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Effect of *Salix tetrasperma Roxburgh* Leaf Extracts on Central Nervous System Activities.

J.H. Virupaksha[1]*, Rama Rao Nadendla[2], M. Sathish Kumar[2] and Kavya S[2].

[1]National College of Pharmacy, Shivamogga, Karnataka, India.
[2]Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India.

ABSTRACT

The present study deals with investigation of leaf extracts of *Salix tetrasperma Roxburgh* was assessed for its CNS activities using neuropharmacological experimental models in mice. These activities are screened for ethanol and aqueous extracts at dose of 200 mg/kg and 400 mg/kg. Locomotor activity was measured by means of actophometer and skeletal muscle relaxant effect was evaluated by using rota rod apparatus. The results of the present study revealed both test extracts exhibited significant (P <0.001) activities in dose dependent manner in locomotor and muscle relaxant activity. From study it can be concluded leaf extracts of Salix tetrasperma Roxburgh possesses wide range of CNS activities.

Keywords: *Salix tetrasperma Roxburgh*, muscle relaxant, rota rod, actophotometer

Corresponding author

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INTRODUCTION

Natural products have been contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost. *Salix tetrasperma Roxburgh* commonly called Indian Willow (Family: Salicaceae) is a medium sized tree of wet and swampy places and growing throughout India. The dried leaves are reported to possess cardiotonic and neurotonic activity [1, 2]. The leaves and bark of the Indian willow tree have been mentioned in ancient texts from Assyria, Sumer and Egypt as a remedy for aches and fever [3]. After an extensive literature search, it was reported that work has been done on the diuretic and laxative [4], hypoglycaemic [5], Anti-inflammatory antioxidant [6, 7], antibacterial activities [8]. Whereas research work on leaf very limited pharmacological work has been reported. So we planned to assess some neuropharmacological activities of Salix *tetrasperma Roxburgh* leaf extracts in experimental rodents.

MATERIALS AND METHODS

Plant Material

The leaves of Salix tetrasperma Roxburgh were collected from the Bommalapura village of Koppa Taluk, Chikmagalur district of Karnataka and were authenticated by Dr. E.Kumara Swamy Udupa, Professor and H.O.D Dept. of Botany S.J.C.B.M. College Sringeri, Karnataka. A voucher specimen (NCP/ 15/2010-11) has been deposited at Pharmacognosy department for further reference.

Extraction

The leaves of *Salix tetrasperma Roxburgh* were dried in shade and powdered was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, chloroform, ethanol as solvent and the marc left was refluxed with aqueous extraction by maceration for 48 hr. All the extracts were vacuum dried to produce petroleum ether (2.4%), ethanol (15%) and Aqueous (2.3%) extracts respectively. The extracts were stored in airtight containers stored in refrigerator further use.

Animals

All the experimental studies were carried out under standard conditions in animal house of National College of Pharmacy, Shivamogga according to Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), and Institutional Animal Ethical Committee (Ref No.NCP/IAEC/CL/05/2012-13). All the animals were procured from Central Animal House National College of Pharmacy Shivamogga.

All the animals were housed in standard polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hr. light and dark cycle for each 24 hr. period at a temperature of approximately 25°C ± 2°C. They were given free access to water and standard feed.

Acute Toxicity Test

Toxicity test studies conducted as per internationally accepted protocol drawn under OECD guidelines. 425. (OECD guidelines. 425 modified, adopted March 23, 2006) in Swiss albino mice.

Phytochemical Analysis

Preliminary phytochemical studies of both the extracts were performed for steroids, glycosides, flavonoids, triterpenoids, tannins and phenolic compounds using standard procedures.[9]

Drugs and Chemicals

The following drugs and chemicals were used. Drugs: Diazepam (East India Pharma, Kolkata) Chemicals: Pet. ether (Spectra chem Ltd, Mumbai,) ethanol (Nice chemicals Ltd, India).

CNS Depressant Activity[10]

Two standard neuropharmacological experimental methods viz. motor coordination and locomotor methods were employed to determine the CNS depressant activity.

Effect on Motor Coordination

Digital rota rod apparatus (INCO - Ambala, India) was used to evaluate the muscle relaxing and sedative effects in the extract and vehicle treated mice. The animals were trained to remain for 3 min on the rod rotating at a speed of 25 rpm. Only animals performing up to the required parameter were included in the test and divided into six groups. Group I served as control and received only vehicle. Group II received reference standard diazepam at a dose of 4 mg/kg i.p. 30 min before the test. Groups III, IV, V and VI were treated oral route with Ethanol, aqueous extracts of *Salix tetrasperma Roxburgh* respectively at a dose of 200 mg/kg and 400 mg/kg respectively. All animals were subsequently assessed for their performance on the rota rod after 30 min. The difference in the fall off time from the rotating rod between the control and treated rats was taken as an index of muscle relaxation.

Effect on Locomotor Activity

The spontaneous locomotor activity was assessed with the help of Actophotometer (INCO - Ambala, India). Each animal was observed for a period of 5 min in a square closed field arena (30 x 30 x 30 cm) equipped with 6 photocells in the outer wall. Interruptions of Photocell beams (locomotor activity) were recorded by means of a 6 digits counter.

