

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

Anti –Nociceptive Activity of Methanolic Extract of Pinus Roxburghii in Male Wistar Rats

Dr.Renuka Chandresh¹, Dr.Ashawani Upadhayaya², Dr.Dinesh³, Dr.Savita Katwal⁴

Ayurvedic Medical officer HP Government, MD Dravyaguna, India¹

Proffesor & HOD, PG Deptt. of Dravya Guna, RGGPG Ayurvedic College Paprola Distt.Kangra (H.P.), India²

Associate Proff., Department of Pharmacology, COVAS Palampur, India³

Lecturer, Rog Nidan Evum Vikriti Vigyan Deptt., SKSS Ayurvedic College Sarabha, India⁴

ABSTRACT: In the present study anti-nociceptive activity of methanolic extract of Saral (Pinus roxburghii) heart wood powder is evaluated in male wistar rats .Anti-nociceptive activity was evaluated by Hot plate, Tail immersion & Acetic acid induced writhing. Methanolic extract of Pinus roxburghii was administered orally in all these tests. Extract when given to the rats showed significant anti-nociceptive activity. The effect was somewhat similar to that of Aspirin.The result indicated anti-nociceptive activity of Pinu roxburghii heart wood powder and support ethnomedical use of this plant in different painful conditions.

KEYWORDS: Hot plate test, Tail immersion tests, Acetic acid induced writhing

I. INTRODUCTION

Pinus roxburghii is an important medicinal plant which is cultivated in various parts of the world for its medicinal value.¹ It contains flavonoids, Alkaloids and some essential amino acids .Various research activities were reported on P.roxburghii such as anti-inflammatory,analgesic,wound healing,spasmolytic,antimicrobial,anticancerous and hepatoprotective .²

II. MATERIALS AND METHODS

Drugs

Heart wood of P.roxburghii were collected from the local area of Paprola in March 2016.The authenticity of this samples was confirmed at DTL Jogindernagar .Heart root was washed ,shade dried ,pulverized to prepare powder ,sieved through #80 mesh ,and preserved in an air –tight glass bottle.

Mehod of preparation of ethanolic extract

An accurate weighed 20 g heart wood of P.roxburghii was macerated in 500 m of methanol procured from research aid Palampur, in a conical flask for 24 hr, shaken frequently during the first 6h and then allowed to stand for 18 hr. Taking precaution against the loss of solvent, it was filtered and filtrate was evaporated to a dried form.

Animals

Wistar albino rats (Rattus norvegicus) male weighing between 200± were taken .The animals were obtained from the animal house of IIIM jammu .The rats were exposed to natural day and night cycles under ideal ambient laboratory conditions .The experimental protocols were approved by the Institutional Animal Ethics Committee) in accordance with the guidelines formulated by CPCSEA ,India.

Dose calculation

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

The human therapeutic dose mentioned in Ayurvedic Pharmacopoeia of India for the powder is 3-6 gm. The dose for experimental study was calculated by extrapolating the human dose based on the body surface area ratio using the table of Paget and Barnes. The dose of extract of *P. roxburghii* was fixed on the basis of results of acute toxicity study and efficacy studies reported by previous authors.

III. ANALGESIC ACTIVITY

HOT TEST PLATE METHOD

Wistar rats weighing between 200 $\pm$ 5 g were divided into five groups containing five rats in each group. Group I was control and was administered distilled water. 1ml dose, second group was given aspirin at the dose of 300mg/kg BW, third group was given Pinus extract at dose of 100 mg/kg BW, Fourth group was given Pinus extract at dose of 300 mg/kg BW, Fifth group was given Pinus extract at the dose of 500 mg/kg BW. Rats were pre-selected on the hot plate apparatus at 55°C. Animals showing a reaction time (defined as the latency for licking the hind feet or jumping) greater than 20 seconds was discarded. Selected rats were pre-treated orally through gavaging with distilled water 1 ml, Pinus extract (100,300,500 mg/kg), Aspirin (300mg/kg). Each animal was placed on the heated surface of the plate maintained at 55°C and the latency to a discomfort reaction (licking of the paws or jumping) was recorded at 30, 60, 90 and 120 min after the administration of the distilled, extract and Aspirin. A cut-off time of 20 s was chosen to indicate complete analgesia and to avoid tissue injury. The latencies for paw licking or jumping were chosen to indicate complete analgesia and to avoid tissue injury. The latencies for paw licking or jumping were recorded for each animal.

