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Sedative and Antinociceptive Properties of *Lindera obtusiloba*

Yong Jae Lee[1], Dong Keon Lee[1], Jong Soo Kim[1], Kyoung Jae Park[1], Dong Seok Cha[1], Dae Keun Kim[1], Jin Kwon[2], Chan Ho Oh[3], Kang San Kim[4], and Hoon Jeon[1,*]

[1]*College of Pharmacy, Woosuk University, Jeonbuk 565-701, Republic of Korea*
[2]*Department of Prosthetics and Orthotics, Korea National College of Rehabilitation and Welfare, Pyong-tack 459-070*
[3]*Department of Biotechnology, Graduate School, Woosuk University*
[4]*Department of Internal Medicine, College of Oriental Medicine, Wonkwang University*

Abstract – The stem of *Lindera obtusiloba* (Lauraceae), has been widely used as a traditional medicine for the treatment of abdominal pain, bruise and hepatocirrhosis. In the present study, antinociceptive and sedative properties of the methanol extract of *L. obtusiloba* (MLO) were evaluated. MLO demonstrated strong and dose-dependent antinociceptive activities on various experimental pain models including thermal nociception and chemical nociception, compared to tramadol and indomethacin, reference drugs. In combination test using naloxone, the diminished analgesic activity of MLO was observed, indicating the relation with opioid receptor. Moreover, MLO also decreases pentobarbital-induced sleep latency and increases sleeping time suggesting its hypnotic and sedative action. The present results indicate that MLO could be used as valuable antinociceptive and sedative agent for the treatment of various diseases.
Keywords – *Lindera obtusiloba*, Sedative, Antinociceptive

Introduction

Lindera obtusiloba Blume. (Lauraceae) is widely distributed in Korea and China. In Korea, *L. obtusiloba* have been used as a traditional medicine with beneficial effects for the treatment of inflammation, chronic liver diseases and improvement of blood circulation (Yook, 1989). Various phytosterols (Komae *et al*., 1972), obtusilactone derivatives (Niwa *et al*., 1975) and Lignans (Kwon *et al*., 1999) were analyzed from *L. obtusiloba*, and some of them have been found to possess anti-allergic, antifibrotic, antiplatelet, neuroprotective, and anti-inflammatory activities (Kim *et al*., 2009; Ruehl *et al*., 2009; Lee *et al*., 2010; Freise *et al*., 2010).

Despite of recent advances in pain control, there is still a need for effective painkillers. In this regards, new drugs originated from natural products has been receiving a lot of attention and many plant-derived compounds present effective antinociceptive activities (Calixto *et al*., 2000). The twigs of *L. obtusiloba*, was traditionally used as painkillers. Moreover, antinociceptive activities of *Lindera angustifolia* and *Lindera aggregata* which belonging to the lindera genus were previously reported (Li *et al*., 1997; Zhao *et al*., 2006). Nevertheless, there is no scientific report available in the literature on the antinociceptive and sedative activities of *L. obtusiloba*. Therefore, the present study was designed to validate the antinociceptive and sedative effects of the methanol extract of *L. obtusiloba* using various pain models and pentobarbital-induced sleeping model, respectively.

*Author for correspondence
College of Pharmacy, Woosuk University, Jeonbuk 565-701, Republic of Korea
Tel: +82-63- 290-1577; E-mail: hoonj6343@hanmail.net

Experimental

Plant material – The plant materials were purchased from Hainyakupsa (Chonbuk, South Korea) in June 2010. The plant was identified by Dr. Dae Keun Kim, College of Pharmacy, Woosuk University, Republic of Korea. A voucher specimen (WME068) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University.

Extraction of plant material – An extract was obtained twice from the dried sample (1000 g) with 12,000 ml of MeOH under sonification for 2 h. The resultant methanol extract was concentrated into 36.5 g (Yield : 3.65%) using a rotary evaporator. The sample was lyophilized and then stored at −20 °C until use.

Animals – ICR mice (6 weeks old, males and females)

weighing 20 - 25 g were supplied by Damul Science (Dajeon, Korea). All animals were housed at 22 ± 1 ºC 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 MLO.

