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ANALGESIC ACTIVITY OF *TAXUS BACCATA* LINN. BARK EXTRACT

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ABSTRACT

In the traditional medicine, *Taxus baccata* Linn. has been used as analgesic, anticonvulsant, and anti-inflammatory remedy. In the present study, bark of plant was extracted with 95% ethanol and ether at room temperature and evaluate for their analgesic activity in mice using the tail-flick and acetic acid-induced writhing method. Ether extract exhibits potent analgesic activity in tail-flick test at 200mg/kg two hours after administration in compare with ethanolic extract, whereas ethanolic extract exhibits significant analgesic activity in acetic acid-induced writhing test at 200mg/kg. The extracted compounds exhibited analgesic activity against chemically and thermal noxious stimuli on both early and late phases of pain by the extracts (100 and 200 mg/kg doses). The observed pharmacological activities provide the scientific basis for the folkloric use of the plant in treating acute pain.

KEYWORD

Taxus baccata, Flavonoids, Analgesic and Diclofenac sodium.

INTRODUCTION

Pain is defined as neuralgia, an unpleasant sensory experience associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause, or persist long after the precipitating injury has healed[1]. It can also occur as a consequence of brain or nerve injury[2]. Pain can constant (chronic) or fleeting and come and go (acute). There are many herbs that are very useful and effective for pain relief. Many are safe for everyone but some should be avoided during pregnancy or while nursing[3]. In historical documents from the Roman period, *Taxus baccata* (L) was used as an analgesic, antimalarial, antirheumatic, emmenagoque, sedative, antispasmodic, aphrodisiac and anti-asthmatic[4]. It was also listed in Avicenna's cardiac drugs, namely Zarnab. So far, the isolation of

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a large number of taxoids as well as lignans, flavonoids, steroids and sugar derivatives has been reported from different parts of various *Taxus* species[5]. In this paper, we would like to describe the extraction and evaluation of analgesic activity of *Taxus baccata* (L).

MATERIALS AND METHODS

Drug and Plant Material:

Reference Standard Diclofenac sodium was procured from Cipla Pharmaceuticals, Mumbai, as a gift sample. The bark of *Taxus baccata* (L) was collected from Tawang forest Divisions of Arunachal Pradesh, India in the month of September. The plant was identified and authenticated by the Botanist of Shibpur Botanical Garden, Kolkata, India (voucher specimen no. SBG/08-005).

Preparation of Plant Extracts:

Fresh bark was cleaned, dried under shade at temperature 40 ± 2°C and powdered by a mechanical grinder. Then the dried and powdered bark of *Taxus baccata* (L) was extracted with 95% ethanol and ether at room temperature. Then the extract was concentrated and the concentrate thus obtained was suspended in H_2O and extracted with $CHCl_3$ which left residue. The $CHCl_3$ extract was purified by preparative TLC by using n-butanol and glacial acetic acid as mobile phase[6].

Preliminary Phytochemical Screening:

Phytochemical screening was carried out in order to find out the various constituents present in the extracts. The extract was tested for the presence of glycoside, alkaloids, steroids, flavonoids, tannins, mucilages, fixed oils, phenolic compounds, proteins and sterols standard qualitative chemical tests[7, 8].

Experimental Animals

Swiss albino mice of either sex weighing 25 to 30 g maintained in our college animal house were used for the study. The selected animals were maintained by giving pelleted diet, water *adlibitum* and kept in 12 hrs/12 hrs light/dark cycle. The animals were divided into six groups each containing mice. All the animal experiments were performed following the approval of study protocols by the Institutional Animal Ethics Committee (HPI/08/60/IAEC, 0026).

Acute Toxicity Study:

The study was carried out according to OECD[9] (Organization of Economic Co-operation and Development) guidelines 423. Nine female Wistar albino rats weighing 150-200 g were taken and extracts were administered orally to animals at a dose of 2000 mg/kg in 0.3% w/v Carboxy Methyl Cellulose Sodium. Then the animals were observed for mortality and morbidity at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hr. Food was given to the animals after 4 hr of dosing and the body weight was checked at 6 hr after dosing. Morbidity like convulsions, tremors, grip strength, lethargy, ptosis and pupil dilation were observed. The animals were observed twice daily for 14 days and body weight was noted.

Analgecic Activity:

Analgesic activity was assessed by Tail-Flick method[10] and acetic acid- induced writhing method[11].

