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KASHMIRI PINUSROXBURGHII - ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

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Abstract:
The Chir Pine, Pinusroxburghii, named after William Roxburgh, is a pine native to the Himalaya. Pinusroxburghii Sarg. (Pinaceae) is traditionally used for several medicinal purposes in India. As the oil of the plant is extensively used in number of herbal preparation for curing inflammatory disorders, the present study was undertaken to assess analgesic and anti-inflammatory activities of its bark extract. Dried and crushed leaves of Pinusroxburghii Sarg. Were defatted with petroleum ether and then extracted with alcohol. The alcoholic extract at the doses of 100 mg/kg, 300 mg/kg, and 500 mg/kg body weight was subjected to evaluation of analgesic and anti-inflammatory activities in experimental animal models. Analgesic activity was evaluated by acetic acid-induced writhing and tail immersion tests in Swiss albino mice; acute and chronic anti-inflammatory activity was evaluated by carrageenan-induced paw oedema and cotton pellet granuloma in Wistar albino rats. Diclofenac sodium and indomethacin were employed as reference drugs for analgesic and anti-inflammatory studies, respectively. In the present study, the alcoholic bark extract of Pinusroxburghii Sarg. demonstratedsignificant analgesic and anti-inflammatory activities in the tested models.

Keywords: *Pinusroxburghii, analgesic, anti-inflammatory activities, Diclofenac sodium, indomethacin*

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INTRODUCTION:

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries [1]. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease [3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions [4]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes [5–7].

Pinusroxburghii Sarg. is the only tree with an ornamental specimen and having different medicinal values found in the Himalayan region of Bhutan, Nepal, Kashmir, Sikkim, Tibet and other parts of North India [8]. The plant is belonging to family Pinaceae commonly known as Chir Pine [9]. It consists of 110–120 species distributed throughout temperate regions of the Northern Hemisphere, and more than 40 taxonomic treatments have been recognized of several major divisions within the genus [10-13].

Pinusroxburghii Sarg.has many medicinal uses, the wood is aromatic, deodorant, haemostatic, stimulant, anthelmintic, digestive, liver tonic, diaphoretic, and diuretic. It is useful in eye, ear, and pharynx diseases, foul ulcers, haemorrhages, haemoptysis, worn infections, flatulence, liver diseases, bronchitis, inflammations, skin diseases, pruritus, and giddiness [14].The chief chemical constituents of turpentine oil from Pinusroxburghii Sarg. are -pinene, -pinene, car-3-ene and longifolene [15] hydrocarbons (d- and l-pinene), resin acids, camphene, fenchene, dipentene, and polymeric terpenes [16, 17].

EXPERIMENTAL

Plant Material

The stems bark of Pinusroxburghii Sarg. were collected from the hilly region of Ramso, District Udampur, Jammu, India,

Preparation of Extract

Shade dried coarse powdered bark of Pinusroxburghii Sarg. in a quantity sufficient as per the volume of extractor was packed in thimble (made of filter paper sheet). A sufficient volume of alcohol was added to the reservoir, and hot continuous extraction process in a Soxhlet extractor was started. This extraction process was continued for about 48 hours or until alcohol coming down the siphoning tube became colourless. The excess of alcohol was distilled under reduced pressure using rotatory vacuum evaporator. (HeidolphLaborota 4011, digital). A brown residue was recovered from flask with 12% yield.

HPLC Analysis

Samples of alcoholic bark extract of Pinusroxburghii Sarg. wereanalysed without any treatment.The HPLC system (Shimadzu, Japan) consisted of a diode array detector (SPDM10AVP), solvent delivery module (LC-10ATVP), online degasser (DGU-14A), an autoinjector (SIL-10ADVP), flow channel system (FCV-14AH), system controller (SCL-10AVP), and a reversed-phase HPLC column (RP-18, 250 mm × 4.6 mm, 5 μm particle size, Sigma, USA). The flow rate of the HPLC was 1 ML/min, and the mobile phase 0.05% TFA in ACN: 0.05% TFA in water (gradient) for 70 min. Standards of chlorogenic acid, rutin and querctin were injected separately (10 ML). Chemical compounds in the samples were identified by comparison of their retention times (Rt) with the standards. Data analysis was carried out using Class VP V6.12 SP2 software (Shimadzu, Japan).

