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Anticonvulsant, analgesic and antipyretic activities of *Taxus wallichiana* Zucc.

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Abstract

*Taxus wallichiana* Zucc. (Himalayan Yew) is often used in northern areas of Pakistan for the treatment of pyrexia, acute pains and epilepsy. We have investigated certain pharmacological activities of the methanol leaf extract against convulsion, nociception and pyrexia induced in rodents. The aim was to justify and explore its folk uses in these pathological conditions, on scientific basis. The studies were carried out using acetic acid-induced nociception and pentylenetetrazole-induced convulsions in mice, while formalin test and yeast-induced pyrexia in rats. Significant analgesic (67.77 and 74.29%) effect was found in acetic acid-induced model at doses of 100 and 200 mg/kg, i.p. respectively. Crude extract exhibited significant (*P* < 0.05) inhibition of the formalin noxious stimulation on both early and late phases of pain by the extracts (100 and 200 mg/kg doses). In case of yeast-induced pyrexia model, 200 mg/kg dose showed very significant (*P* < 0.01) inhibition while 50 and 100 mg/kg dose caused a significant (*P* < 0.05) inhibition. Plant extract has controlled the pentylenetetrazole-induced convulsions in mice. 100 and 200 mg/kg i.p doses of the extract significantly (*P* < 0.05) inhibited the micon and clonus while inhibition of tonus and hind limb tonic extension (HLTE) was highly significant (*P* < 0.01). The anticonvulsant activity of this plant has been reported for the first time throughout the whole genus. The observed pharmacological activities provide the scientific basis for the folkloric use of the plant in treating epilepsy, pyrexia and acute pain.

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Keywords: *Taxus wallichiana* Zucc.; Anticonvulsant; Analgesic; Antipyretic; Taxaceae

The genus *Taxus* (Taxaceae) is well known for the famous anticancer agents with emphasis on taxol and docetaxel. The taxol and related bioactive taxoids have been reported from the various species of the genus, including *Taxus wallichiana* Zucc. (Wani et al., 1971; Bala et al., 1999; Madhusudanan et al., 2000; Prasain et al., 2001). Apart from anticancer activities, the genus *Taxus* have not been widely studied pharmacologically. Several pharmacological studies on this genus revealed the isolation of variety of taxoids shown to possess immunomodulatory (Chattopadhyay et al., 2006) histamine release inhibitory activity (Koyama et al., 2006). Küpeli et al. (2003) described not only antinociceptive activity but also isolated anti-inflammatory compounds from *Taxus* species. In one study, antifungal activities were also reported (Tachibana et al., 2005). Yin et al. (2006) reported a lignan which showed improvement in postmenopausal osteoporosis in the ovariectomized rats. The ethanol extract of the heartwood of *Taxus baccata* and its chloroform fraction were reported to possess antiplatelet and vasorelaxant activities (Erdemoglu et al., 2004). Similarly, the aqueous-methanol extracts of the wood of *Taxus yunnanensis* displayed DPPH radical scavenging and nitric oxide (NO) inhibitory activities (Banskota et al., 2003).

*Taxus wallichiana* Zucc. (Himalayan Yew) is a small to medium-sized evergreen tree, growing 10–20 m tall, exceptionally up to 28 m. The leaves are flat, dark green, arranged spirally on the stem. It is one of the oldest native plants known in the northern area of Pakistan. Literature survey revealed that this plant is used traditionally for treatment of high fever and acute painful conditions (Kaul, 1997). Leaves of the plant are used to make herbal tea for indigestion and epilepsy (Baquar, 1995; Ahmed, 1997).

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However, there is no report to our knowledge on its analgesic, antipyretic and anticonvulsant activities. Keeping this in view, the present study was undertaken to investigate the analgesic, antipyretic and anticonvulsant potential of the methanol leaf extract of *T. wallichiana* Zucc. in experimental animal models.

1. Materials and methods

1.1. Plant material

Plant material was collected from ‘Hazara’ division of the North-western Frontier Province, Pakistan, in March 2005, authenticated by Prof. Dr. Jahandar Shah a taxonomist and Vice-Chancellor, University of Malakand, Dir, Pakistan. A specimen having voucher number MU-027 (2006) was deposited in the National herbarium of the same institution. The aerial parts of the plant were air-dried under shade for 2 weeks at room temperature. The dried plant material was later on chopped, pulverized and stored in a polyethylene bag under refrigeration for further experimentation.

1.2. Extraction of plant material

The dried and powdered leaves (2.5 kg) were macerated in methanol for a period of 48 h. After filtration, the process was repeated three times using 2.5 L methanol each time. The crude methanol extract (357 g, 14.28% (w/w)).