ACETIC ACID INDUCED WRITHING SYNDROME

The method employed was that of Koster (1959), which is a modification of phenylquinone writhing syndrome of Siegmund et al (1957), as described by Witkin et al (1961). The dose of the acetic acid (0.9% v/v) used was 1 ml per 100 gm body weight. Acetic acid in aqueous solution in the above mentioned dose produces a writhing syndrome characterized by intermittent contraction of the abdomen, twitching and turning of the trunk and extension of hind limbs, beginning 3-10 minutes after the injection of acetic acid and persisting for 3-4 hours. Only those mice who exhibited this syndrome were used. 30 Wistar rats were taken and grouped into five groups after weighing carefully. All the rats were given acetic-acid in aqueous solution by intra-peritoneal route at a dose of 1ml per 100 gm body weight, 45 minutes after test drug administration.

TAIL IMMERSION TEST

The procedure is based on the observation that aspirin-like drugs are selectively capable of prolonging the reaction time of typical tail withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55°C.

The lower 5 cm portion of the tail is marked. This part of the tail is immersed in to the water bath of exactly 55°C. Within a few seconds the rats reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by stopwatch. After each determination the tail is dried carefully. The reaction time is determined before and periodically after oral administration of the test and standard substance. The cut off time is 15 sec. Wistar male rats were divided into 5 groups with 6 animals in each group. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 min, the observations were recorded and at the time interval of 90,120 min. The results of tail immersion test in rats were tabulated in following table. Selected rats were pre-treated with distilled water; Pinus extract (100,300,500 mg/kg, Aspirin (300mg/kg BW)

IV. STATISTICAL ANALYSIS

The obtained data have been presented as mean $\pm$ standard error of mean difference between the groups, statistically determined by ANOVA followed by Dunnett t-test to assess the statistical significance between the groups. The value $P < 0.005$ is considered as statistically significant.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

V. RESULT

The test drug was evaluated for analgesic activity by noting their effect by three tests:

- 1) Acetic acid induced writhing.
- 2) Hot plate test.
- 3) Tail immersion test.

In Acetic induced writhing the test drug was evaluated for analgesic activity by noting their effect on rats. Expression of the writhing syndrome with acetic acid is reported to be dependent on the continuous formation and release of phlogistic mediators especially Prostaglandin E ; thus it is considered to be an ideal model for assessing peripheral analgesic agents like non-steroidal anti-inflammatory agents.

In the nervous system pain is aroused by stimulation of the peripheral pain receptors (end organs) and the impulse thus produced is carried along the sensory nerves, via spinothalamic tracts of the spinal cord, to the lateral nucleus of the thalamus, where integration and awareness of pain occurs. The pain sensation is finally perceived in the sensory areas of the cerebral cortex, which is necessary for localization and evaluation of intensity of pain. The analgesics possibly act by raising the threshold of pain stimuli and prevent nerve impulse carrying pain sensation to reach consciousness .This depressant effect is presumably accompanied by some temporary alteration in the biochemical activity of the cells, responsible for the reception of pain sensation in the brain, by means of analgesic drugs. Pinus extract here did not produce any significant result.³

In present studies done on *Pinus roxburghii* extract did not produce any significant analgesic activity. Our results are in contrast with O.A.Oyedapo Analgesic , antioxidants & anti-inflammatory related activities of 2'-hydroxy -2,4'-dimethoxychalone 4- hydroxychalone in mice who while working on *Pinus roxburghii* bark also found significant analgesic activity.⁴

Hot plate test represented the thermal method of inducing pain which is known to be of both central and peripheral origin. Pain of thermal origin was induced by placing the rat on hot floor at 50°C .It is one of the most common tests for evaluating the analgesic efficacy. Paws of rats are very sensitive to heat at temperature which does not damage the skin. It was found that the time taken to react pain was decreased in Pinus extract treated rats The action of NSAID Aspirin was also same as that of Pinus extract treated rats.⁵

Pinus roxburghii extract produce significant analgesic activity. Our results are in agreement with Dhirender Kaushik et al who while working on *Pinus roxburghii* alcoholic bark extract also found significant analgesic activity.⁶

Tail immersion method of analgesia is effective in estimating the efficacy and potency of centrally acting analgesics. It is considered to be selective to examine compounds acting through opioid receptors. The extract increase the pain threshold which indicates that it may act via centrally mediated analgesic mechanism, Narcotic analgesic inhibit both peripheral & central mechanism of pain, while NSAID'S inhibit only peripheral pain.⁷

Extract inhibits pain with central mechanism , suggesting that the plant extract may act as a peripheral analgesic .This was evident in this study wherein the pain threshold increases significantly during the period of observation in all the four drug treated groups, with maximum effect observed in ASPIRIN group .Though Aspirin has a central component of action ,it predominately produces analgesia through a peripheral action; hence, maximum analgesic action of Aspirin cannot be evident in this method which may be reason that analgesic activity of *Pinus roxburghii* was comparable to Aspirin.

