

# Anti-Nociceptive and Anti-Inflammatory Activity of *Araucaria bidwillii* Hook

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## ABSTRACT

The effect of alcoholic extracts of leaf from *Araucaria bidwillii* Hook. [ABH] (Family: Araucariaceae) was evaluated in experimental models of pain and inflammation. Oral administration of 100, 200 and 300 mg/kg of leaf extracts of ABH were used for the above study. The leaf extract at 300 and 200 mg/kg showed significant reduction in acetic acid induced writhings in mice with a maximum effect of 65.1% reduction at 300 mg/kg dose. In hot plate method the percentage of pain inhibition was found to be 81.69% and 66.1% with both the tested dose of the leaf extract respectively. The effect produced by the alcoholic extract at the highest dose was comparable to that of acetyl salicylic acid at 100 mg/kg (91.52%). The alcoholic extracts of *A. bidwillii* showed significant inhibition in carrageenan (18.61%, 32.12% and 45.64%) and serotonin (32.81%, 38.68% and 40.75%) induced hind paw oedema in rats at 100, 200 and 300 mg/kg of the ABH extract respectively. The anti-inflammatory effects showed by the extract were comparable to that of standard indomethacin 5 mg/kg (68.51% and 63.28%). The results suggest that the anti-inflammatory and analgesic effect of the extracts as claimed in folklore medicine, which may be mediated via both peripheral and central mechanisms.

**Keywords:** *Araucaria bidwillii* Hook, Ethanol extract, Inflammation, Anti-nociceptive, Paw oedema

A scientific evaluation of herbs according to their traditional methods of use in various diseases management can incorporate into the complementary and alternative medicine (CAM) system elsewhere. The plant *Araucaria bidwillii* Hook. (Family: Araucariaceae) popularly known as monkey-puzzle is a evergreen plant distributed in high altitudes hills areas of Western Ghats of Nilgiris and northern part of Himalayan regions in India [1, 2]. The leaves and oleoresins of this plant is traditionally used to treat pain, inflammation and mental stress in thoda tribes of Nilgiris district, Tamil Nadu [0]. The decoction of the oleoresins is traditionally used in Thailand for treatment of insomnia [4]. Anti-ulcer and anti-pyretic activity has been reported for leaf and oleoresins of this plant [5-7]. The genus *Araucaria* is rich in biflavones and many of them have been isolated from the leaves. In order to evaluate the pharmacological basis for the use of *A. bidwillii* Hook. in folk medicine for treatment of pain and inflammation, the present study was designed to investigate the antinociceptive and anti-inflammatory effect of alcoholic extract of the leaves on inflammation induced edema and pain behavior in animals.

## MATERIALS AND METHODS

### Plant Material

The fresh leaves of *A. bidwillii* were collected from the Botanical Garden, Udhamandalam, Nilgiris District, Tamil Nadu. The plant materials were authenticated at survey of medicinal plants and collection unit, Government of India, Nilgiris District. A voucher specimen (JUNPSL 2002-01) of this plant material has been retained in the School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

### Preparation of Extracts

The shade dried, coarse powdered leaves of *A. bidwillii* were extracted separately in the soxhlet extraction apparatus using ethanol (95%). The resultant alcoholic extract was concentrated using rotary vacuum evaporator. The extracts were then freeze-dried and stored in vacuum desiccator (yield of the leaf extract was 13.16% w/w).

### Phytochemical Character of the Extract

The hydroalcoholic extracts of leaf was subjected to qualitative analysis where the presence of major phytoconstituents like flavanoids were found. The leaf extract was chromatographed on precoated silica gel GF254 plate using mixture of toluene: ethyl acetate: formic acid: water (35:50:10:5) as mobile phase. Silica gel plate was first observed under UV 254 nm, UV 366 nm and then sprayed with natural product – polyethylene glycol reagent (NP-PEG) to detect the presence of the flavonoids [8, 9]. A number of various yellow–green spots were observed with  $R_f$  value between 0.16-0.60. This characteristic spots are in accordance with the earlier phytochemical investigation of *Araucaria bidwillii* Hook, in which several biflavones have been reported to be present in the leaf extracts [10, 11]. Further isolation of the various phytoconstituents from leaf is under progress in our laboratory.