***Tail-flick method*:**

Swiss albino mice (25-30 g) of either sex (reaction time: 3-4 sec) were divided into groups of 6 each. Diclofenac sodium (50 mg/kg, p.o.) was used as standard. The tail-flick latency was assessed by the analgesiometer (Techno, India). The magnitude of the current which was passing through the naked nichrome wire was kept constant at 6 ampere. The tail skin was kept at a distance of 1.5 cm from the heat source. The radiant heat (55°C±2) in the tail was applied and maintained at 2.5 cm measured from the root of the tail. In order to avoid the tissue damage, the cut of reaction time was kept at 10-13 sec. The

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mean scores in control, standard (Diclofenac sodium), and test groups were recorded and tabulated in Table 1

***Acetic-acid induced writhing method*:**

In the writhing test adult Swiss albino mice (25-30 g) of either sex were used in six groups of 6 each. Diclofenac sodium (50 mg/kg, p.o.) was used as standard. 1% w/v gum acacia was used as control and the 95% ethanol and ether (100 and 200 mg/kg, p.o.) were used as treatment for other four groups. Then 1% v/v aqueous acetic acid was administered intraperitonially to all the groups to produce writhe. Test substances were administered 30 minutes before injection of acetic acid. Animals were kept individually under glass jar for observation immediately after acetic acid injection for 20 minute period. Onset on writhes was noted and the number of abnormal constrictions, trunk twist response and extension of hind limbs were recorded. The mean writhing scores in control, standard (Diclofenac sodium), and test groups and the percentage of writhing were calculated and tabulated in Table 2

Statistical Analysis:

Values are expressed as mean ± SEM and data was analyzed by ANOVA followed by Dunnet's test. $P<0.05$ was considered as significant.

RESULTS:

The results of analgesic activity of the bark extracts of *Taxus baccata* (L) are expressed in the Table 1 and 2.

Table 1

Effect of Taxus baccata bark extract on thermally induced nociception in mice

Sl. No.	Group	Dose/kg body wt.	Reaction time in sec. (Mean ± SEM)		
			Tail flick at 0.5h(sec.)	Tail flick at 1.0h(sec.)	Tail flick at 2.0h(sec.)
1	Control	20 ml	3.28±0.01	3.60±0.03	3.70±0.02
2	Diclofenac sodium	50mg	9.07±0.02*	10.55±0.02*	11.22±0.02*
3	95% Ethanolic extract	100mg	9.02±0.01*	10.02±0.01*	11.98±0.02*
4	95% Ethanolic extract	200mg	7.98±0.03*	8.25±0.09*	9.34±0.09*
5	Ether extract	100mg	7.63±0.008*	8.25±0.06*	8.36±0.37*
6	Ether extract	200mg	9.01±0.008*	10.83±0.008*	12.62±0.07*

*n=6 in each group. * P< 0.01 compared to control*

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Table 2

Effect of Taxus baccata bark extract on acetic acid – induced writhing in mice

Sl. No.	Group	Dose/kg body wt.	Number of writhing (Mean ± SEM)	% inhibition of writhing
1	Control	20 ml	73.33±2.37	------
2	Diclofenac sodium	50mg	24.17±1.78*	67.04
3	95% Ethanolic extract	100mg	55.33±3.89*	24.55
4	95% Ethanolic extract	200mg	36.50±4.26*	50.23
5	Ether extract	100mg	40.67±1.89*	44.54
6	Ether extract	200mg	38.50±1.38*	47.49

