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Analgesic activity of ethanolic extract of roots of *Prosopis cineraria* (I.) Druce

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ABSTRACT

Prosopis cineraria (L.) Druce is a deep rooted, nitrogen fixing, multipurpose tree endemic to the hot deserts of India. Its synonym is *Prosopis spicigera*. It belongs to the family *Leguminosae* and subfamily *Mimosoideae*. In view of its medicinal importance, the present research was focused on the analgesic properties of roots of *P. cineraria* by *in vitro* approach in rats. The analgesic activity of root of *Prosopis cineraria* was studied using hot-plate method and tail-immersion method in rats. Doses of the ethanolic extract of 200mg/kg & 300mg/kg, orally were selected for analgesic activity. The extract at all the doses used and the Diclofenac sodium significantly inhibited both the analgesic activity for hot plate and tail immersion method. The present study demonstrates the potential analgesic effect of ethanolic extracts of *Prosopis cineraria* roots. The dose of 200mg/kg b.w is very effective than 300mg/kg b.w in both above pharmacological models.

KEYWORDS: Prosopis cineraria, Roots, Analgesic, Hot plate and Tail immersion.

INTRODUCTION

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world (Sharma et al., 2008). A promising multipurpose tree species commonly found is Prosopis cineraria (L.) Druce. The tree, locally called as Jandi or Khejri is a boon to the people due to its myriad virtues. It holds an important place in the rural economy in the northwest region of Indian subcontinent. The genus Prosopis comprises about 44 species distributed mainly in dry regions of Southwest Asia, Africa and, predominantly America from western North America to Patagonia (Puri & Kumar, 1995). It is a small thorny, irregularly branched tree, 5-10 in high. Evergreen or nearly so, it forms an open crown and has thick, rough gray bark with deep fissures. Rooting can be very deep; the tap root of *P. cineraria* may penetrate vertically up to 20 m or more (Mohaney, 1990). The leaves are good fodder for camels, goats and donkeys. The pods are used as a vegetable. Sangri - green pods - are boiled and dried. The flowers are useful for honey production. Khejri is also used for soil improvement and sand dune stabilization. The wood is ideal for domestic heating. The bark of the tree has abortifacient and laxative properties. Khejri is reputed for the treatment of asthma and worm (Kirtikar & Basu, 1984). The purpose of study of this plant was its abundant occurance in nature and there was no analgesic activity on root part of the plant was noticed.



The medicinal importances of this plant encourage for evaluation of analgesic activity on this plant and results proved that it has good analgesic activity.

MATERIALS AND METHODS

Plant Material

The roots of *Prosopis cineraria* (L.) were collected from Shami Vatika near CIMAP, Lucknow (U.P.) in the month of November. Specimen herbarium of the plant was submitted and authenticated by Dr. Tariq Husain from N.B.R.I, Lucknow, Uttar Pradesh, India. (The accession no. for the specimen is 97844.)

Extraction

The roots of *Prosopis cineraria* were washed with distilled water to remove dirt and soil. It was further shade dried and then coarsely powdered by mechanical grinder. This coarse powder was extracted with ethanol using soxhlet apparatus. These extracts were concentrated at reduced temperature and pressure using rotary evaporator. Yields for ethanol extract were 8.59% (w/w).

Animals

Young wistar rat (165-190gm body weight) of either sex were selected. The animals were maintained at room temperature of $25\pm2^{\circ}$ C with relative humidity of $75\pm5\%$ under 12 hours dark and light cycle. The animals maintained under standard husbandry conditions and had free access to diet and water. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animal was devoid of water and food 12 hours before the administration of treatment. The animals were divided into four groups each consist of six animals were fasted overnight prior to the experiments. Two groups were for 2 dose strengths (200 and 300 mg/kg b.w.) of the test drug (Ethanolic extract of root) while one each for Standard drug and control respectively. The ethical committee of the institute approved the protocol of the study.

Drugs and Chemicals

Drugs

Diclofenac Sodium (Voveran) as standard drug purchased from commercial source. Chemicals: Ethanol (RFCL Ltd. India) & Carboxy methyl cellulose as suspending agent were used from Institute laboratory.

Analgesic activity

Two standard methods viz. Tail immersion and Hot plate methods were employed to determine the analgesic activity. (Animal Ethical letter : CPCSEA/ORG/CH/2007; 1113/2515)

Tail immersion method (Vogel & Vogel, 1997)

Young Wistar rats (165-185 gm body weight) of either sex were used. Healthy albino rats of the either sex (160-190 g) were divided into 4 groups of 6 animals each. They were fasted for 18 h prior to the test, with free access to water. Group I received the vehicle (0.8ml of 0.5% CMC suspension, orally, and served as the control group. The groups II, rats were administrated with standard drug Diclofenac sodium at dose 25 mg/kg body weight. Orally, Group III & IV were treated with, ethanolic extract (200 mg/kg & 300 mg/kg b.w.) respectively. All drugs/vehicle were administered orally. They are placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test drug, e.g., after 0.5, 1, 2, 3 and 4 hours. The cut off time of the immersion is 15 seconds. The withdrawal time of untreated animals is between 1 and 5.5 seconds. A withdrawal time of more than 6 second therefore is regarded as a positive response. The analgesic activity data are presented in Table 1.