OBSERVATIONS AND RESULTS

GROUPS	DOSE mg/kg BW	30 min	60 min	90 min
Control	Distilled Water	10.5±0.96	9.0±0.51	8.5±0.34
Aspirin	300	8.0±0.51*	6.0±0.57**	5.66±0.55
Pinus Extract	100	6.0±0.51	4.66±0.42**	4.33±0.42**
Pinus Extract	300	5.66±0.65**	4.5±0.56**	4.00±0.44**
Pinus Extract	500	4.00±0.42*	3.83±0.87	3.5±0.71

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

Data was analyzed by ANOVA TEST followed by Dunnet's post hoc test. Each value is Mean $\pm$ SEM n=5
*P < 0.05 **P< 0.001 when compared with control

In hot plate test after 30 min of drug administration Aspirin showed significant (P<0.05) analgesic activity and Pinus extract @ 300 mg/kg BW & 500 mg/kg BW also showed significant analgesic activity. After 60 min of drug administration Aspirin & Pinus extract @ 100 & 300 mg/kg BW showed significant (P< 0.01) analgesic activity. Pinus extract @ 100 & 300 mg/kg BW showed significant (P < 0.01) analgesic activity after 90 min of *Pinus roxburghii* administration.

GROUPS	DOSE mg/kg BW	NO OF WRITHS $\pm$ SEM
Control	Distilled Water	4.2 $\pm$ 11.6
Aspirin	300	4.2 $\pm$ 1.0
Pinus extract	100	10.4 $\pm$ 4.8
Pinus extract	300	17.4 $\pm$ 1.9
Pinus extract	500	18.0 $\pm$ 2.0

Data was analyzed by ANOVA TEST followed by Dunnet's post hoc test. Each value is Mean $\pm$ SEM n=5
*P < 0.05 **P< 0.001 when compared with control group.

In acetic induced writhing test no significant difference was observed as compared to the control group.

GROUPS	DOSE mg/kg BW	30 min	60 min	90 min
Control	Distilled Water	10.5 $\pm$ 0.96	9.0 $\pm$ 0.51	8.5 $\pm$ 0.34
Aspirin	300	8.0 $\pm$ 0.51*	6.0 $\pm$ 0.57**	5.66 $\pm$ 0.55
Pinus Extract	100	6.0 $\pm$ 0.51	4.66 $\pm$ 0.42**	4.33 $\pm$ 0.42**
Pinus Extract	300	5.66 $\pm$ 0.65**	4.5 $\pm$ 0.56**	4.00 $\pm$ 0.44**
Pinus Extract	500	4.00 $\pm$ 0.42*	3.83 $\pm$ 0.87	3.5 $\pm$ 0.71

Data was analyzed by ANOVA TEST followed by Dunnet's post hoc test. Each value is Mean $\pm$ SEM n=5
*P < 0.05 **P< 0.001 when compared with control

In hot plate test after 30 min of drug administration Aspirin showed significant (P<0.05) analgesic activity and Pinus extract @ 300 mg/kg BW & 500 mg/kg Pinus extract @ 100 & 300 mg/kg BW showed significant (P< 0.01) analgesic activity. Pinus extract @ 100 & 300 mg/kg BW showed significant (P < 0.01) analgesic activity after 90 min of *Pinus roxburghii* administration.

VI. CONCLUSION

The present study suggests that powder form of *P.roxburghii* has central analgesic effect in rats and extract of test drugs have shown significant effect through peripheral mechanism in rats .The results of the present study corroborate with the classical use and traditional claims made on Saral i.e. *P.roxburghii* for the management of painful condition.

REFERENCES

1. Prof P.V.Sharma, Dravyaguna Vigyan Part II, Page No.307, Reprint year 2005, Chaukhamba Publications.
2. Dr.KM Nadkarni, Indian metrica Medica, page no.300, Reprint year 2002, Popular Prakashan .



ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 8, Issue 3, March 2019

- 3.F.Ahmed, M.H.Hossain, A.A.Rahman et al .2006."Anti-nociceptive & sedative effects of the bark of *Cerberao dollam* Gaertn.'" International Journal of Oriental Pharmacy and Experimental medicine Vol 6 P.344-348
- 4.O.A.Oyedapo et al; 2008 ,Analgesic , antioxidants & anti-inflammatory related activities of 2'-hydroxy -2,4'-dimethoxychalcone 4-hydroxychalone in mice " Journal of Biological Science ,vol.8 ,P.131-132
5. Bhakthavatsalam,Gosh M N; 1987 .A role for dopaminergic receptors in amphetamine anorexic tolerance .J PharmacologyExp. P.240:196-202
6. DhirenderKaushik et al.(2012) Analgesic & Anti-inflammatory activity of *Pinusroxburghii*, Research article fromPubmed p.4
7. E.Elisabetsky,T.A.Arnador,R.R.Albuquerque et al, Analgesic activity of *Psychetria colorata* (willd .ex. R.S.) Muell Arg. Alkaoid ," Journal of Ethanapharmacology, vol.48