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The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models

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ABSTRACT

Aim of the study: The current study was designed to establish the pharmacological rationale for the traditional use of the rhizomes of *Polygonatum verticillatum* in the treatment of painful conditions and as a plant diuretic.
Materials and methods: The crude methanolic extract of the rhizomes of *Polygonatum verticillatum* (PR) was tested in various established pain models in rodents at 50, 100 and 200 mg/kg i.p. while the diuretic activity was assessed at 300 and 600 mg/kg p.o. in rats.
Results: PR demonstrated significant reduction (14–72%) in the number of writhes induced by acetic acid in a dose-dependent manner. When nociceptive threshold was measured in the formalin test, PR strongly attenuated the formalin-induced flinching behaviour in both phases (6–30% in first phase while 12–72% in second phase). Central involvement in the analgesic profile of PR was confirmed by the hot plate test, in which PR elicited a significant ($P < 0.01$) analgesic activity by increasing latency time. However, an opioid receptor antagonist, naloxone (2 mg/kg s.c.) strongly antagonized the antinociceptive activity of PR. As a plant diuretic, PR showed mild but statistically insignificant diuretic activity at 300 mg/kg. The crude extract and solvent fractions of the plant contained reasonable quantity of total saponin and alkaloid contents.
Conclusions: The mechanisms underlying the analgesic action of PR shows that the opioid dependant central mediation has synergistic effect by enforcing the peripheral analgesic effects. Interestingly, our findings not only substantiated the folk use of the plant as an analgesic but also reported for the first time in the whole genus.

1. Introduction

*Polygonatum (*King Solomon's-seal, Solomon's Seal) is a genus of about 57 species belongs to family Liliaceae or Convallariaceae. It is widely distributed in East Asia, mainly China and Japan where 40 species of *Polygonatum* are found (Tamura, 1993; Monika et al., 2006). Solomon's seal has been used for thousands of years in herbal medicine. The rhizomes are antiperiodic, antitussive, cardiotonic, demulcent, diuretic, energizer, hypoglycemic, sedative, tonic and are used in the treatment of dry coughs and pulmonary problems, including tuberculosis (Jiang, 1977, 1986; Flora of China, 1980). The antibacterial and antifungal activity of the *Polygonatum* has been reported (Alluri et al., 2006). *Polygonatum* reduced blood sugar level by different mechanisms including α-glucosidase inhibition and increased insulin sensitivity (Li et al., 2004; Hong et al., 2008). The cytotoxic activity with human MCF-7 breast cancer cells of various steroidal saponins isolated from the Rhizomes of *Polygonatum* is also on the record (Mi-Jeong et al., 2006).

Polygonatum verticillatum [L.] All. (Nooreallam) is a perennial rhizomatous herb. The rhizomes are usually shortly branched and 0.7–1.5 cm thick. Stem is usually erect and the leaves are four to eight in a whorl; the flowers are hermaphrodite. The syrup of fresh rhizome is used in the treatment of pain, pyrexia, burning sensation and for phthisis (Amrit, 2006). It is also used in combination with other herbs to promote urine discharge (diuretic) and removes painful urine (Ballabh et al., 2008). Other ethnobotanical uses of the plant include as emollient, aphrodisiac, vitiated condition of pitta and vata, appetizer and tonic, galactagogue (increases milk release), weakness (Ghayur, 2004). It is also used as a substitute of *Polygonatum cirrhifolium* (Parveen et al., 2004). Keeping in view

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the ethnobotanical use of the plant in the treatment of painful conditions, the current study was designed to provide scientific background to this claim.

2. Materials and methods

2.1. Plant material

Polygonatum verticillatum All was collected from District Swat (Gabral and Miandam valleys), N.W.F.P, Pakistan, in July–August 2007. The botanical identity of the plant material was done by the Taxonomy Department of PCSIR Laboratories Peshawar and a specimen with catalogue No.: 9970 (PES) was submitted to the herbarium of PCSIR Laboratories Peshawar.

2.2. Plant extraction and fractionation

The air dried rhizomes of the *Polygonatum verticillatum* (8 kg) was ground to fine powder and extracted by maceration with methanol at room temperature for 14 days with occasional shaking (Khan et al., 2007, 2009). The methanol soluble residue was filtered off and concentrated under vacuum at low temperature (40 °C) using rotary evaporator, yielded a dark greenish semisolid material (2.2 kg, 27.50%, w/w). The crude methanol extract (1.6 kg) was dissolved in distilled water and sequentially fractionated with hexane, chloroform, ethyl acetate and butanol, yielding hexane (258 g, 16.13%, w/w), chloroform (219 g, 13.69%, w/w), ethyl acetate (226 g, 14.13% w/w), butanol (265 g, 16.56%, w/w), and aqueous (501 g, 31.31%, w/w). These fractions were then screened for various pharmacological and phytochemical analysis.