Table- 1: Analgesic Activity (Tail immersion Method) of ethanolic extract of roots of *Prosopis cineraria* in rats.

	Dose	Mean Reaction Time In Tail Immersion Method mean reaction time (sec.) ± SEM						
Group								
		30 min.	60 min.	120 min.	180 min.	240 min.		
I (Control)	0.5%	2.02	2.71	2.94	2.22	2.21		
	CMC	± 0.29	± 0.12	± 0.07	± 0.62	± 0.05		
II (Standard)	25 mg/kg	4.97 ±0.47	6.43 ± 0.60**	7.30 ± 0.81**	6.37 ± 0.35**	4.43 ± 0.05		
III	200	3.78	3.89	4.05	3.80	3.26		
(P. cineraria)	mg/kg	± 0.05	± 0.03	$\pm 0.04*$	± 0.05	± 0.24		
IV	300	3.24	3.94	4.20	3.90	3.33		
(P. cineraria)	mg/kg	±0.26	± 0.01	$\pm 0.05*$	± 0.12	± 0.23		

Table- 2: Analgesic activity (Hot plate method) of ethanolic extract of roots of *prosopis cineraria* in rats.

		MEAN REACTION TIME IN HOT PLATE METHOD							
		MEAN REACTION TIME (sec.) ± SEM							
GROUP	DOSE	30 min.	60 min.	120 min.	180 min.	240 min.			
I (Control)	0.5% CMC	4.32±0.06	4.54 ± 0.24	$4.52{\pm}0.07$	4.7 ± 0.19	4.59±0.27			
II (Standard)	25mg/kg	4.63±0.75	5.11±0.39	$7.33 {\pm} 0.59$	9.01±0.12*	8.55±0.48			
III (P. cineraria)	200mg/kg	6.23±0.05	9.55±0.28*	10.01±0.04*	9.075±0.24*	7.77±0.28			
IV (P. cineraria)	300mg/kg	7.06±0.14	8.68±0.18*	9.32±0.08*	$6.46{\pm}0.04$	5.07±0.18			

Data are expressed as Mean \pm S.E.M, n = 6 in each group, Statistical analysis done by one way ANOVA followed by Dunnett's test. The percent inhibition for each group was calculated by comparison with the control group. Experimental group were compared with control P value (<0.05). P*<0.05 and P**<0.01, Mean significant difference from control.

Hot plate method

The method described by Awouters *et al* was followed. Healthy albino rats of the either sex (160-190 g) were divided into 4 groups of 6 animals each. They were fasted for 18 h prior to the test, with free access to water. Group I received the vehicle (0.8ml of 0.5% CMC suspension, orally, and served as the control group. The groups II, rats were administrated with standard drug Diclofenac sodium at dose 25 mg/kg body weight. Orally, Group III & IV were treated with, ethanolic extract (200mg/kg & 300mg/kg b.w.) respectively. All drugs/vehicle were administered orally. Rats were kept on a hot plate $(55\pm0.5^{\circ}c)$, the time for forepaw licking or jumping was taken as the reaction time. Rats showing reaction time between 3-5 sec. were selected. Animals not responding in this period were discarded. The reaction time was recorded at 30, 60, 120, 180 & 240 minutes following administration of the test compounds or the standard drug to determine the onset and duration of action. One hour after the administration of vehicle, standard drug and extracts treated rats were individually placed on the hot plate of the analgesiometer maintained at 55°c. Analgesic activity was determined by comparing with the control group. The analgesic activity data are presented in Table 2.

Statistical Analysis

The results are expressed as mean \pm SEM. The Dunnett's test was used to make a statistical comparison between groups. Result with P**<0.01 and P*<0.05 were considered significant.

RESULTS AND DISCUSSION

The analgesic screening was done using Tail immersion test and Hot plate method in rats. The ethanolic extracts were administered orally at the acute dose of 200 and 300 mg /kg b.w. Several activities on these doses have already been reported. Both the dose showed significant ($p^{*}<0.05 \& p^{**}<0.01$) analgesic activity. One important conclusion of this activity was that 200mg/kg b.w. shows better activity than 300mg/kg b.w. Analgesic activity of ethanolic extract of root of *Prosopis cineraria* may be

due to the presence of Alkaloids, Tannins and Steroids. Majority of constituents present in root are Alkaloids and Tannins. The result of this study demonstrated the justified use of roots of *prosopis cineraria* as analgesic traditionally. Thus the plant can be key contributor in home based remedy in the treatment of pain and can play more important role as preventive therapy. These results indicate a significant analgesic activity at both dose levels studied. The analgesic activity shown by *Prosopis cineraria* root extract in various models indicate that the plant extract might possess centrally and peripherally mediated analgesic Properties.

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