### Chemicals and Drugs

Serotonin, and carrageenan were purchased from Sigma Chemical Co., St. Louis, MO. Acetylsalicylic acid, indomethacin, carboxy methyl cellulose and acetic acid were purchased from Ranbaxy laboratories, New Delhi, India. Other reagents were of analytical grade and were procured from SISCO Research Laboratories Pvt Ltd, Mumbai, India.

### Test Samples and Standards

Suspension of the ethanol extract of the leaf (AEL) of *A. bidwillii* was prepared in sodium carboxy methyl cellulose (CMC, 0.3%) using distilled water. Acetylsalicylic acid (100 mg/kg) and indomethacin (5 mg/kg) were used as standards. Gastric administration of all drugs was accomplished via oral gavage.

### Test Animals

Wister rats (200–250 g) and albino mice (20–25 g) of either sex were used in this investigation. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. Experiments on animals were performed based on animal ethics guidelines of Institutional Animal Ethics Committee. Albino mice (n=6, per group) were used for anti-nociceptive activity and Wister rats (n=6, per group) were used for anti-inflammatory screening and divided into five different groups. First group served as control animals they were treated with 0.3% CMC. Three groups of animals were treated with the alcoholic leaf extract (AEL) at three different doses (100, 200 and 300 mg/kg). Fifth group of animals were treated with standard drugs. The standard drugs acetyl salicylic acid 100 mg/kg (anti-nociceptive activity) or indomethacin 5 mg/kg b.wt (anti-inflammatory activity) were used.

### Acute Toxicity Study in Mice

Acute toxicity was carried out using 50% mortality till 24 h following oral administration of extracts in Swiss albino mice and the LD<sub>50</sub> was calculated [12]. Animals were divided into different groups. The control

animal group received the vehicle (0.3% CMC) while the test group treated with various graded dose of leaf extract orally. Animals were observed individually after dosing. Observation includes mortality and gross behaviors like body positions, locomotion, rearing, tremors, gait was observed. The effect of leaf extract on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, were also assessed [13].

### Anti-Nociceptive Activity

**Hot Plate Method.** Albino mice were placed in aluminum hot plate kept at a temperature of  $55 \pm 0.5$  °C for a maximum time of 30 s [14]. Reaction time was recorded when animals licked their fore, hind paws and jumped at before 0, 15, 30, 45 and 60 min and after oral administration of AEL (100, 200 and 300 mg/kg). Acetyl salicylic acid 100 mg/kg was used as a reference drug.

**Acetic Acid-Induced Writhing Test.** Antinociceptive response of the extract AEL (100, 200 and 300 mg/kg) was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) induced by 1% acetic acid solution (1mL: 100 g) in mice [15]. Number of writhes per animal was counted during 30 min test period, beginning 3 min after the injection of acetic acid. Acetyl salicylic acid 100 mg/kg b.wt was used as a reference drug.

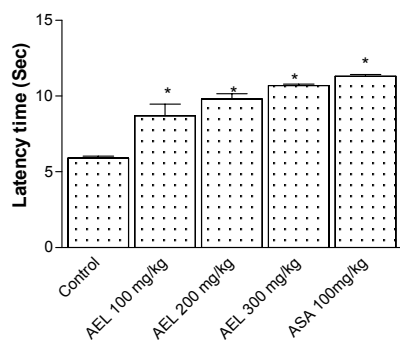
### Anti-Inflammatory Activity

**Carrageenan Induced Rat Paw Oedema.** Oedema was induced by subplanter injection of 0.1 mL of 1 % freshly prepared suspension of carrageenan into the right hind paws of the rats of all groups. The volume of the injected paws and contra-lateral paws were measured at 1, 2, 3, 4 and 5 hours intervals using Plethysmometer [16]. The AEL (100, 200 and 300 mg/kg) extracts was administered to three groups of animal and remaining two groups of animals received 0.3% CMC (Control 10 mL/kg) and indomethacin 5 mg/kg as standard drug respectively.