Animals

Wistar rats (150–250 gm) and Swiss albino mice (20–25 gm) of either sex, brought from National Institute of Pharmaceutical Education and Research Hyderabad (A.P).

Anti-Inflammatory Activity (Carrageenan Induced Paw Edema Method)

Carrageenan-induced paw inflammation was produced according to the method described by Winter et al. [18]. One hour after oral administration of the alcoholic extract of Pinusroxburghii Sarg. (100, 400, and 500 mg/kg), reference drug (indomethacin, 10 mg/kg) or vehicle (tween 80 (5%)), an injection of 0.1 ML of carrageenan (1% carrageenan suspended in 0.9% NaCl) was made into the right hind limb of each rat under the subplantaraponeurosis.

Measurement of paw volume was done by means of volume displacement technique using

plethysmometer (UgoBasile no. 7140) immediately after carrageenan injection and after 1, 2, 3, and 4 hr.

Cotton Pellet Granuloma Method

Cotton pellet granulomas produced according to the method described by Winter and Porter [19]. Sterile cotton pellets (20 ± 0.5 mg) were implanted subcutaneously in the abdomen region of the rats. The animals received alcoholic bark extract of Pinusroxburghii Sarg. (100, 300 and 500 mg/kg), reference drug (diclofenac sodium, 50 mg/kg) or vehicle (tween 80 (5%)), orally, once a day through an oral cannula over seven consecutive days. On the 8th day, the rats were sacrificed, the cotton pellets removed, pellets dried up to constant weight at 60°C and the net dry weight, that is, after subtracting the weight of the cotton pellets, was determined.

Analgesic Activity (Acetic Acid Induced Writhing Test Method)

The method used in this test has been described by Koster et al. [20]. The total number of writhings following intraperitoneal administration of acetic acid solution (1%, 10 mL/kg) was recorded over a period of 10 min, starting 5 min after acetic acid injection. The mice were treated with the alcoholic bark extract of Pinusroxburghii Sarg. (100, 300, and 500 mg/kg), or vehicle (tween 80 (5%)) or standard drug (diclofenac sodium, 50 mg/kg), 30 min before administration of acetic acid. The number of writhings and stretching was recorded and permitted to express the percentage of protection.

Tail Immersion Test in Rats

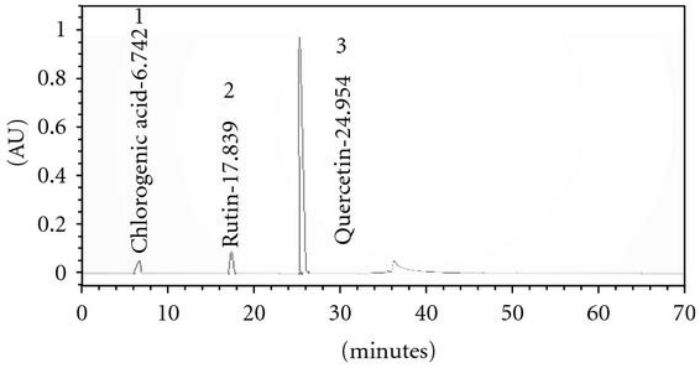
The procedure described by Aydin et al. [21] was used to conduct this test. 3 cm of the tail was introduced in hot water at a temperature of 55 ± 0.5°C. Within a few minutes, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. The animals were treated by alcoholic extract of PinusroxburghiiSarg. (100, 300 and 500 mg/kg), or water (vehicle) or standard drug (diclofenac sodium, 50 mg/kg), 30 min before the immersion of the tail. The time reaction is taken at 1, 2, 3, and 4 after administration of different preparations

Acute Toxicity Test of Plant Extract

Alcoholic extract of the plant Pinusroxburghii Sarg. was found safe at the dose of 5000 mg/kg according to OECD guidelines 425.

