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Antinociceptive Activity of different extracts of Leaves of *Salvadora Persica L.*

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ABSTRACT

*Salvadora persica L. (*family- *Salvadoraceae*) is an evergreen small tree commonly known as Pilu, Jal and toothbrush tree. It is used in the treatment of pain, low fever, toothache, nose trouble, piles, scabies, inflammation, scurvy, gonorrhoea, chest disease and boils. The present study was undertaken to evaluate the antinociceptive activity of the successive extracts (chloroform, ethyl acetate, ethanol and aqueous extracts) of powdered leaves of *Salvadora persica L.* at the dose of 500 mg/kg b. w. using eddy's hot plate method. The results of the statistical analysis showed that chloroform and ethyl acetate extracts have significant antinociceptive activity. From the results of antinociceptive effects it can be concluded that the chloroform extract of the powdered leaves of *Salvadora persica* has shown significant activity ($P < 0.05$) at 30 min and significant activity ($P < 0.01$) at 60 and 120 minutes. The ethyl acetate extract has shown significant activity ($P < 0.01$) at 30, 60 and 120 minutes when compared to the control group. While the standard drug (Morphine Sulphate) shown significant activity ($P < 0.01$) at 30, 60, 120 and 180 minutes. Other successive extracts (ethanol and aqueous extracts) could not produce the significance of the difference from the control as antinociceptives. Hence present investigation reveals the antinociceptive activity of chloroform and ethyl acetate extracts of leaves of *Salvadora persica L.*

Key Words: *Salvadora persica* L. antinociceptive activity, chloroform and ethyl acetate extracts

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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 (Figure-1). 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. *Salvadora persica* L. is a large much-branched evergreen shrub or small tree of 6 to 7 m in height; with soft whitish yellow wood; main trunk erect or trailing with profusely branched, wide crown of crooked, straggling and dropping branches; young branches are green in color; bark slightly rough, grayish-brown on main stem paler elsewhere. Leaves are oblong-elliptic to almost circular, light to dark green, rather flashy, sometimes with wart like glandular dots and dense, loose hairs; apex broadly tapering to rounded, sharp-tipped; base broadly tapering; margin entire; petiole up to 10 mm long; leaves in opposite pairs. The Flowers are greenish to yellowish, very small, in loose, slender-branched axillary or terminal panicles, up to 10 cm long. The Fruits are spherical, fleshy, 5-10 mm in diameter, pink to scarlet when mature.

Figure-1: *Tree of Salvadora persicaL. (Salvadoraceae)*

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, gonorrhea and pain. The bark is useful in the treatment of low fever and amenorrhea. The root is useful in the treatment of toothache, chest disease and boils. [1-4]

Miswak extract showed high content of sodium chloride and potassium chloride as well as salvadourea and salvadorine, unidentified alkaloids, saponins, tannins, vitamin C, silica, and resin in addition to cyanogenic glycoside and benzylisothiocynate[5]. Anticonvulsant and sedative effects have been reported from stem extract of S. persica L[6]. There is no report on the

antinociceptive activity of different extracts 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 antinociceptive effect of successive extracts (Chloroform, ethyl acetate, ethanol and aqueous extracts) of the leaves of *Salvadora persica L.* on laboratory animals.

MATERIAL AND METHODS

Plant Material

Collection of leaves of the *Salvadora persica L.* plant was done personally from the campus of Central Arid Zone Research Institute, Jodhpur (Raj.) in the last week of Maech 2009. Taxonomic identification of the *Salvadora persica L.* was done by Botanical Survey of India, Arid Zone Circle, Jodhpur (Raj.) as per letter ref no. BSI/AZC/A.19014/SE-1/Estt. Dated on 02-04-2009 and the institutional code no. of voucher specimen is JNU/PH/2009/S-1. A voucher specimen has been also preserved in Dept. of Pharmacognosy, F. O. P S. Jodhpur National University, Jodhpur for further reference. The plant material was 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 and collected coarsely powdered material.

Preparation of extracts

The powdered plant material (500 gm) of dried leaves of *Salvadora persica L.* was subjected to continuous hot exhaustive extraction with various solvents in increasing order of polarity *viz* petroleum ether (60-80°C), chloroform, ethyl acetate, ethanol (99.5%) and aqueous in succession using Soxhlet extractor. After each extraction the solvent was recovered and each extract was concentrated in vacuum to yield a semi solid mass. These extracts were used for the study of antinociceptive activity [7-10].

Animals used

Albino mice of either sex; weighing between 16-22 g body weight was provided by Animal Housing Facility of F. O. P. S., Jodhpur National University, Jodhpur, India. Animals housed in standard cages in light- controlled room at 25 ± 3° (12 hour light/ 12 hour dark cycle and 50 ± 5 % RH) were given a standard pellet diet and aqueous 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 is 1258/ac/09/CPCSEA of F. O. P S. Jodhpur National University, Jodhpur 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 antinociceptive activity; the test samples (chloroform, ethyl acetate) were dissolved in 5% acacia solution, the other test samples (ethanol and aqueous extracts) were dissolved in distilled water. The standard drug, Morphine Sulphate (Troikaa Pharmaceutical Ltd., Thol, Gujrat) was prepared in water for injection. The 0.1% Carboxy Methyl Cellulose was prepared in distilled water as control group.

