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RESEARCH ARTICLE

INVESTIGATION OF ANTI-INFLAMMATORY AND INVITRO ANTIOXIDANT ACTIVITIES OF HYDROALCOHOLIC EXTRACT OF BARK OF SALIX TETRASPERMA ROXB

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Abstract:
Present study was to investigate antioxidant and anti inflammatory effects of salix tetrasperma Roxb in rats. The Hydroalcoholicic extract of salix tetrasperma Roxb (100 and 200 mg/kg body weight) was studied for anti-inflammatory in animal models. The activity was studied in carragenan induced rats paw edema against indomethacin as standard, and it showed significant anti inflammatory activity. On the basis of the results we conclude that extracts have significant Anti oxidant and Anti inflammatory of salix tetrasperma Roxb. The results supported the traditional use of this plant in some painful and inflammatory conditions.

Keywords: salix tetrasperma, Anti oxidant, Anti inflammatory.

Introduction

Medicinal herbs have been known from millennia and are highly esteemed all over the world as a rich source of therapeutic agents for prevention of diseases. Always medicinal plants are at the main stay for the treatment of ailments in India from ancient time. In this connection there is always need of research and many species of plants have been exploited and are being screened for their medicinal efficacy. Among them Salix species have been exploited for economical, medicinal and aesthetic values. Salix species includes shrubs, distributed in the warmer part of the world[1-3]. For the present investigation Salix Tetrasperma, Roxb, has been selected for evaluation of antioxidants and anti inflammatory properties in view of its wide use in traditional medicine against various common diseases. *Salix tetrasperms* Roxburgh (Family: Salicaceae), commonly called Indian Willow, For the present investigation *Salix Tetrasperma*, Roxb, has been selected for the screening of phytochemical constituents and evaluation of antioxidants, Wound healing and anti inflammatory properties in view of its wide use in traditional medicine against various common diseases.

Earlier workers on this plant have extensively used the aerial parts of the plant, whereas the bark being one of the important parts of the plant where in most of the active constituents are stored has not been subjected for systematic investigation. They are no reports to show the use of bark of *Salix tetrasperma* for the screening of phytochemical analysis and pharmacological investigations. Hence, the bark served as the core material for the investigations carried out in the present study.

MATERIALS AND METHODS

Plant Material:

Salix tetrasperma Roxb were collected from local area and authenticated from department of Pharmacognosy, GCOP, Hyderabad. The bark of the plant were collected, chopped and grounded into coarse powder. The powdered materials were used for extract preparations. The powdered material of bark of S. tetrasperma. Roxb was refluxed successively with the equal quantities of alcohol and water in a soxhlet extractor for 12 hrs[4-6]. The solution so obtained was transferred to china dish and then allowed for drying.

Experimental Animals:

Albino Wistar rats (200-250 g) of either sex were used for the study and were maintained under standard conditions (temperature 22 ± 2oC, relative humidity 50±5% and 12 h light/dark cycle). The experimental protocol was initially approved from the Institutes animal ethics committee and then experimental studies were undergone according to their rules and regulations[7]. The animals were housed under standard environmental conditions and standard pellet diet and water *ad libitum.*

Acute toxicity studies

Animals were starved over night and divided into 5 groups (n=5) .They were fed orally with the bark extracts of *Salix tetrasperma* Roxb in increasing does levels of upto 2000mg/Kg body weight and even at the dose of 2000mg/kg no sign of toxicity observed.

Anti-inflammatory activity

Acute inflammatory condition is produced in the animals by adopting the method of Carrageenan-induced paw edema inflammation[8-9]. The animals were randomly divided into four groups of six animals each. Group I received only vehicle i.e., saline. Group II served as positive control and was treated with indomethacin (5 mg/kg i.p). Groups III & IV were treated with different concentrations of hydroalcoholic extract of Bark of Salix tetrasperma (Roxb). Two hours after the administration of extracts, paw inflammation was induced by injecting 0.1 ml of 1% w/v carrageenan in 0.9% sodium chloride into a plantar surface of the right hind paw. Paw volumes were measured using a Plethysmometer at different time intervals of 0, 3 and 6 h. the reduction in the paw volume was calculated[10-12].

The percentage inhibition of edema was calculated using the following formula:

% Inhibition of Edema = [1 – (Vt/Vc)] x 100

Where, Vt is edema volume of the drug treated group and Vc is the edema volume of the control group.

