Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTINOCICEPTIVE AND ANTIPYRETIC ACTIVITIES OF *SALIX TETRASPERMA ROXBURG*

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ABSTRACT

The present study was designed to evaluate antinociceptive and antipyretic activities of leaf extracts of *Salix tetrasperma Roxburgh* in rodents. The ethanolic and aqueous leaf extracts were tested for antinociceptive and antipyretic activities. Antinociceptive activity was evaluated using thremoceptive models namely eddy's Hot plate and tail flick by analgesiometer. The antipyretic activity was evaluated using brewer's yeast induced method. Retreatment with ethanolic and aqueous extracts of *Salix tetrasperma* leaves at 200 and 400 mg/kg showed significant dose dependent antinociceptive activity in the tested models. Both the extracts lowered the elevated rectal temperature in the brewer's yeast induced pyretic model in a dose dependent manner. The findings in this study suggest that the ethanolic and aqueous leaf extracts of *Salix tetrasperma* possesses antinociceptive and antipyretic activity. Thus it provides a scientific evidence for its traditional claim.

KEY WORDS: **Antinociceptive, antipyretic, Eddy's Hot plate, Analgesiometer, Salix *tetrasperma*.**

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INTRODUCTION

In spite of the advancement in medical research for the past decades, the treatment of many serious diseases is still challenging. Today the plant-based drugs continue to play an essential role in health care. It has been estimated by the World Health Organization that 80% of the population of the world rely mainly on traditional medicines for their primary health care.[1] Currently, at least 119 chemicals, derived from 90 plant species, can be considered as drugs with therapeutic potency in one or more countries.[2]Presently, drugs which are available for the management of pain are either narcotics analgesics (e.g.: opioids), NSAIDs (e.g. salicylates) and corticosteroids (e.g. hydrocortisone). These synthetic drugs are expensive and also possess serious side effects. Non-steroidal anti-inflammatory drugs (NSAID) causes gastric lesions and opiates induce tolerance and dependence, the use of these drugs as analgesic agents have not been successful in all cases.[3, 4]Since time immemorial people have been using plants to protect themselves against several diseases and also to improve health and longevity. Plant and phytomedicine symbolizes safety and are serving several purposes whether health, protection from disease or nutrition. Therefore, attention is being focused on the investigation of traditionally used drugs of plant origin.[5]*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 cardio tonic and neurotonic activity. [6, 7] The leaves and bark of the willow tree have been mentioned in ancient texts from Assyria, Sumer and Egypt as a remedy for aches and fever. [8] The paste of both leaf and root is applied externally in scorpion stings, bug bites, for sores and warts; the decoction of the dried root is taken orally for the treatment of hepatitis. [9]After an extensive literature search, it was noted that a lot of work has been done on the diuretic and laxative, [10] hypoglycaemic[11], Anti-inflammatory antioxidant, [12, 13] antibacterial[14]activities whereas, research work on its leaves is scarcely available. We therefore, planned to explore the presence of antinociceptive and antipyretic activity in the leaf extracts. Hence, the present study aims to investigate the potential benefit of *Salix tetrasperma Roxburgh* by thermoceptive and antipyretic screening models.

MATERIAL AND METHODS

Drugs: Pentazocine Inj. (Fortwin Ranbaxy Pharmaceuticals Ltd.), Ethanol (NICE chemicals), Brewer's yeast (Loba Chem, Mumbai), Diclogesic (Torrent Pharma).

Instruments: Eddy's hot plate (INCO, Ambala, India), Analgesiometer (INCO, Ambala, India) and Digital thermometer.

Plant Material: The leaves of *Salix tetrasperma Roxburgh* were collected from the Bommalapura village of Koppa taluk, Chikmagaluru 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 (No. NCP/15/2010-11) has been deposited at herbarium of pharmacognosy department for further reference. Leaves was dried under shade, coarsely powdered and stored in airtight container for further use.

Preparation of extract: The leaves of *Salix tetrasperma* were dried in shade and powdered. Dried powder (100 g) was subjected to successive extraction in soxhlet extractor as per standard procedure using petroleum ether (40-60^{o}C) and ethanol at their boiling point for 48hr.The excess of ethanol was distilled under pressure using rotary flash evaporator. The marc obtained from the ethanol extraction utilized for aqueous extraction, by maceration for 48 hr. During maceration period, few drops of chloroform were added to avoid fungal growth. The extract was filtered, concentrated and the solid marc was weighed and percentage yield was calculated. The percentage yields of petroleum ether extract of *Salix tetrasperma* was 0.6 g, ethanolic extract was 5.1 g and aqueous extract was 2.3g and the extracts were stored in airtight containers and kept in refrigerator till use. Both the extracts were subjected to phytochemical and pharmacological screening[16].