Acute toxicity test – In order to evaluate possible toxicity, the acute toxicity test was carried out. Mice (n = 6) were tested by administering different doses of MLO (p.o.) by increasing or decreasing the dose, according to the response of animal (Bruce, 1985), while the control group received only the 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 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 ºC. The reaction time, necessary for the mice to withdraw the tail, was measured 0, 30, 60, 90 and 120 min after administration of MLO (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.

Hot plate 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 ºC. Only mice that showed initial nociceptive responses (licking of the forepaws or jumping) between 7 and 15 s were used for the further experiment. The selected mice were pre-treated with MLO (250, 500 mg/kg; p.o.) or vehicle (D.W.) and the measurement was started. A 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 damages. The reaction time was calculated as described in tail immersion test.

Acetic acid-induced writhing test – Antinociceptive activity of MLO was detected as previously described (Olajide *et al*., 2000). The response to an intraperitoneal injection of acetic acid solution (1% in 0.9% saline), consisting of abdominal constrictions and hind limbs stretchings, was measured for each mice, starting 5 min after acetic acid injection during the following 15 min period. Each experimental group of mice was treated orally with vehicle (D.W.), MLO (250, 500 mg/kg) or indomethacin (10 mg/kg) 1 h prior to acetic acid injection.

Formalin test – In formalin test (Santos and Calixto, 1997), groups of mice were treated orally with vehicle (D.W.) and MLO (250, 500 mg/kg). After 60 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 tiem (s) 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 a positive control drug, which was administrated 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, tramadol, indomethacin and MLO were investigated in groups of mice pretreated with naloxone (5 mg/kg; i.p.) 15 min prior to drug administration.

Evaluation of sleep latency and sleeping time using pentobarbital-induced hypnosis model – The sleeping time in mice was studied by the method of Dandiya and Collumbine (1959) with minor modification. The mice received diazepam (2 mg/kg i.p.), a positive control or different doses of the plant extract (250 and 500 mg/kg, p.o.). After 60 min, sodium pentobarbital (50 mg/kg i.p.) was administered and the sleep latency and sleeping time were recorded. The time required to induce loss of righting reflex was recorded as the sleep latency, while the time elapsed between the loss and recovery of righting reflex was considered as sleeping time. All experiments were carried out between 1:00 p.m. and 6:00 p.m.

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

Results and Discussion

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

Table 1. Effect of MLO on the nociceptive response in tail immersion test

Treatment	Dose (mg/kg)	Latency Period (min)				
		0	30	60	90	120
Vehicle	–	4.09 ± 0.23	4.07 ± 0.27	3.95 ± 0.22	3.83 ± 0.20	3.82 ± 0.30
Tramadol	10	3.92 ± 0.13	7.89 ± 0.31**	5.77 ± 0.44*	5.13 ± 0.50	4.34 ± 0.33
MLO	250	3.93 ± 0.17	4.73 ± 0.28	5.14 ± 0.59	4.04 ± 0.22	3.71 ± 0.27
MLO	500	3.77 ± 0.12	4.75 ± 0.17	6.36 ± 0.57**	5.06 ± 0.41	4.19 ± 0.22

Values expressed as mean ± S.E.M. and units are in seconds. (n = 8)
Differences between groups were statistically analysed by Student-*t* test.
*$p < 0.01$ and **$p < 0.001$ compared to vehicle-treated group.

Table 2. Effect of MLO on the nociceptive response in hotplate test

Treatment	Dose (mg/kg)	Latency Period (min)				
		0	30	60	90	120
Vehicle	–	11.85 ± 0.84	12.66 ± 0.90	12.43 ± 0.42	12.45 ± 0.88	12.95 ± 1.14
Tramadol	10	12.12 ± 0.46	23.86 ± 1.11**	20.11 ± 1.66**	16.40 ± 0.91*	15.05 ± 0.87
MLO	250	11.91 ± 0.44	13.45 ± 0.90	15.32 ± 0.80*	14.17 ± 0.89	13.87 ± 0.79
MLO	500	12.06 ± 0.56	16.17 ± 1.24	17.99 ± 1.08**	16.69 ± 0.86*	12.19 ± 0.79

Values expressed as mean ± S.E.M. and units are in seconds. (n = 8 - 9)
Differences between groups were statistically analysed by Student-*t* test.
*$p < 0.01$ and **$p < 0.001$ compared to vehicle-treated group.