Antioxidant Activity

Free scavenging activity was measured by a decrease in absorbance at 516 nm of a methonolic solution of colored DPPH. A stock solution of DPPH (1.3 mg/ml in methonolic) was prepared such that 75 µl of it in 3 ml methonolic gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract at different concentrations was noted after 15 min. EC50 (i.e. the concentration of the test solution required to give a 50% decrease in the absorbance compared to that of blank solution) was calculated from percent inhibition[13]. A blank reading was obtained using methonolic instead of the extract. Ascorbic acid was used as standard[13].

The percentage inhibition of antiradical activity was calculated using the formula,

%inhibition = [Absorbance of blank – Absorbance of test sample/ Absorbance of blank] x 100

Statistical analysis:

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance was carried out[15].

Results & Discussion:

The evaluation of anti-oxidant and anti-inflammatory activity of Salix tetrasperma (Roxb) has yielded positive results. This plant has been widely reported to have several medicinal properties in traditional form of medicine mainly, anti pyretic, anti-inflammatory and anti rheumatic properties[16]. In anti-inflammatory activity study, in acute inflammation model, the carrageenan induced paw edema was significantly reduced in the animals pretreated with hydroalcoholic extract of bark of *S. tetrasperma* which was in a dose and time- dependent manner. The percentage inhibition of paw edema volume was found to be significant ($p < 0.05$) when compared to control group of animals (Table-1). In the *in* vitro antioxidant studies hydroalcoholic extract exhibited potent antioxidant activity with lower EC50 values in DPPH, Superoxide anion inhibition assays (Fig. 1).

CONCLUSION

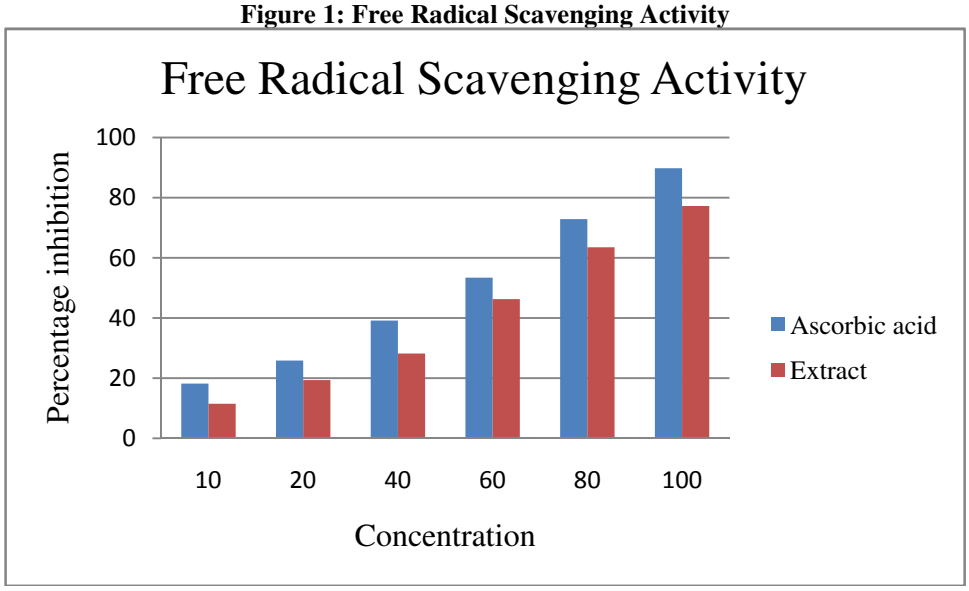
The present studies provide the scientific evidence for the presence of several beneficial medicinal properties in the plant material *Salix tetrasperma* Roxb. The hydroalcoholic extract was also found to possess *in vivo* healing activity in two wound models which are supported by biochemical & wound photographic image observations and also shown to possess *in vitro* antioxidant property. Thus, the studies carried out provide a supportive scientific evidence for the medicinal use of *Salix* tetrasperma, Roxb. against various diseases, thereby justifying its use in the Indian traditional system of medicine.

Table 1: Carrageenan induced Paw edema- Salix tetrasperma (Roxb)

GROUPS	Change in paw volume (ml) mean±SEM & % inhibition		
	1 hr	3 hr	6 hr
control	0.49±0.02	0.54±0.02	0.62±0.03
Indomethacin	0.19±0.01 (61.22)	0.17±0.02 (68.51)	0.18±0.02 (70.96)
Extract 100 mg/kg	0.25±0.02 (48.97)	0.31±0.02 (42.59)	0.24±0.01 (61.29)
Extract 200 mg/kg	0.29±0.01 (40.81)	0.28±0.01 (48.18)	0.29±0.01 (53.22)

All values are mean ±SEM (n=6); *p< 0.05 when compared to control.

Figure 1: Free Radical Scavenging Activity



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