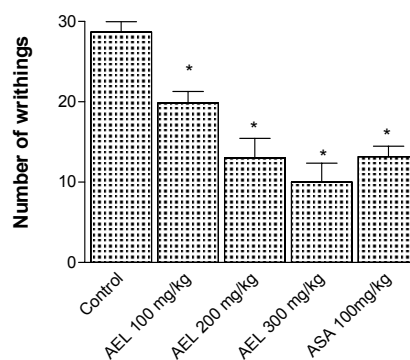
**Serotonin Induced Rat Paw Oedema.** The paw oedema was induced in the right hind paw by sub planter injection of 0.05 mL of 1 % freshly prepared solution of serotonin [16]. The volume of injected paws and contra-lateral paws were measured at 1, 2, 3, 4 and 5 hours intervals using plethysmometer. AEL (100, 200 and 300mg/kg) extracts was administered to three groups of animal and remaining groups of animals received 0.3% CMC (Control 10 mL/kg) and indomethacin 5 mg/kg (Standard) respectively for assessing comparative pharmacological significance.

### Statistical Analysis

Results are reported as mean  $\pm$  SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *p*-Value was found statistically significant ( $p < 0.05$ ), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS® Inc., USA).



**Fig 1.** Effect of ethanol extracts from *Araucaria bidwillii* and acetyl salicylic acid on mice exposed to hot plate. Values are mean  $\pm$  SD. \* Indicates statistically significant reduction compared to control,  $p < 0.05$ ; AEL = Ethanol extract of *A. bidwillii*; ASA = Acetyl Salicylic Acid.



**Fig 2.** Effect of ethanol extracts from *Araucaria bidwillii* on acetic acid-induced writhing responses in mice. Values are mean  $\pm$  SD. \* indicates statistically significant reduction compared to control,  $p < 0.05$ . AEL = Ethanol extract of *A. bidwillii*; ASA = Acetyl Salicylic Acid.

## RESULTS

### Effect of Hydro Alcoholic Extracts of ABH on Acute Toxicity Test in Mice

Animal treated with 5000 mg/kg of alcoholic extract of leaf (AEL) was observed for 24 hrs and showed no changes in behavior, a fact indicating low toxicity of the AEL tested at various dose levels.

### Hot Plate Test

Fig 1 shows the results of the hot plate test. Three doses of extracts of *A. bidwillii* increased the reaction time in a dose-dependent manner to the thermal stimulus. The highest nociception inhibition of thermal stimulus was exhibited at a higher dose of the extracts 300 mg/kg of leaf AEL (81.69%), which is comparable to the acetylsalicylic acid (91.52%).

### Acetic Acid-Induced Writhing Test

Dose dependent antinociceptive effect was noted with the extract at the tested dose levels (Fig 2). Maximum percentage of inhibition of writhing response exhibited by the AEL extract at 300 mg/kg was 65.1%, while the same at 200 and 100 mg/kg showed 54.64 and 30.8% reduction in acetic acid induced writhing response respectively, which was comparable to that of standard acetylsalicylic acid (100 mg/kg) that caused 54.08% pain inhibition.

### Carrageenan-Induced Edema Test

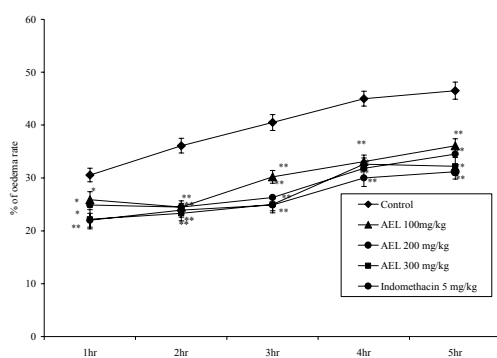
Effect of the extracts and reference drug on paw edema induced by carrageenan, has been shown in Fig 3. Paw edema in rats reached its peak at 4 hrs after carrageenan administration. Administration of various doses of AEL produced a significant inhibition of the oedema at the end of 3 hrs with carrageenan administration. Maximum percentage of inhibition of oedema exhibited by the AEL extract at 300 mg/kg was 45.64%. This effect was comparable to that standard indomethacin.