1.3. Phytochemical tests

The extracts of the plant material were screened for various classes of natural products using standard qualitative methods as described by Harborne (1973). The test for tannins was carried out by subjecting 1 g of each plant extract in 2 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For alkaloids, the test was carried out by subjecting 1 g methanolic extract in 10 ml 1% HCl, boiled, filtered and Mayer’s reagent. The extract was subjected to frothing test for the identification of saponins. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnins, and potassium hydroxide solution. Diterpenoids were detected by spraying TLC with ceric sulphate reagent. Steroids were screened by adding 1 ml of acetic anhydride to 0.25 g methanolic extract of each sample with 1 ml H2SO4. The color changed from violet to blue or green in some samples indicating the presence of steroids. The test for anthraquinones was performed by adding 1 g of extract to 2 ml benzene, filtered and ammonia solution added. For detecting coumarins, a piece of filter paper was moistened in NaOH and then kept over a test tube with boiling plant extract solution. If the filter paper later showed any yellow fluorescence under UV light, that was taken to indicate a positive test for coumarins. Detection for any sterols and terpenes in the extract involved treatment of the extract with petroleum ether and followed by extraction with CHCl3. The subsequently acquired CHCl3 layer was treated with acetic anhydride and concentrated HCl. The change of pink to purple and green to pink colors was indicative of presence of terpenes or sterols, respectively.

1.4. Animals

Adult Wistar rats (180–260 g) and Swiss albino mice (18–25 g) of either sex were maintained at the Animal House at HEJ Research Institute of Chemistry, University of Karachi, under standard environmental condition of temperature (25 °C) and light/dark cycles (12/12 h). Experiments were performed according to the guide for the care and use of laboratory animals, from the US Department of Health and Human Services, Institute of Laboratory Animal Resources, Washington, DC, 1985.

1.5. Drugs and reagents

Acetic acid (Hopkin and Williams, England); pentylenetrazole (PTZ), diazepam, paracetamol (Sigma Chemical Co., USA); aspirin, formalin and sodium chloride (Merck, GmbH); and yeast (Vahine Professional, France) were used. Sterile normal saline (10 ml/kg, i.p.) was used as control in all studies while the dose levels of the extract (50, 100 and 200 mg/kg, i.p. and s.c.) prepared in distilled water were employed for the test groups. Aspirin (150 mg/kg, i.p) diazepam (7.5 mg/kg) and paracetamol (20 mg/kg, i.p.) were used as the reference drugs.

1.6. Antinociceptive activities

1.6.1. Acetic acid-induced writhing

The acetic acid abdominal constriction test (Koster et al., 1959) was used with modification according to Adzu et al., 2001. Mice were divided into five groups of six mice each and starved for 18 h. Group 1 serving as control received saline 10 ml/kg, i.p.; groups 2, 3 and 4 received the test extract 50, 100 and 200 mg/kg, i.p. respectively; group 5 received acetylsalicylic acid ASA: 150 mg/kg, p.o. After half an hour, all mice received a 0.7% aqueous solution of acetic acid 10 mg/kg, i.p. and writhings were counted for 10 min after the acid injection.

1.6.2. Formalin test

The method of Dubisson and Dennis (1977), as modified by Tjolsen et al. (1992), was used. Briefly, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the rat hind paw, 30 min after treating the animals with the extracts (50, 100 and 200 mg/kg i.p.). Behavioral responses detected were recorded as scores in the following manner: rat walking or can stand on injected paw, 0; paw partially elevated, 1; total elevation of injected paw, 2; injected paw licking or biting, 3. Scores of the first 10 min after formalin were recorded as the first phase of algesia, while the period between 15 and 60 min was recorded as the late phase of pain.

1.7. Antipyretic activity (yeast-induced pyrexia)

The procedure of Al-Ghamdi (2001) was used for the antipyretic studies. The test was performed in rats by inject-
ing 10 ml/kg s.c. of 15% aqueous solution of yeast (Vahine Professional, France) to induce pyrexia. Rectal temperature of each animal was taken before and 24 h after the yeast injection using digital clinical thermometer (Hartmann, Germany). Animals that did not show a minimum increase of 0.5 °C in temperature 24 h after yeast injection were discarded. The selected animals were grouped into five \( n = 6 \) and treated with saline, extract or paracetamol (Vimala et al., 1998). The rectal temperature of each animal was again recorded at 0.5, 1, 1.5 and 2 h after treatment. Antipyretic effect was rated as the ability of test articles to reverse the induced pyrexia.

1.8. PTZ-induced convulsions

A 90-mg/kg dose of PTZ was administered intraperitoneally 30 min after the intraperitoneal or subcutaneous injection of the plant extract (50, 100, and 200 mg/kg) to mice that were grouped into eight \( n = 6 \). Latencies to the onset of mioclonic (twiches), clonic (jerks), tonic (seizers), tonic–clonic seizures or hind limb tonic extension (HLTE) and the mortality were evaluated during 30 min after PTZ injection (Swinyard and Kuperberg, 1985). Control animals were administered with vehicle and diazepam (7.5 mg/kg) as reference drug.

1.9. Data analysis

Results of the study were expressed as mean ± S.E.M Student t-test was used to analyse data between groups and analysis of variance (ANOVA) among groups followed by Dunnet’s test for multiple comparisons. \( P < 0.05 \) was considered significant in all cases.