Animals: Wister rats (150-200 gm.) and Swiss albino mice (20-25 gm.) of either sex, obtained from Central Animal House, National College of Pharmacy, Shivamogga. Animals were housed at standard conditions of temperature (22± 1º C) and 12/12 hr. light/dark cycle. They were fed with standard food pellets and water *ad libitum*. Six animals are used in each group. Permission for conducting of these experiments was obtained from Institutional Animal Ethical committee (IAEC) with Ethical clearance (NCP/IAEC/CL/05/2012-13).

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.[15]

ASSESSMENT OF ANTINOCICEPTIVE ACTIVITY [16]

Eddy's Hot Plate Method

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1^{o}C. Responses such as jumping, withdrawal and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal/normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the

animals were then noted at 30, 60, 90 and 120 minutes interval after drug administration.

TAIL FLICK TEST BY ANALGESIOMETER[17]

Analgesic activity was assessed by tail flick model using Analgesiometer. The instrument has a nichrome wire, which would be heated to the required temperature (55ºC) and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. A cut off time of 15 sec was planned to avoid any tissue damage in the animal. The ethanolic and aqueous leaf extracts of *Salix tetrasperma* were made suspended in Tween 80(1% v/v) treated for 7 days and on 7th day standard drug diclofenac sodium in 10 mg/kg is given 1 hr before study. The reaction time for each group was measured at 30, 60, 90 and 120 minutes using analgesiometer.

ANTIPYRETIC ACTIVITY [16]

Brewer's yeast induced pyrexia is a standard and well established model for screening of anti-pyretic activity of any investigational product. Anti-pyretic activity on albino rats was studied with fever induced by 20% w/v of brewer's yeast. Healthy wistar strain albino rats weighing about 150-200gms were taken. They were fasted overnight with water *ad libitum* before inducing pyrexia. The animals were allowed to quite in the cage for some time and after that their basal rectal temperature was measured by using clinical thermometer by insertion of thermometer to a depth of one inch into the rectum. After taking temperature, pyrexia was induced by injecting subcutaneously 20% w/v suspension of Brewer's yeast in 0.9% saline solution at a dose of 20 ml/kg body weight. After 18 hr of brewer's yeast injection the rise in rectal temperature was recorded. Only rats which were shown a rise in temperature with an increase of at least 0.6^0 C (or 1^0 F) was used for further experiment. The animals were divided in to six groups each groups contain six animals in each. The different groups were treated with ethanolic and aqueous extracts (200 and 400 mg/kg), and standard drug, Paracetamol (100 mg/kg, p.o.). Tween 80 (1% v/v) was used as suspending agent. The rectal temperature was then recorded over a period of 4 hr.

Statistical analysis: The data were expressed as mean standard error mean (S.E.M). The data were analysed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by multiple comparisons by Dennett's tests, p values less than 0.05 were considered as significance.