2.3. Animals

Swiss albino mice (20–25 g) and Wistar rats (210–270 g) of either sex were used in various experimental models. Animals were housed 10 per cage and were fed laboratory diet *ad libitum* and allowed free access to drinking water under standard environmental condition of temperature (25 °C), relative humidity 60 ± 10% and light/dark cycles (12/12 h). Experiments were performed according to ethical principles established in 1979 for laboratory animals at the service of mankind Lyons, France.

2.4. Drugs and reagents

The chemicals used in this study include: aspirin (Reckitt and Colman, Pakistan), acetic acid, formalin, morphine sulphate, naloxone hydrochloride, sodium chloride hydrochlorothiazide (Sigma chemicals company, St. Louis, USA) Sterile normal saline was used as control in all studies and the crude methanol extract (PR) used in various studies were prepared in normal saline.

2.5. Acute toxicity studies

The acute toxicity test for PR was carried out to evaluate any possible toxicity. Swiss albino mice ($n = 6$) of either sex were tested by administering different doses of PR by increasing or decreasing the dose, according to the response of animal (Bruce, 1985). The dosing patron was 500, 1000 and 2000 mg/kg p.o., while the control group received only the normal saline. All the groups were observed for any gross effect or mortality during 24 h.

2.6. Antinociceptive activity

2.6.1. Visceral pain model (acetic acid-induced abdominal constriction)

The peripheral nociceptive activity was investigated by using the acetic acid-induced abdominal constriction test (Koster et al., 1959; Adzu et al., 2001). Briefly, the prescreened animals were divided into five groups ($n = 6$). The writhes were induced by intraperitoneal injection of 1.0% acetic acid (v/v, 0.1 ml/10 g body weight). Group I was used as control, received normal saline (10 ml/kg, i.p.); groups II, III and IV were treated with PR (50, 100 and 200 mg/kg, i.p.), respectively; group V received aspirin (100 mg/kg i.p.), as a standard drug. The number of muscular contractions was counted over a period of 20 min after acetic acid injection. The number of writhes in each treated group was compared with control (saline treated group) and the percent inhibition of the writhes was calculated.

2.6.2. Formalin test

The method used in our study for the assessment of formalin-induced flinching behaviour in normal rats was described previously (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). In this method, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the right hind paw, 30 min after treating the animals with the extracts (50, 100 and 200 mg/kg i.p.). Nociceptive behaviour was quantified as rat walking or can stand on injected paw; paw partially elevated; total elevation of injected paw, injected paw licking or biting. Formalin injection induced a stereotyped response characterized by two well distinct phases; phase I started almost immediately and was short lasting (0–5 min) followed, by prolonged tonic phase II lasting (15–30 min). Morphine (3 mg/kg s.c.) was used as a standard drug.

2.6.3. Thermal nociception (hot plate test)

In thermal nociception (hot plate test) mice were screened by placing them on a hot metal plate maintained at 50 ± 0.05 °C (Dar et al., 2005). The mice were treated either with vehicle (10 ml/kg i.p.), PR (50, 100 and 200 mg/kg i.p) or morphine (10 mg/kg s.c.) an opioid analgesic as a standard drug. Thermal nociception was estimated by measuring withdrawal response latency in the form of jumping, withdrawal of the paws or the licking of the paws. In the pretreatment session, mice were tested on two separated occasions, each 30 min apart and then only those mice were selected for the study, which responded within 15 s and which showed comparatively similar results. The response latencies were recorded at 0, 30, 60, 90 and 120 min with a cut off period of 30 s to avoid damage to the paw in the absence of response. In order to investigate the participation of the opioid system in the antinociceptive effect of PR, naloxone hydrochloride (2 mg/kg s.c.), a non-selective opioid receptor antagonist was injected, 15 min prior to the administration of test samples, as explained above and the hot plate latencies were sequentially measured at 0, 30, 60, 90 and 120 min.

2.7. Diuretic activity

The diuretic activity of PR was determined by method previously established (Jabeen et al., 2009). Male Albino rats were divided in to four groups ($n = 6$). The animals were fasted for 24 h and were fed laboratory diet *ad libitum* and allowed free access to drinking water. On the day of experiment, the animals of group I was treated with saline (15 ml/kg p.o) and this group served as control. Similarly, the animals of group II, III and IV were administered hydrochlorothiazide (10 mg/kg p.o) as standard drug, PR (300 and 600 mg/kg p.o), respectively. The extract was dissolved in saline and was suitably diluted for administration. Immediately after the drug treatment, the animals were placed in metabolic cages (1 animal in each metabolic cage). Urine was collected in graduated cylinders and its volume was recorded at 2, 3 and 6 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g body weight.