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**IN VITRO ANTI INFLAMMATORY EVALUATION OF HYDROALCOHALIC LEAVES
EXTRACT OF *PINUS ROXBURGHII* BY HRBC METHOD**

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ABSTRACT

Pinus Roxiburghii (family: Pineceae) also known as Chir-Pine. The present work aims at evaluating the anti-inflammatory activity of *Pinus Roxiburghii* by HRBC membrane stabilization. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. The anti-inflammatory activity of the hydroalcoholic extract, Hexane extract, Chloroform, ether extract and Mixture extract of leaves part of *Pinusroxiburghii* were compared to that of the standard drug Diclofenac. The percentage protection of lysis for Standard Diclofenac 50 µg is 67.27%, Standard Diclofenac 100 µg is 61.64%, hydroalcoholic extract 100µg is 68.55%, hydroalcoholic extract 200µg is 65.64%, hexane extract 100µg is 75.46%, Hexane extract 200µg is 78.37%, chloroform extract 100 µg is 78.73% and chloroform extract 200µg is 81.46%. The hydroalcoholic extract of *Pinus Roxiburgii* significant anti-inflammatory activity in comparison to chloroform extract and hexane extract of the same plant and with standard drug Diclofenac.

KEYWORDS

Anti-inflammatory, *Pinus roxiburghii* and HRBC membrane.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells^{1, 2}. Although it is a defense mechanism that helps body to protect it-self against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases³. Drugs that are currently used for the management of pain are opioids or

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nonopioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids⁴. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapies⁵. Opioids are the commonly used drugs for the management of acute postoperative pain⁶. *Pinus roxburghii* (Family: Pinaceae) commonly known as Chir Pine. The Chir Pine, *Pinus roxburghii*, named after William Roxburgh, is a pine native to the Himalaya. *Pinus roxburghii* Sarg. (Pinaceae) is traditionally used for several medicinal purposes in India⁷⁻⁹. The plant is extensively used in number of herbal preparation for curing various disorders, the present study was undertaken to assess analgesic and anti-inflammatory activities of its leaves extract¹⁰⁻¹².

MATERIAL AND METHOD

Collection of drug

Fresh leaves of plant *Pinus roxburghii* was collected from the local forest of Dehradun, Uttarakhand and authenticated by Forest research Institute (FRI), Dehradun.

Preparation of extract

The collected plants were immediately shade dried and then powdered. The powdered plant material extracted through following solvents as chloroform, hydroalcoholic, hexane, ether and mixture (chloroform, methanol and water) at room temperature. The extract was pooled, filtered through a whatmann filter paper and the solvent was removed on a vacuum under reduced pressure to get the dried extract. The dried extract was stored.

In vitro Anti-inflammatory activity

HRBC method was used for the estimation of anti-inflammatory activity *in vitro*¹³. Blood was collected from healthy volunteers and was mixed with equal

volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37 or 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection= 100- (OD sample/ OD control) x 100

RESULTS AND DISCUSSION

The ash value of powder of the leaves of *Pinus roxburghii* was calculated and the total ash, acid insoluble ash, acid soluble ash, water soluble ash, water insoluble ash was found out to be 11.6%, 8.62%, 91.37%, 63.79%, 36.20% respectively^{14, 15}. The extractive value of powder of the leaves of *Pinus roxburghii* was calculated and the extract of Hydroalcoholic, Chloroform, Ether, hexane, mixture (chloroform, methanol, water [86:14:1] was found out to be 22%, 9%, 7%, 4%, 4% respectively. The moisture content was also calculated and the percentage of loss of drying was found out to be 2.6%.

The HRBC Membrane stabilization method was used for the in-vitro anti-inflammatory activity of the hydroalcoholic extract of powdered leaves of *Pinus roxburghii*. The HRBC Membrane stabilization activity of the extract at concentration 200 µg/ml showed 65.64±1.24% inhibition of denaturation in hypotonic solution as the comparison of the standard Diclofenac 50µg/ml showed 67.27±0.98% inhibition of denaturation.

Table No.1: In vitro anti-Inflammatory activity of *Pinus Roxburghii* by HRBC Membrane Stabilization Method

S.No	Type of extract	Concentration (µg/ml)	Absorbance	% Inhibition of denaturation
1	Control	----	0.55±0.23	---
2	Hydro alcohol (Test)	100	0.173±0.31	68.55±1.05
3	Hydro alcohol (Test)	200	0.189±0.26	65.64±1.24
4	Chloroform	100	0.117±0.12	78.73±1.12
5	Chloroform	200	0.102±0.21	81.46±1.16
6	Ether	100	0.126±0.18	77.1±1.17
7	Ether	200	0.115±0.19	79.1±1.09
8	Hexane	100	0.135±0.14	75.46±1.05
9	Hexane	200	0.119±0.20	78.37±1.12
10	Mixture	100	0.123±0.22	77.64±1.09
11	Mixture	200	0.111±0.28	79.82±1.06
12	Diclofenac	50	0.180±0.32	67.27±0.98
13	Diclofenac	100	0.211±1.20	61.64±1.79

CONCLUSION

Hydro alcoholic extract of *Pinus roxiburghii* leaves exhibited membrane effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane^{16, 17}. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage from the above study it was concluded that the Hydro alcoholic extract of *Pinus roxiburghii* has significant membrane stabilization property¹⁸.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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