2. Results

2.1. Phytochemical tests

The crude extract was found to be positive for the presence of alkaloids, diterpenes, steroids, saponins, tannins, flavonoids (phenols and polyphenols), and anthraquinones. However the crude extract was found negative for the presence of coumarins.

2.2. Effect on acetic acid-induced writhing

The extract 50, 100 and 200 mg/kg, i.p. significantly reduced the acetic acid-induced writhing by 32.93, 67.77 and 74.29% respectively (Table 1).

2.3. Effect on formalin test

The extract showed a significant \( P < 0.05 \) inhibition of the formalin noxious stimulation on both early and late phases of pain at doses of 50, 100 and 200 mg/kg, respectively, in rats (Table 2).

2.4. Antipyretic effect

The results of the antipyretic study showed that intraperitoneal administration of the plant extract at 50 and 100 mg/kg caused a significant \( P < 0.05 \) while 200 mg/kg showed very significant \( P < 0.01 \) inhibition of the pyrexia induced by yeast (Fig. 1). The antipyretic effect of 200 mg/kg plant extract was comparable to that of paracetamol (20 mg/kg i.p.) between 30 and 60 min after treatment.

2.5. Anticonvulsant effect

PTZ (90 mg/kg) induced mioclonic, clonic and tonic and tonic–clonic seizures (HLTE) convulsions in 100% of control
mice. Pretreatment with methanol extract both intraperitoneally and subcutaneously at doses 50, 100 and 200 mg/kg significantly reduced the latency of Mioclonus (P < 0.05). In case of clonus, the extract (100 and 200 mg/kg) delayed the onset of first clonus (P < 0.01), and tonus (by 200 mg/kg s.c. dose, P < 0.01) induced by PTZ. The extract in all three doses through both routes fully protected the mice from tonic–clonic seizurs (HLTE). Percentage of animals showing mortality was also decreased. Diazepam showed a remarkable anticonvulsant activity at 7.5 mg/kg (P < 0.01). It is evident from the data (Table 3), that the subcutaneously administered treatments produced better results when compared with the intraperitoneal route.

3. Discussion

This study has investigated the scientific reasons behind the folkloric use of T. wallichiana Zucc. in the management of painful conditions, pyrexia and epilepsy. The results indicated that the methanol leaf extract of the plant was active against all the experimentally induced laboratory models of pain. Acetic acid-induced writhes is a sensitive procedure in detecting analgesic effect of medicinal agents (Collier et al., 1968). This pain mechanism is believed to involve, in part, local peritoneal receptors (Bentley et al., 1983) caused by peritoneal fluid concentration of PGE2 and PGF2α (Deraedt et al., 1980). The results of the formalin study, showed that both the aphasis (early) and tonic pain (late phase) were blocked by the extract.

It is, therefore, assumed that central mechanisms may be involved in the observed phenomenon since the extract could elicit activities against both models of pain. Agents that exhibit these activities are believed to act primarily on the central nervous system. The peripherally acting substances would inhibit only the aphasis pain (Tjolsen et al., 1992). The ability of the extract to have effect against both phases of pain suggests that both central and peripheral pain inhibition mechanisms are likely to be involved. The extract caused a significant hypothermal activity against yeast-induced pyrexia in rats. Subcutaneous injection of yeast induces pyrexia by increasing synthesis of prostaglandin and is used to screen agents for antipyretic effect (Al-Ghamdi, 2001). The Antinociceptive and antipyretic activities may be attributed to the presence of alkaloids, phenols, polyphenols, saponins, tannins, anthraquinones, steroids and especially the diterpenes (i.e. taxoids) found in crude extract and the fractions thereof. The anticonvulsant activity of T. wallichiana Zucc. was also significant, which strongly supports its folkloric use for the treatment of epilepsy. PTZ is the most frequently used substance in a preliminary screening to test potential anticonvulsant drugs (Swinyard and Kupferberg, 1985). The mechanism by which PTZ is believed to exert its action may be attributed to by acting as an antagonist at the GABA A receptor complex (Ramanjaneyulu and Ticku, 1984). Drugs protecting against tonic–clonic seizurs induced by PTZ are considered to be useful to control mioclonic and absence seizurs in humans.

The anticonvulsant effect observed in the model experiment scientifically reinforces the use of this plant for the treatment of convulsions in traditional medicine (Ahmed, 1997). This study seems to be very interesting in the sense that it is reported for the first time throughout genus of this plant. Furthermore, anticonvulsant effect of T. wallichiana was compared with that produced by the GABA A agonist diazepam, a potent antiepileptic drug, highly effective to prevent convulsions induced by PTZ (Gasior et al., 2000). The benzodiazepine site in the GABA A receptor and even T-type Ca2+ currents could be targets for future studies to learn about the mechanisms of action of the T. wallichiana extract and/or its constituents.

In conclusion, this study demonstrates that the methanol extract of T. wallichiana leaves possesses analgesic, antipyretic and anticonvulsant activities which may explain the folkloric use of the plant in epilepsy, pyrexia and acute pain.

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References


