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Antinociceptive Effect of the Stem of *Polygonum multiflorum*

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Abstract – Polygoni multiflori Ramulus, the stem of *Polygonum multiflorum* Thunb., has been widely used as a traditional medicine for the treatment of many diseases. Presently, antinociceptive tests of the butanolic fraction of *P. multiflorum* (SPB) were performed using several thermal and chemical pain models. SPB had strong and dose-dependent antinociceptive activities, both thermal and chemical, compared to the reference drugs Tramadol and Indomethacin. In combination with naloxone, the analgesic activity of SPB was unchanged indicating that the antinociceptive activity of SPB was not due to action as an opioid receptor agonist. The present results indicate the potential of SPB as an analgesic agent for pain control.
Keywords – *Polygonum multiflorum*, antinociceptive, analgesic

Introduction

The International Association for the Study of Pain has defined the pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979). However, there is still a need for effective painkillers. In this regard, new drugs obtained from natural products have been receiving a lot of attention and many plant-derived compounds present effective antinociceptive activities (Calixto *et al*., 2000).

Dried stems of *Polygonum multiflorum* Thunb. have been widely used as oriental medicine for the treatment of insomnia and hyperhidrosis. The major chemical constituents of the root of *P. multiflorum* are stilbenes and anthraquinones. *P. multiflorum* root possess anti-oxidant activity (Chiu *et al*., 2002), myocardial protective activity (Yim *et al*., 1998), neuroprotective activity (Wang *et al*., 2006; Li *et al*., 2005), anti-aging activity (Xiao *et al*., 1993), estrogenic activity (Kang *et al*., 2006; Zhang *et al*., 2005), cognitive enhancing activity (Um *et al*., 2006; Chan *et al*., 2003), anti-mutagenic activity (Zhang *et al*., 1999), and can relieve arthritic pain and skin infections (Li *et al*., 2003). Recently, we reported that the methanolic extract of the stem of *P. multiflorum* has inhibitory activity on the inflammatory mediators production in lipopolysaccharide (LPS)-stimulated murine macrophages (Cha and Jeon, 2009). In addition to its anti-inflammatory properties, the stem of *P. multiflorum* has been traditionally used as a painkiller. Nevertheless, there is no scientific report on the antinociceptive activities of the stem of *P. multiflorum*.

Thus, we evaluated the antinociceptive action of the butanolic fraction of the stem of *P. multiflorum* (SPB) using experimental models of pain evaluation including thermal nociception, such as tail immersion test and hotplate test, and chemical nociception induced by intraperitoneal acetic acid and subplantar formalin in mice. We also performed combination test with naloxone to verify the relation to opioid receptor.

Experimental

Plant material – The plant materials were purchased from Hainyakupsa (Jeonbuk, South Korea) in October 2009. A voucher specimen (WH005) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University.

Extraction and solvent fractionation – An extract was obtained twice from the dried sample (3 kg) with 12,000 mL of methanol (MeOH) under sonification for 2 h. The resultant methanolic extract was concentrated into 60.7 g (yield 3.035%) using a rotary evaporator. The sample was then subjected to successive solvent partitioning to give *n*-hexane (0.83 g), CH_2Cl_2 (16.4 g), ethyl acetate (EtOAc) (21 g) and *n*-butanol (BuOH) (15.4 g) soluble fractions. Each fraction was lyophilized and then stored at

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–20 °C until use. Preliminary experiments showed that, among the four fractions, the *n*-BuOH fraction (SPB) had the most potent pharmacological potential. Thus, further studies were conducted using SPB.

Experimental animals – ICR mice (6 weeks old, males and females) weighing 20 - 25 g were supplied by Damul Science (Daejeon, Korea). All animals were housed at 22 ± 1 °C with a 12 h light/dark cycle and fed a standard pellet diet with tap water *ad libitum*. The experimental protocols complied with the recommendations of International Association for the study of pain (Zimmermann, 1983).

Grouping and drug administration – Animals were randomly assigned into several groups, each consisting of 8 or 10 mice for analgesic tests. Negative controls were treated with the same volume of distilled water which was used for reconstitution. Positive controls were treated with standard drugs: Tramadol (i.p.) or Indomethacin (p.o.). Treatment groups in each test were treated orally with different doses of SPB.