Acute toxicity studies

Swiss albino mice of either sex weighing 18-25 g were used for the acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423. The successive extracts were administered orally to different groups of over night fasted mice at the doses of 300-5000 mg/kg body weight. After oral administration of the extracts, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week. No adverse effects or mortality was observed therefore the successive extracts are safe to use even at the doses of 5000 mg/kg of body weight orally. From the study, the dose for antinociceptive activity was fixed to be 500 mg/kg b. w. for the comparison of activity in different successive extracts and selection of more active extract [11].

Antinociceptive activity

Antinociceptive activity of successive extracts (chloroform, ethyl acetate, ethanol and aqueous extracts) of leaves of *Salvadora persica L.* was studied by eddy's hot plate method.

Eddy's hot plate method

The animals were divided into six groups of 6 animals each. Group I served as control and treated orally with 0.1% CMC (5 ml/kg b. w.). Group II served as standard and were injected morphine sulphate (2 mg/kg) s. c. Group III, IV, V and VI were treated orally with chloroform, ethyl acetate, ethanol (95 %) and aqueous extracts at the dose 500 mg/kg b. w. respectively. The animals were individually placed on the hot plate maintained at 55°C, before the drug and one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first before the drug and at 0, 30, 60, 120 and 180 minutes . A cut off period of 15 seconds was considered as maximal latency to avoid injury to the paws (Table-1) [12-14].

RESULTS AND DISCUSSION

The results of antinociceptive activity of successive extracts of powdered leaves of *Salvadora persica L.* by EDDY'S HOT-PLATE Method are mentioned in the Table 1 and Figures 2& 3.

The data were calculated per group as mean ± SEM. The significance of the difference between 'control' and 'drug treated' means were determined by One-way ANOVA followed by Dunnett's Standard test. The result in the table 1 showed that only the chloroform and ethyl acetate extracts have considerable antinociceptive activity as compared to control and standard.

Table 1: Antinociceptive effect of different extracts of *salvadora persica* leaves subjected to the eddy's hot-plate method.

Treatment	Dose	Reaction Time in seconds at time (minutes)					
		Before drug	0 min	30 min	60 min	120 min	180 min
		1	2	3	4	5	6
Control Group	5 ml/kg(b.w).	4.392± 0.131	4.138± 0.249	4.27±0. 233	4.352±0.2 52	4.227±0. 203	4.072±0. 141
Std (Morphine Sulphate)	2 mg/kg(b. w).	4.322± 0.25	4.313± 0.228	8.848± 0.43**	12.1±0.19 1**	11.15±0. 327**	8.375±0. 31**
SPL $CHCl_3$ Extract	500 mg/kg(b. w).	3.825± 0.175	3.685± 0.155	5.63±0. 241*	6.548±0.3 78**	4.727±0. 070**	4.305±0. 141**
SPL EtOAc Extract	500 mg/kg (b. w).	4.332± 0.075	4.258± 0.086	6.303± 0.38**	8.295±0.8 93**	6.783±0. 886**	5.143±0. 663
SPL EtOH Extract	500 mg/kg (b. w).	4.033± 0.075	3.95±0. 156	5.212± 0.234	5.795±0.4 52	5.118±0. 332	4.828±0. 517
SPL Aq. Extract	500 mg/kg(b. w.)	3.972± 0.07	3.997± 0.063	4.23±0. 198	4.047±0.2 65	4.39±0.2 07	4.197±0. 194

Each value is the Mean± S.E.M. for 6 mice *P<0.05; **P<0.01; compared with control data were analyzed by using One-way ANOVA followed by Dunnett's, Standard: Morphine sulphate (2 mg/kg b. w.), SPL $CHCl_3$: Chloroform extract of *Salvadora persica L.* Leaves at dose 500 mg/kg b. w., SPL EtOAc Extract**:** Ethyl Acetate extract of *Salvadora persica L.* leaves at dose 500 mg/kg b. w., EtOH Extract: Ethanol extract of *Salvadora persica L.* leaves at dose 500 mg/kg b. w., Aq. Extract: Aqueous extract of *Salvadora persica L.* leaves at dose 500 mg/kg b. w.

From the results of antinociceptive effects it can be concluded that the chloroform extract of the powdered leaves of *Salvadora persica* has shown significant activity ($P < 0.05$) at 30min and significant activity ($P < 0.01$) at 60 and 120 minutes. The ethyl acetate extract has shown significant activity ($P < 0.01$) at 30, 60 and 120 minutes when compared to the control group. While the standard drug shown significant activity ($P < 0.01$) at 30, 60, 120 and 180 minutes.