To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for 5 minutes. The basal activity score for all the animals was noted. Group I served as control and received only vehicle. Group II received reference standard diazepam at a dose of 4 mg/kg i.p. 30 min before the test. Groups III, IV, V and VI were treated oral route with Ethanol, aqueous extracts of Salix tetrasperma Roxburgh respectively at a dose of 200 mg/kg and 400 mg/kg respectively and 1 hour retesting, activity score for 5 minutes observed. The difference in the activity, before and after drug administration was noted. Percentage decrease in motor activity was calculated.

Statistical Analysis

All results were expressed as the mean ± S.E.M. The results were analysed for obtained from present study were analysed using One Way ANOVA followed by Dunnett's tests. Data were considered statistically significant $P < 0.001$.

RESULTS

Preliminary phytochemical analysis revealed the presence of flavonoids, steroids, tannins, saponins, glycosides and polyphenolic compounds in STEtOH and STAQ extracts. The ST extracts was found to be safe in Swiss albino mice up to the dose of 2000 mg/kg body weight p.o. Hence 1/10 th (200 mg/kg) and 1/5 th (400 mg/kg) of this was selected for this further studies.

In locomotor activity study, it was found that ST extracts significantly ($P<0.001$) depressed the locomotor activity in mice in a dose and time-dependent fashion. The activities increased as time approached to 60 minutes. The results are summarized in Table - 1.

In muscle relaxant study, the ST extracts at both doses significantly ($P<0.001$) and dose dependently decreased the fall off time in mice demonstrating its skeletal muscle relaxant property. The effect was most prominent after 60 minutes of administration. The results are summarized in Table - 2.

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Table 1: Effects ethanolic and aqueous leaf extracts of *Salix tetrasperma Roxb* for locomotor activity using actophotometer.

Groups	Dose mg/kg	Mean motor activity in 10 min		After 60 min treatment	% reduction	
		Before treatment	After 30 min trial	After 60 min	After 30 min	After 60 min.
Control	----	206.66±5.86	206.33±5.71	207.66±2.75	--	--
Diazepam	4mg/kg	206.16±4.90	96.83±3.50	23.51±2.67	53.19	75.72
ST EtoH	200 mg/kg	205.50±5.23	182.17±9.0	113.5±4.11***	11.35	37.69
ST EtoH	400 mg/kg	205.17±4.34	179.15±7.84	64.33±3.37***	12.68	64.10
ST AQ	200 mg/kg	204.50±3.16	181.50±6.03	119.16±1.96***	11.25	34.36
ST AQ	400 mg/kg	206.00±5.71	177.50±7.23*	95.50±7.25***	13.81	46.19

Values are expressed mean ± SEM. * P<0.05, *** P<0.001, (n= 6) when compared to control, statistical analysis done by One Way ANOVA followed by Tukey comparison tests.

Table 2: Effects ethanolic and aqueous leaf extracts of *Salix tetrasperma Roxb* for motor coordination using rota rod.

Groups	Dose mg/kg	Before treatment	10 min treatment trial		% decrease in fall off time	
			30 min	60 min	After 30 min	After 60 min
Control	---	22.25±0.30	17.61±0.64	17.0±0.80	------	-----
Diazepam	4mg/kg	21.51±0.60	5.85±0.32	3.67±0.39	72.80	82.93
ST EtoH	200 mg/kg	21.33±0.57	16.56±1.14	15.03±1.08	22.36	29.53
ST EtoH	400 mg/kg	21.30±1.09	6.96±0.45	4.47±0.34	67.32	79.06
ST AQ	200 mg/kg	20.08±0.79	17.13±0.48	15.96±0.93	14.70	20.51
ST AQ	400 mg/kg	20.97±0.72	11.15±0.50	8.76±0.87	46.82	58.22

DISCUSSION

In this work, most of centrally acting agents influence the locomotor activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property [11]. Locomotor activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as result of reduced excitability of the CNS [12]. The results of the present study showed significant influence in locomotor activity of mice by ST extracts treatment demonstrating decrease in locomotor activity and hence indicating CNS depressant activity in mice. In muscle relaxant activity the ST extract induced decrease in fall off time was due to loss of muscle grip implying skeletal relaxation. Demonstration of marked muscle relaxant effect by rota rod study indicated that ST induced neurological deficit accompanied with taming or calming effect in mice, thereby further supporting its CNS depressant effect [13].

Polyphenolic compounds like flavonoids, tannins and phenolic acids commonly found in higher plants have been reported to possess multiple biological effects [14]. Previous research showed that plants containing flavonoids, saponins, and tannins are useful in many CNS disorders [15]. Many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the CNS, which led to the assumption that they may act as benzodiazepine-like molecules (which act through $GABA_A$ receptor) [16]. In the present study, phytochemical investigations revealed the presence of steroids, glycosides, flavonoids, triterpenoids; tannins and phenolic compounds in the test extracts of *Salix tetrasperma* Roxburgh, therefore presence of these phytocostitutents may be responsible for its CNS depressant activities.

CONCLUSION

From the present preliminary study, it can be concluded that the leaf extracts of *Salix tetrasperma Roxburgh* of possessed promising centrally mediated locomotor and skeletal muscle relaxant effects in the experimental rodent models. The study further revealed that the extracts are devoid of any neurotoxicity and CNS depressant effect. As the present study is based upon the behavioural models without any associated neurochemical estimations, it becomes necessary to carry out the specific binding studies and estimations of

the neurotransmitter levels in the brain to understand the exact mechanism of action and extend these results further.

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