Acute toxicity test – In order to evaluate possible toxicity, an acute toxicity test was carried out. Mice (n = 6) were tested by administering different doses of SPB by increasing or decreasing the dose, according to the response of the animal (Bruce, 1985), while the control group received only an equal volume of distilled water. All the groups were observed for any gross effect or mortality during 24 h.

Tail immersion test – In the present study, the tail immersion test was investigated according to the protocol of Wang *et al*. (2000) with minor modification. Briefly, the lower two-thirds of mice tails were immersed on a water bath set at temperature of 50 ± 0.2 °C. The reaction time for the mouse to withdraw the tail was measured 0, 30, 60, 90 and 120 min after administration of SPB (250, 500 mg/kg; p.o.) or Tramadol (10 mg/kg; i.p.). To avoid tissue injury, cut-off time was chosen as 20 s.

Hotplate test – The hot-plate test (Franzotti *et al*., 2000) was carried out on groups of male and female mice using a hot-plate apparatus, maintained at 55 ± 1 °C. Only mice that showed initial nociceptive responses (licking of the forepaws or jumping) between 7 and 15 s were used for further experiments. The selected mice were pre-treated with SPB (250, 500 mg/kg; p.o.) or vehicle (distilled water) and 30 min later the measurement started. Tramadol (10 mg/kg; i.p.)-treated animal group was included as a positive control. The cut-off time was set at 30 s in order to minimize skin damage. The reaction time was calculated as described in the tail immersion test.

Acetic acid-induced writhing test – Antinociceptive activity of SPB was detected as previously described (Olajide *et al*., 2000). The response to an i.p. injection of acetic acid solution (1% in 0.9% saline), consisting of abdominal constriction and hind limbs stretching, was measured for each mice, starting 10 min after acetic acid injection during the following 10 min period. Each experimental group of mice were treated orally with vehicle (distilled water), SPB (250, 500 mg/kg) or Indomethacin (10 mg/kg) 1 h prior to acetic acid injection.

Formalin test – In a formalin test (Santos and Calixto, 1997), groups of mice were treated orally with vehicle (distilled water), *n*-BuOH fraction of SPB (250, 500 mg/kg) and after 30 min, each mouse was given 20 µl of 5% formalin (in 0.9% saline, subplantar) into the right hind-paw. The duration of paw licking as an index of painful response was determined at 0 - 5 min (first phase, neurogenic) and 20 - 35 min (second phase, inflammatory) after formalin injection. Tramadol and Indomethacin were used as positive control drugs, which was administrated 30 min before the test at the dose of 10 mg/kg, i.p. and p.o., respectively. In order to examine the possible connection of endogenous opioids in the antinociceptive activity, SPB-, Tramadol- and Indomethacin-treated groups were pretreated with naloxone (5 mg/kg; i.p.) 15 min before drug administration.

Statistical analysis – All data are expressed as the mean ± S.E.M. Data was subjected to Student's unpaired 2-tailed *t*-test and the *p*-values < 0.01 were considered to indicate statistical significance.

Results and Discussion

SPB showed strong analgesic activities on both central and peripheral nociception against thermal and chemical nociception tests in mice. First, in order to test possible toxicity of SPB on animals, an acute toxicity test was conducted. Various concentrations of SPB (up to 2000 mg/kg) were administered to animals. Treated mice did not present any behavioural alterations, convulsion and death during the 24 h period of assessment.

Thermal nociception models such as tail immersion and hotplate tests were used for the determination of central antinociceptive activity. SPB showed analgesic activities in both tail immersion and hotplate tests, implicating both spinal and supraspinal analgesic pathways. In both tests, Tramadol exhibited rapid effect with a maximum peak in a short time, similar to the action of opioid agonists (e.g. morphine), whereas SPB reached highest analgesia level at 60 min after administration (Figs. 1 and 2). This difference in the maximum analgesic point could be explained by the methods of drug administration (i.p. or