In the present study, the chloroform and ethyl acetate extracts (500 mg/kg) of leaves of *Salvadora persica L.* significantly increased the reaction time in hot plate test, suggesting their central analgesic activity. The central analgesic activity may be due to the presence of reported alkaloids and glycosides. The ethyl acetate extract significantly increased more the reaction time than the chloroform extract, it could be suggested that ethyl acetate extract has more central analgesic activity than chloroform extract (figure 2 & 3).

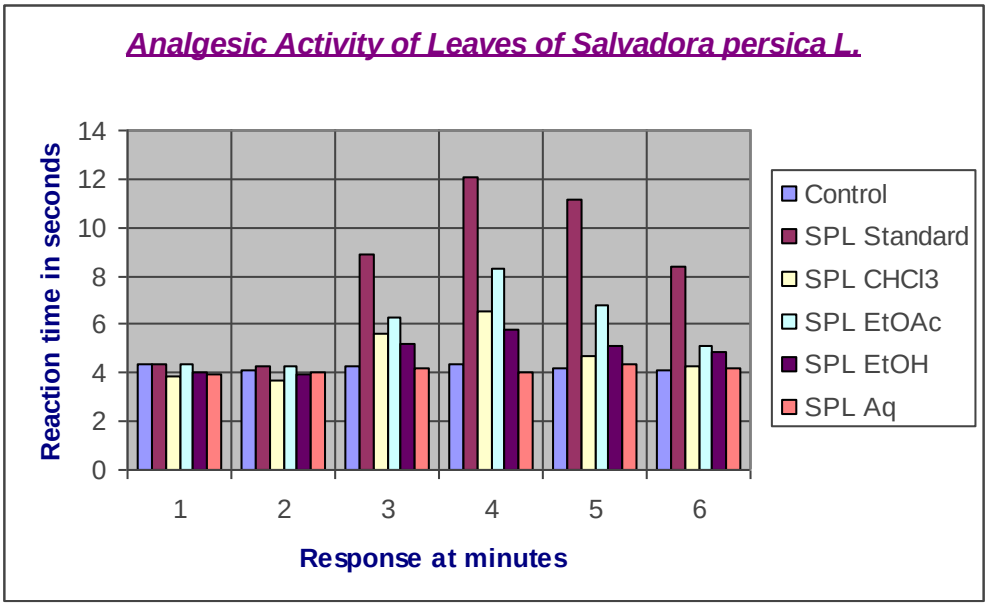

Figure 2: Column Chart of Antinociceptive activities of Leaves *Salvadora persica L.*

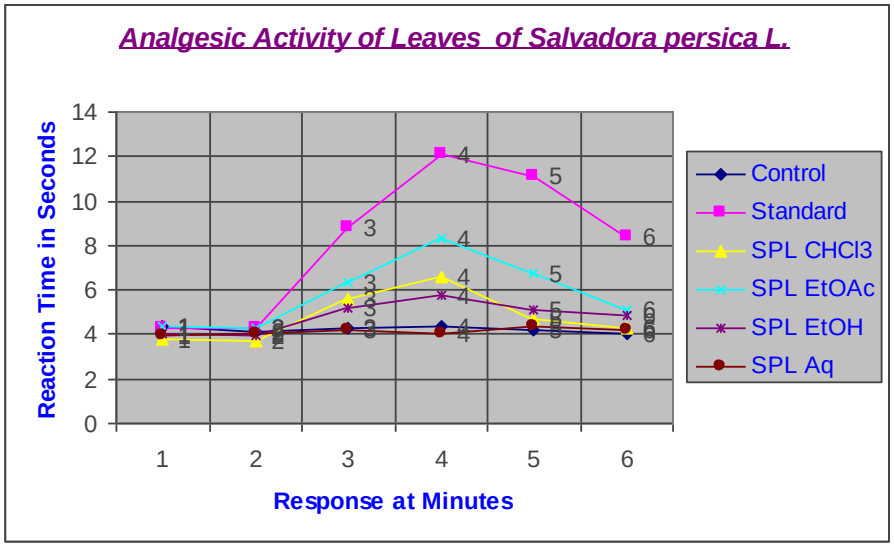

Figure 3: Line Chart of Antinociceptive activities of Leaves *Salvadora persica L.*

CONCLUSION

On the basis of the results obtained in the present study, it was concluded that the chloroform and ethyl acetate extracts of leaves of *Salvadora persica L.* possesses the significant antinociceptive

activity. According to column and line chart of response reaction time at different time it can be concluded that the ethyl acetate extract has more antinociceptive activity than chloroform extract. Other successive extracts could not produce the significance of the difference from the control as antinociceptive. These finding suggest that this plant is a potential source of natural antinociceptive activity. Further studies are warranted for the isolation and characterization of antinociceptive components and also *in vivo* studies are needed for understanding their mechanism of action as an antinociceptive better.

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