*n= 6 in each group. * P< 0.01 compared to control*

Figure 1

Effect of Taxus baccata bark extract on thermally induced nociception in mice

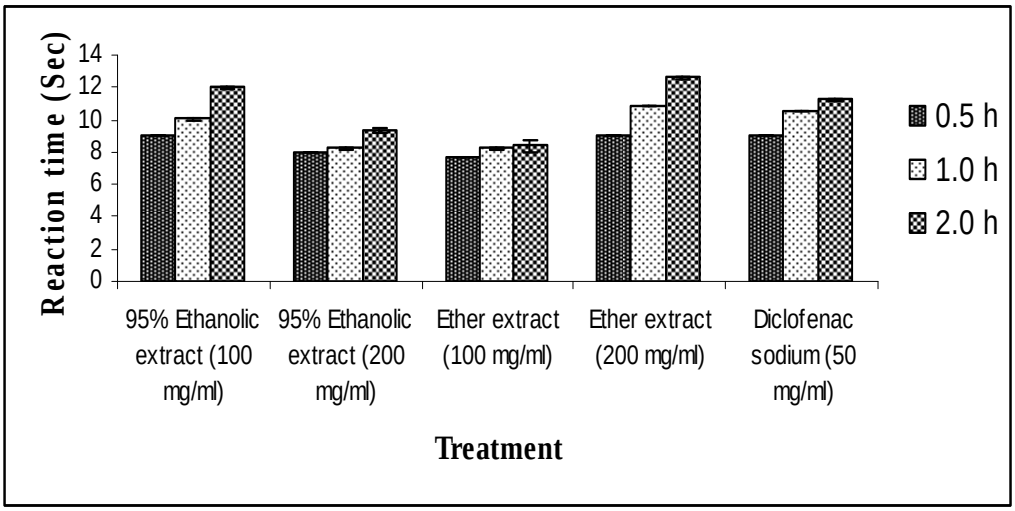

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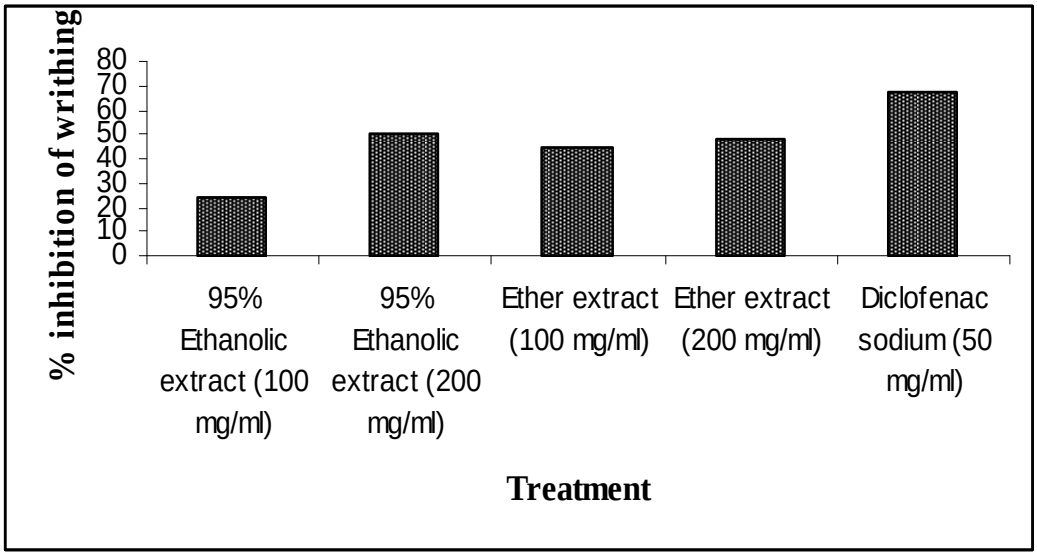
Figure 2

Effect of Taxus baccata bark extract on acetic acid – induced writhing in mice



In the both tail-flick and acetic acid-induced writhing method, 95% ethanol and ether (100 and 200 mg/kg) and standard drug showed significant results as compared to control group. In the tail-flick model, 30 minutes after drug administration reaction time was increased significantly for the test and standard drugs when compared to the predrug reaction time. The extracts suppressed the acetic acid induced writhing response significantly on mice. Ether extract exhibits potent analgesic activity in tail-flick test at 200 mg/kg two hours after administration in compare with ethanolic extract, whereas ethanolic extract exhibits significant analgesic activity in acetic acid-induced writhing test at 200 mg/kg. The analgesic activity was found to be significant on the tail-flick model (P<0.01), the acetic acid induced writhing model (P<0.01) and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

DISCUSSION

The results of the present study revealed the antinociceptive effect of *Taxus baccata* bark extract in both the experimental pain models. The tail-flick test are considered to be selective to examine compounds acting through opioid receptor, the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism[12, 13]. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids[14]. The acetic acid

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induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells[15], acid sensing ion channels[16] and the prostaglandin pathways[17].

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic. Such a mode of action is proposed for opioid analgesic such as morphine. It is also reported that the inhibition of pain could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents[18] and also steroidal constituents[19]. There are also reports on the role of flavonoid in analgesic activity primarily by targeting prostaglandins[20, 21]. So, it may be due to the similar type of constituents like flavonoid, steroids, phenolic constituents present in the extract. It needs further evaluation to trace the bio-molecular mechanism of *Taxus baccata* (L) bark extract.

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