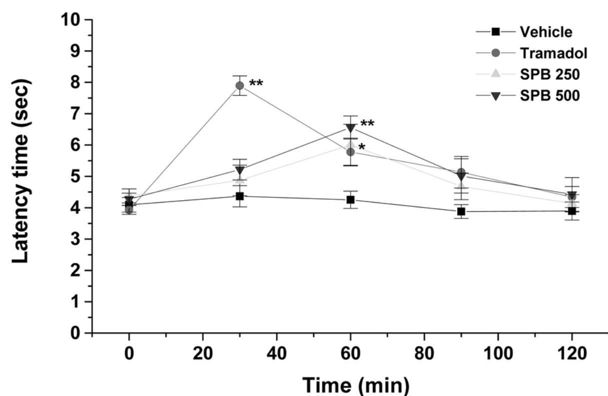

Fig. 1. Effect of SPB on the nociceptive response in tail immersion test. Mice were treated with Tramadol (10 mg/kg; i.p.), SPB (250, 500 mg/kg; p.o.), or distilled water. Values expressed as mean ± S.E.M. and units are in seconds (n = 10 - 12). Differences between groups were statistically analyzed by Student-*t* test. *$p < 0.01$ and **$p < 0.001$ compared to vehicle-treated group.

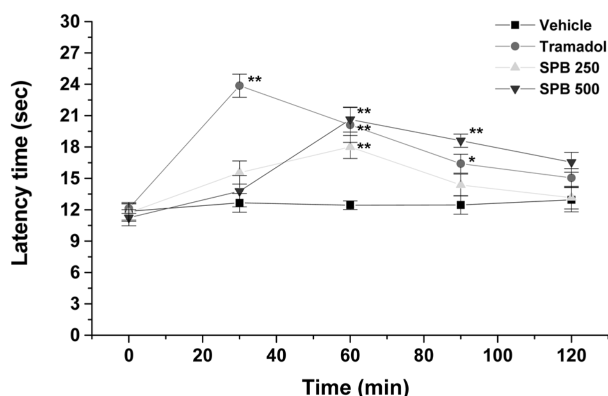

Fig. 2. Effect of SPB on the nociceptive response in hotplate test. Mice were treated with Tramadol (10 mg/kg; i.p.), SPB (250, 500 mg/kg; p.o.), or distilled water. Values expressed as mean ± S.E.M. and units are in seconds. (n = 11 - 15) Differences between groups were statistically analyzed by Student-*t* test. *$p < 0.01$ and **$p < 0.001$ compared to vehicle-treated group.

p.o.) or metabolic rate of each drug.

Effect of SPB on peripheral nociception was determined using acetic acid-induced writhing model which is frequently used to estimate both central and peripheral analgesic effects of drugs (Fukawa *et al.*, 1980). The acetic acid-induced writhing test has been associated with increased level of prostaglandins (PGs), especially PGE_2, in peritoneal fluids (Derardt *et al.*, 1980). PGs induce abdominal constrictions via activation and sensitization of peripheral chemosensitive nociceptors (Dirig *et al.*, 1998), which has been linked to the development of inflammatory pain (Bley *et al.*, 1998). Therefore, one of the possible analgesic mechanisms of this test may be connected to the inhibition of the enzyme cyclooxygenase (COX). Non-

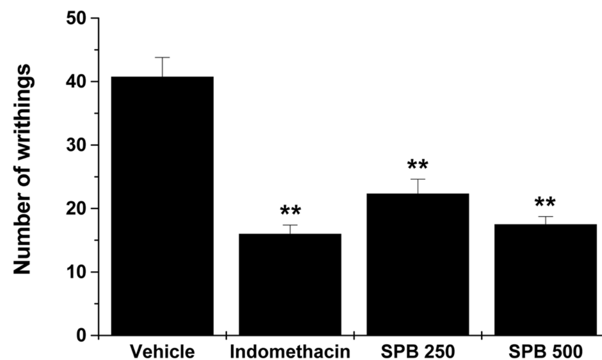

Fig. 3. Effect of SPB on the nociceptive response in acetic acid-induced writhing test. The mice were treated with Indomethacin (10 mg/kg), SPB (250, 500 mg/kg), or distilled water orally. Values expressed as mean ± S.E.M. (n = 8) Differences between groups were statistically analyzed by Student–*t* test. **$p < 0.001$ compared to vehicle-treated group.

steroidal anti-inflammatory drugs (NSAIDs) exert their peripheral analgesic potential consequent inhibition of PG synthesis; presently, Indomethacin produced a significant decrease in the writhing response. SPB also showed potent inhibition on acetic acid-induced abdominal constriction in a dose-dependent manner (Fig. 3). Since we previously reported the suppressed expression of COX-2 by MeOH extract of the stem of *P. multiflorum* (Cha and Jeon, 2009), this peripheral antinociceptive potential of SPB can be related to its inhibitory action on COX-2, at least in part.