Table 1

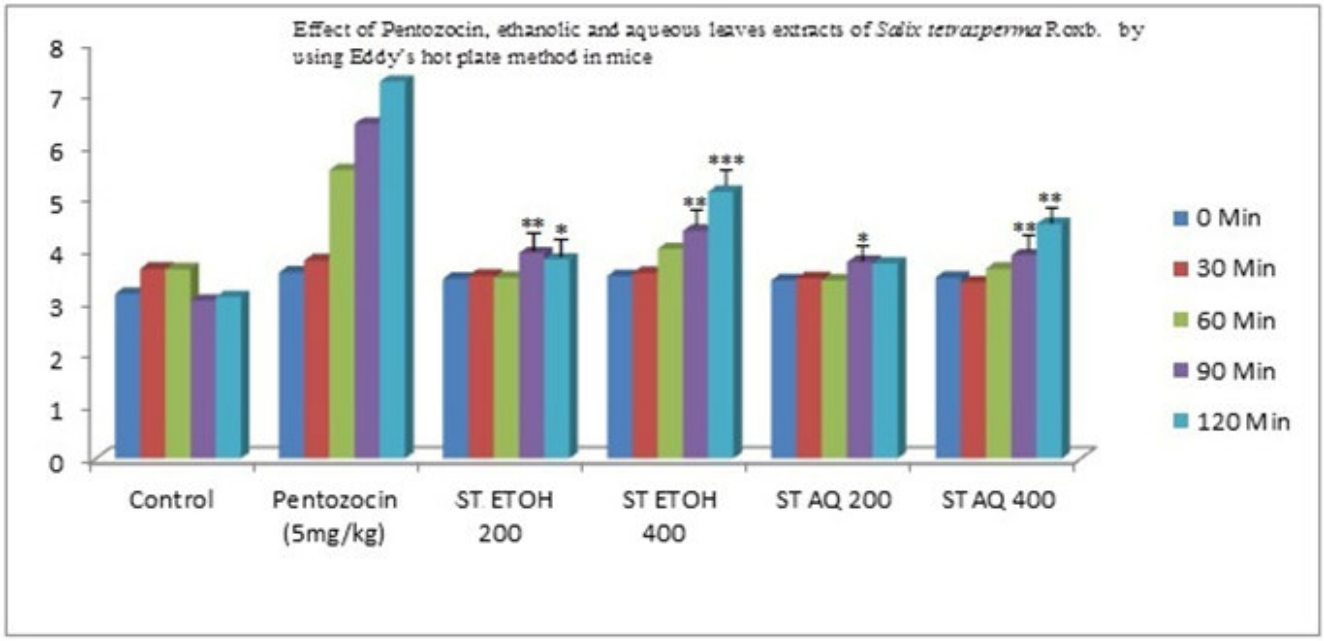
Effects ethanolic and aqueous leaf extracts of Salix tetrasperma Roxb on basal reaction of rats using Eddy's hot Plate method

		Basal reaction time in Seconds				
S.No	Treatment	0 Min	30 Min	60 Min	90 Min	120 Min
1	Control	3.17±0.03	3.65±0.06	3.64±0.03	3.04±0.07	3.11±0.04
2	Pentozocin (5mg/kg)	3.58±0.03	3.81±0.56	5.55±0.12	6.44±0.14	7.25±0.07
3	ST EtOH 200	3.46±0.06*	3.52±0.45	3.48±0.09	3.95±0.15**	3.83±0.17*
4	ST EtOH 400	3.51±0.70	3.57±0.04	4.03±0.06	4.38±0.01**	5.13±0.12***
5	ST AQ 200	3.43±0.08	3.47±0.58	3.43±0.08	3.78±0.17*	3.75±0.15
6	ST AQ 400	3.48±0.01	3.39±0.12	3.65±0.11	3.91±0.19**	4.51±0.23***

Values are mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001, n= 6 animals in each group; when compared to control

Fig 1

Effects ethanolic and aqueous leaf extracts of Salix tetrasperma Roxb on basal reaction of rats using Eddy's hot Plate method.



Values are mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001, n= 6 animals in each group; when compared to control

Table 2

Effects of ethanolic and aqueous leaf extracts of Salix tetrasperma Roxb on tail flick in rats using Analgesoimeter.

Time	0 min	15 min	30 min	45 min	60 min	120 min
Control	3.25±0.14	3.5±0.12	3.86±0.17	3.69±0.20	3.46±0.10	3.53±0.20
Diclofenac Sodium 10mg/kg	3.51±0.18	5.8±0.32	5.23±0.30	10.36±0.29	11.6±0.51	12.71±0.34
STEtOH 200	3.34±0.29	4.86±0.34*	4.96±0.17*	5.58±0.15	5.36±0.24**	5.95±0.58*
STEtOH 400	3.08±0.27	5.25±0.25*	5.2±0.26**	9.43±0.48***	11.27±0.37***	11.31±0.68***
ST AQ 200	3.27±0.15	4.16±0.42	4.65±0.18	5.55±0.29**	5.06±0.19*	5.76±0.32*
ST AQ 400	3.06±0.15	4.80±0.21*	4.88±0.19*	9.12±0.22***	7.45±0.31***	7.2±0.49**

Values are mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001, n= 6 animals in each group; when compared to control

Table 3

Effects of ethanolic and aqueous leaf extracts of Salix tetrasperma Roxb against Brewer's yeast induced in wistar rats

