols, alkaloids in ethanol extract and tannins, saponins and amino acids in water extract. Also it had been seen that majority of the phytoconstituents were extracted in ethyl acetate and water extract of *Salvadora persica L.* These constituents may be responsible for reported pharmacological activities of this plant.

The analgesic activity of ethyl acetate extract is shown **Table 1**. The ethyl acetate extract showed significant analgesic activity (P < 0.01) at 45, 60 and 120 minutes when compared to the control group. While the standard drug (Pentazocine) has shown significant activity (P < 0.01) at 30, 45, 60 and 120 minutes.

The anti-inflammatory activity of ethyl acetate extract is shown **Table 2**. The ethyl acetate extract showed significant

activity (P < 0.01) at 2 and 3 hr when compared to the control group. The percent inhibition of paw edema exhibited by ethyl acetate extract were 13.24%, 45.16%, 33.32% and 12.69% at 1, 2, 3 and 4 hr respectively; while Indomethacin treated animals showed maximum percent inhibition of paw oedema (51.61%) at 2 hr. Carrageenin induced oedema is mediated by release of histamine and 5HT followed by the prostaglandin kinin and has frequently used to assess the anti-inflammatory effect in natural products. The study therefore revealed that the leaves of *Salvadora persica L.* possess analgesic and anti-inflammatory activities.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Raj Singh, Sr. Scientist, CAZRI, Jodhpur for providing plant material and Dr. P. J. Parmar, Joint Director, Botanical Survey of India, Jodhpur for authenticating the plant. The authors are also thankful to Dr. B. P. Nagori, Director, L. M. College of Sc. & Tech., Jodhpur, Rajasthan, for his help in carrying

out biological activities.

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