Because the acetic acid-induced writhing test was not a distinct test to indicate if the potential resulted from central or peripheral, formalin test was conducted. The subcutaneous injection of formalin as a peripheral noxious stimulus causes biphasic nociceptive responses involving two different mechanisms (Hunskaar and Hole, 1987). The first phase (neurogenic pain) is caused by the direct chemical stimulation of nociceptive afferent fibers, predominantly C fibers, which can be suppressed by opiates like morphine (Amaral *et al.*, 2007). On the other hand, the second phase (inflammatory pain) results from the action of inflammatory mediators such as prostaglandins, serotonin, histamine and bradykinin in the peripheral tissues (Hunskaar and Hole, 1987), and of functional changes in the spinal dorsal horn (Dalal *et al.*, 1999). The results of the present study show that Tramadol, a central analgesic drug, is effective in preventing both the early and late phases of formalin-induced nociception, while Indomethacin, a NSAID, suppressed mainly in the later phase. These results are quite reasonable, since many reports suggest that the drugs which primarily act on the central nervous system inhibited equally in both phases,

Table 1. Effect of SPB on the nociceptive response in formalin test

Treatment	Dose (mg/kg)	Naloxone	Early Phase (0 - 5 min)		Late Phase (20 - 35 min)	
			Licking time (s)	Inhibition (%)	Licking time (s)	Inhibition (%)
Vehicle	–	–	116.36 ± 2.30	–	117.62 ± 8.75	–
Tramadol	10	–	60.31 ± 5.78	48.16**	26.49 ± 7.56	77.47**
	10	+	89.45 ± 6.86	23.12*,#	75.15 ± 7.20	36.10**,##
Indomethacin	10	–	122.82 ± 5.32	–5.55	57.97 ± 5.11	50.71**
	10	+	121.22 ± 5.00	–4.17	60.31 ± 5.15	48.72**
SPB	250	–	79.87 ± 6.30	31.36**	82.25 ± 7.20	30.07*
	500	–	71.00 ± 6.26	38.97**	56.43 ± 6.72	52.02**
	500	+	72.21 ± 6.94	37.94**	55.96 ± 7.63	52.42**

Values expressed as mean ± S.E.M. (n = 8 - 10)
Naloxone (5 mg/kg) was treatment was performed 15 min prior drug administration.
Differences between groups were statistically analyzed by Student–*t* test.
*$p < 0.01$ and **$p < 0.001$ compared to vehicle-treated group, while $^{\#}p < 0.01$ and $^{\#\#}p < 0.001$ compared to naloxone-untreated group.

while peripherally acting drugs, such as steroids and NSAIDs, mostly cause slight inhibition of the early phase of the formalin test (Vontagu *et al*., 2004; Hunskaar *et al*., 1985; Trongsakul *et al*., 2003). Presently, SPB could reduce the duration of the paw licking time obviously in both the first phase and the second phase representing suppressive properties on neurogenic as well as the inflammatory nociception (Table 1). These data provide further confirmation on the central effect of SPB apparent in the tail-flick and hotplate tests. Furthermore, in agreement with the results from the acetic acid test, SPB (500 mg/kg) also displayed better peripheral analgesic activities than that of Indomethacin.

In a combination test using naloxone, a non-selective opioid receptor antagonist, the antinociceptive action of Tramadol was reduced but still remained under the influence of naloxone. These results correlate with the previous studies that Tramadol is a partial opioid agonist having both opioid and non-opioid properties (Yalcin and Aksu, 2005). However, naloxone did not affect the antinociception of Indomethacin in the second phase. Interestingly, naloxone was totally unable to antagonize the analgesic action of SPB, which indicates that SPB-induced antinociception is affected by a non-opioid mechanism of action. Thus, these results show that the antinociceptive action of SPB is not related to opioid receptor. Based on these findings, it may be concluded that SPB has excellent antinociceptive properties involving central and peripheral mechanisms.

In summary, SPB has potent antinociceptive activities on both central and peripheral mechanisms. In addition, a combination test with naloxone revealed that SPB acts as a non opioid receptor agonist. Based on these results, SPB may hold great promise for use in many diseases as an effective painkiller.

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