Treatment	Initial rectal temperature 0 ^{0} C	Rectal temp at 18 hours after yeast induction	Rectal temperature (^{0} C) after treatment with extracts			
			1 hr	2 hrs	3 hrs	4 hrs
Control	37.07±0.25	39.25±0.22	39.21±0.15	39.20±0.15	39.18±0.15	39.16±0.16
Paracetamol 100 mg/kg	37.10±0.18	39.27±0.18	37.18±0.44	37.30±0.36	37.03±0.50	37.01±0.50
ST EtOH 200	37.10±0.29	39.20±0.14	37.76±0.62	37.96±0.46	37.42±0.44	37.30±0.47*
ST EtOH 400	37.08±0.25	39.28±0.13	37.05±0.29*	37.16±0.23**	36.71±0.26**	36.63±0.27**
ST AQ 200	37.08±0.17	39.35±0.33	37.56±0.53	37.51±0.55	37.42±0.55	37.30±0.56*
ST AQ 400	37.10±0.26	39.30±0.28	37.25±0.58	37.41±0.47**	36.78±0.48**	36.62±0.50**

Values are expressed as mean ± S.E.M., n=6 values are statically significant at *p< 0.05, **p<0.01.

RESULTS

Acute toxicity studies: The ethanolic and aqueous leaf extracts of *Salix tetrasperma Roxburgh* was found safe at 2000 mg/kg. Hence 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this were selected for this further study.

Phytochemical screening: The phytochemical examination of the extracts found the presence of flavonoids, tannins, triterpenes, phenolic compounds, saponins, steroids and sterols.

Results of ST on Eddy's hot plate**:** The result of hot plate indicated that the ethanolic and aqueous extracts of *Salix tetrasperma Roxburgh* shows a significant increase in reaction time at 90 and 120 min in a dose dependent manner, whereas the ST AQ 200 did not shown any antinociceptive activity except at 90 min. Pentozocin showed appreciable antinocicetpive activity throughout the study period. The results are shown in table 1 and Fig 1**.**

Results of ST on tail flick method: The result showed highest significant (p<0.001) increase in latency time in ST EtOH 400 mg/kg and ST AQ 400 mg/kg treated group at 45, 60, 120 min . But ST EtoH and ST AQ at the dose of 200 mg/kg exhibited weak antinociceptive

activity in dose dependent manner. These effects were long lasting, whereas standard drug diclofenac sodium 10 mg/ kg showed most significant activity entire study. The results are given in Fig 2. Results ST on yeast induced pyrexia: Subcutaneous injection of yeast suspension significantly increased the rectal temperature of rats compared to the initial rectal temperature. Administration of ethanolic aqueous extract of *Salix tetrasperma Roxburgh* at 400/kg significantly ($P<0.01$) lowered the yeast induced elevated rectal temperature, this antipyretic effect was maintained from the 2nd to the 4th hr and this effect was comparable to the Paracetamol. The results are shown in Table 3.

DISCUSSIONS

A drug with anti-inflammatory activity usually exhibit antipyretic and analgesic properties. The best examples would be the nonsteroidal anti-inflammatory drugs, which possess all three activities.[18] Inflammation is a defensive reaction of the local microcirculation to tissue injury arising from cell damages due to mechanical trauma, chemical, physical and thermal injury, antigen antibody reactions and infections. The signs and symptoms of inflammation include redness, swelling, heat, pain and loss of function of the affected area. Pain is an unpleasant sensation that is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects. It is therefore, inevitable to search for new less toxic and more effective anti-inflammatory and analgesic agents.[19] Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection or other disease states. Antipyretics are agents which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and drugs like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins which set the thermoregulation centre at a lower temperature.[20]Flavonoids are often used for their antioxidant effect against free radicals. There are also strong indications that they have antiviral, anti-inflammatory and antihypertensive properties.[21]we propose that the antinociceptive and antipyretic activity of the *Salix tetrasperma Roxburgh* could be due to combined effect of flavonoids, saponins, steroids and triterpenoids, which are the major components of the test extracts of this plant. The results clearly indicate that the leaf extracts of *Salix tetrasperma Roxburgh* possess antinociceptive and antipyretic activity.

CONCLUSION

The results of the present study support the folklore use of this plant in pain. Our results provide justification and support for the use of leaf extract of *Salix tetrasperma Roxburgh* as traditional medicine against antinociceptive and antipyretic activity. However, purification of plant extracts and further studies may reveal mechanisms and constituents responsible for activities.

ACKNOWLEDGEMENT

The authors are thankful to the management of National Education Society ® Shivamogga (Karnataka), for providing the necessary facilities through Principal, National College of Pharmacy, Shivamogga.