### Serotonin Induced Rat Paw Oedema

The results of serotonin induced rat paw oedema test were reported in Fig 4. It was observed that leaf extracts of *A. bidwillii* exerted a significant edema reduction from the first hour and remained along the time. Administration of AEL at different doses produced significant inhibition ( $p < 0.05$ ) of oedema at the end of 3 hr with serotonin administration. However higher dose 200 and 300 mg/kg of AEL extracts exhibited maximum inhibition of paw edema (38.68 and 40.75%) respectively as compared to that of control group.

## DISCUSSION

Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases [18-20]. A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation. The AEL extract demonstrated significant anti-nociceptive activity at two different dose levels in various animal models of pain. In hot plate test, nociceptive reaction towards thermal stimuli in mice is a well-validated model for detection of opiate analgesics as well as several types of analgesic drugs from spinal origin [21]. Acetic acid-induced writhing has been used as a model of chemonociception induced pain, which increases  $PGE_2$  and  $PGF_{2\alpha}$  peripherally [22]. Thus the anti-nociceptive activity shown by AEL extracts in hot plate and acetic acid induced writhing test indicate that alcoholic extracts of leaf might possess centrally and peripherally mediated anti-nociceptive properties. It is well known that the carrageenan-induced paw oedema is characterized by a biphasic event, with involvement of different inflammatory mediators: in first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play a role, while in second phase (3–5 h after carrageenan injection) kinin and prostaglandin are also involved [23]. Our results revealed that administration of AEL inhibited the edema starting from the first hour and during all phases



**Fig 3.** Effect of AEL and indomethacin on carrageenin induced paw oedema in rats. Values are mean  $\pm$  SD. \*\* Indicates statistically significant reduction compared to control, \*\*  $p < 0.05$ . AEL = Ethanol extract of *A. bidwillii*.

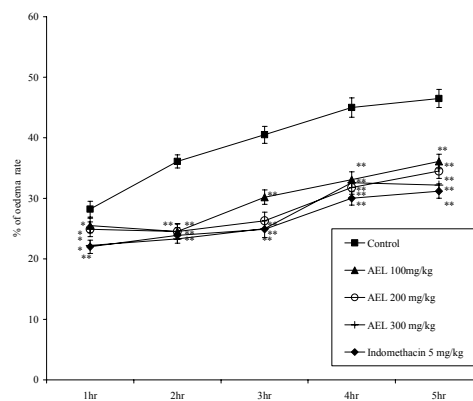
of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. The effects of alcoholic extract of ABH in inflammation process induced by serotonin suggest that they act by affecting a time-delayed system in a similar fashion to glucocorticoids. The ability of the extracts to suppress abdominal writhes, increase pain threshold latency, inhibition of the phases of carrageenan as well as suppression of the serotonin induced inflammation confirm the analgesic and anti-inflammatory properties of the extract. The chemical analysis of the leaf extract showed the presence of biflavones as the major constituents. These findings justify traditional use of this plant in the treatment of pain and other inflammatory conditions and validate its claim of being used for the said purpose in folklore medicine. It can be concluded that alcoholic extracts of ABH possesses analgesic and anti-inflammatory properties, which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms which may be of potential benefit for the management of pain and inflammatory disorders.

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**Fig 4.** Effect of AEL and indomethacin on serotonin induced paw oedema in rats. Values are mean  $\pm$  SD. \*\* Indicates statistically significant reduction compared to control, \*\*  $p < 0.05$ . AEL = Ethanol extract of *A. bidwillii*.

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