HPLC Analysis

A correct assignment to the various compounds was not possible. From UV spectra and retention times of the main peaks, some compound classes contained in the extract have been determined. High-performance liquid chromatography (HPLC) revealed the presence of bioflavonoids, quercetin, chlorogenic acid, and rutin (Figure 1)



HPLC analysis of Pinusroxburghii Sarg. (Pinaceae) showing presence of chlorogenic acid (1), rutin (2) and quercetin (3).

Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema in Rats

In the carrageenan-induced oedema test, the paw volumes and percentages of ages of inhibition by the alcoholic extract of Pinusroxburghii Sarg. and standard drugs are shown in Table 1. Injection of carrageenan was done 1 h after oral administration of the extract (100, 300, and 500 mg/kg), indomethacin (reference drug) and water. Measurement of paw size was taken before carrageenan injection and then 1, 2, 3, and 4 h after carrageenan injection.

Analgesic Activity

Acetic Acid Induced Writhing Test in Mice

The alcoholic extract of Pinusroxburghii Sarg. (100, 300, and 500 mg/kg) dose significantly and dependently reduced the number of abdominal constriction induced in mice by a solution of acetic acid 1%. This dose-dependent protective effect reached a maximum inhibition of 80.95% at the dose of 500 mg/kg. Diclofenac sodium (reference drug) exerted a significant protective effect, with percentage of protection of 90 (Table 3).

Tail Immersion Test in Rats

As presented in Table 4, alcoholic extract of Pinusroxburghii Sarg. in doses of 500 mg/kg () body weight showed a significant elongation of reaction time, 30 minutes after oral administration of the extract. After 60 minutes, the alcoholic extract of Pinusroxburghii Sarg. in doses of 300 mg/kg and 500 mg/kg body weight showed a significant elongation. After 90 minutes alcoholic extract of Pinusroxburghii Sarg. in doses of 300 mg/kg and 500 mg/kg body weight showed a significant elongation of reaction time. After 120 minutes alcoholic extract of Pinusroxburghii Sarg. in doses of 100 mg, 300 mg, and 500 mg/kg body weight showed no significant elongation of reaction time.

DISCUSSION AND MODE OF ACTION:

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [22]

Since the extract/fractions significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by nonsteroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme.

The inflammatory granuloma is a typical feature of an established chronic inflammatory process [23, 24]. The cotton pellet granuloma method has been widely employed to evaluate the transudative, exudative, and proliferative components of chronic inflammation, because the dried weight of the pellets correlates well with the amount of granulomatous tissue [25]. We found a dose-dependent inhibition of granuloma formation in mice, suggesting that the aqueous stem bark extract of Pinusroxburghii Sarg. inhibits chronic inflammation processes during the late phases of acute inflammation.

The brain and spinal cord play a major role in central pain mechanisms. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including substance P, somatostatin, neuropeptide Y, inhibitory amino acid, nitric oxide, endogenous opioids, and the monoamines which are the major targets for pain and inflammation [26]. The tail immersion test was considered to be selective to examine compounds acting through opioid receptor; all the extract/fractions increased pain threshold which means basal latency, which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal anti-inflammatory drugs inhibit only peripheral pain [27]

The results of the present study have shown that the crude extract of the investigated plant exhibited very high anti-inflammatory and analgesic activities. These activities may be linked with the presence of polyphenolic compounds present in the extract. The HPLC analysis of AB extract shows the presence of bioflavonoids, quercetin, and rutin, which are reported to be anti-inflammatory, antiasthmatic, analgesic anti-inflammatory, and antioxidant, and these findings are in concordance with our results. Many plants containing flavonoids have been shown to have diuretic, laxative, antispasmodic, anti-hypertensive, and anti-inflammatory actions [28]

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