REFERENCES

1. Farnsworth NR, Akerele O and Bingel AS. Medicinal plants in therapy. Bulletin of the World Health Organization. 1985; 63(6): 965–981.
2. Arvigo R and Balick M. Rainforest Remedies, One Hundred Healing Herbs of Belzie. Lotus Press Twin Lakes. 1993.
3. Dharmasiri JR, Jayakody AC, Galhena G, Liyanage SSP, Ratnasooriya WD. Anti-inflammatory and analgesic activities of fresh mature leaves of *Vitex negundo*. J.Ethnopharmacol. 2003; 87:199-206.
4. Saikath Sen, Raja Chakraborty, Biplab De, Joy deep Mazumder. Plants and phytochemicals for Peptic ulcer: An overview. Pharmacog.Rev. 2009; 3:270-279.
5. Ahmad F, Khan RA, Rasheed. Study of analgesic and anti-inflammatory from plant extracts of *Lactuca scariola* and *Artemisia absinthium.* Islam J. Acad. Sci.1992; 5: 111-114.
6. Bhakuni DS, Dhar MM, Dhavan BN, Gupta B, Srimali RC. Screening of Indian plants for biological activity. Indian Exp Biol. 1971; 9:91.
7. Kamboj VP, Setty B Khanna VM, Semen coagulation – a potential approach to Contraception. Contraception 1977;15: 601-610.
8. Gupta ML, GuptaTK, Bhargava KP. A Study on antifertility effects of some indigenous drugs. J Res Indian Med.1971; 6:112-116.
9. Valsarj R, Pusshpangadan P, Smitt UW, Andersen A, Nyman U. Antimicrobial screening of Selected medicinal plants from India. J Ethanopharmacol.1997; 58(2):75-83.
10. Modal Sumanta, Hechhu Ramana, Suresh P and Chhetree Rishi Raj. Studies on diuretic and laxative activity of the *Salix tetrasperma Roxburgh*. Int. Res. Journal of Pharmacy. 2010; 1(1):145-149.
11. Chhetree RR, Dash GK, Modal S and Parhi R. Studies on the hypoglycaemic activity of the bark of *Salix tetrasperma Roxburgh.* Int.J.Drug.Dev. & Res.2010; 2(4):799-805.
12. Assem El-Shazly, Afaf El-Sayed and Eman Fikrey. Bioactive Secondary metabolites from

Salix tetrasperma Roxb. Z.Naturforsch.2012; 67C: 353-59.

13. Eman A, El-Wakil, El-Sayed S, Abdel-Hameed, Mortada M, El-Sayed and Ezzat Abdel- Lateef. Identification of the chemical composition of the methanolic extracts of *Salix tetrasperma Roxb* using LC-MS and evaluation of its potential as antioxidant agent. Der Pharma Chemica. 2015; 7(2):168-177.
14. Saiful Islam M, Ronok Zahan. et al. Antibacterial, Insectidal and in vivo cytotoxic activities of *Salix tetrasperma* Roxb. Int. J. Pharm Sc. and Res.2011; 2(8):2103-2108.
15. Dr Khandelwal KR. Practical Pharmacognosy Techniues and experiments.In: Dr.Khandelwal KR .Preliminary phytochemical screeinig.19 th ed.Pune:Nirali Prakashan; 2008. p. 149-56.
16. Gupta MK et al. Evaluation of Analgesic, anti-inflammatory and antipyretic Potential of *Parkinsonia aculeata* Linn. Leaves. IJRPS; 2011; 1(1):100-109.
17. Rahul Chulet, Mahesh Jhajharia, Pankaj Pradhan, Sarwan Sharma. Analgesic and antipyretic activity of *Albizzia Lebbeck*. Pharmacologyonline.2010; 3: 737-749.
18. Swain SR, Sinha BN, Murthy PN. Comparative evaluation of Antipyretic and analgesic activity of *Rungia repens* Needs and *Rungia pectinata* L. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4(2):103-106.
19. Salem TS, Basha SD, Mahesh Rami PV, Kumar NS. Analgesic, anti-pyretic and anti-inflammatory of Dietary Sesame Oil in Experimental animal models. Pharmacologia. 2011; 2(6):172-177.
20. Hullatti KK, Sharada MS. Comparative Antipyretic activity of Patha: An Ayurvedic drug. Pharmacognosy magazine. 2007; 11(3):173-175.
21. Ibrahim B, Sowemimo A, Rooyen A, Venter M. Anti-inflammatory, analgesic and antioxidant activities of *Cyathula prostrate* (Linn.) Blume (Amaranthaceae), J Ethnopharmacol, 2012; 14(1): 282-289.