Table 3. Effect of MLO on the nociceptive response in acetic acid-induced writhing test

Treatment	Dose (mg/kg)	Number of writhings (525 min)	Inhibition (%)
Vehicle	–	43.12 ± 2.74	–
Indomethacin	10	16.00 ± 1.39	62.89**
MLO	250	28.50 ± 1.55	33.91**
MLO	500	21.00 ± 1.24	51.30**

Values expressed as mean ± S.E.M. (n = 8 - 9)
Differences between groups were statistically analysed by Student-*t* test.
**$p < 0.001$ compared to vehicle-treated group.

Effect of MLO 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) in peritoneal fluids (Derardt *et al*., 1980). PGs induces abdominal constrictions via activating and sensitizing of the peripheral chemosensitive nociceptors (Dirig *et al*., 1998) which is largely associated to the development of inflammatory pain (Bley *et al*., 1998). It is well known that non-steroidal anti-inflammatory drugs such as aspirin and indomethacin exert their peripheral analgesic action via consequent inhibition of PG synthesis. MLO showed potent inhibition on the acetic acid-induced abdominal constrictions in a dose dependent manner (Table 3). This peripheral antinociception of MLO could be explained by its anti-inflammatory potential (Freise *et al*., 2010).

Since the acetic acid-induced writhing test was not a distinct test to indicate if the potential resulted from central or peripheral, formalin test was performed. The subcutaneous injection of formalin, as a peripheral noxious stimulus, causes biphasic nociceptive responses involving two different mechanisms (Hunskaar *et al*., 1985). The early phase (neurogenic pain) 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 late phase (inflammatory pain) results from the action of inflammatory mediators such as prostaglandins, serotonin, histamine and bradykinin in the peripheral tissues (Hunskaar *et al*., 1985), and of functional changes in the spinal dorsal horn (Dalal *et al*., 1999). The results of the present study have shown that tramadol, a central

Table 4. Effect of MLO 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**
Tramadol	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**
Indomethacin	10	+	121.22 ± 5.00	–4.17	60.31 ± 5.15	48.72**
MLO	250	–	80.73 ± 3.92	31.26**	76.57 ± 10.24	35.99*
MLO	500	–	68.35 ± 5.23	41.80**	67.16 ± 6.14	43.85**
MLO	500	+	91.98 ± 6.38	21.69*,#	74.77 ± 4.57	37.50**

Values expressed as mean ± S.E.M. (n = 8 - 10)
Naloxone (5 mg/kg) treatment was performed 15 min prior drug administration.
Differences between groups were statistically analysed 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.

Table 5. Sleeping inducing effect of pentobarbital and MLO

Group	Dose (mg/kg)	No. of fall asleep/Total	Sleeping latency time (sec)	Sleeping time (min)
Pentobarbital	40	3/10	170.3 ± 11.0	20.6 ± 14.9
Pentobarbital	45	6/10	157.7 ± 10.3	73.6 ± 6.6
Pentobarbital	50	10/10	119.3 ± 20.1	81.0 ± 25.9
MLO	500	0/10	0	0

Values expressed as mean ± S.E.M. (n = 10)

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, while peripherally acting drugs, such as steroids and NSAIDs are mostly can cause slight inhibition against the early phase of the formalin test (Vontagu *et al*., 2004; Hunskaar *et al*., 1985; Trongsakul *et al*., 2003). In this test, it was observed that MLO 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 4). These data provided a further confirmation on the central analgesic effect of MLO which showed in the tail-flick and hotplate tests. Furthermore, in agreement with the results from the acetic acid test, MLO also displayed a significant peripheral analgesic activity. Moreover, in combination study, naloxone exhibited biphasic blocking on the MLO's analgesic action moderately. Therefore, it was obvious that the opioid system is connected with the central and peripheral antinociceptive action of MLO, at least in part.

Similar to our results, Zhao *et al.* (Zhao *et al*., 2006) also noted anti-nociceptive effect of *Lindera angustifolia*, an allied plant of *L. obtusiloba*. They revealed that aporphine-type alkaloids from *L. angustifolia* are responsible for analgesic activity. Thus, we could speculate that similar alkaloid or alkaloid derivatives might be one of active constituents of *L. obtusiloba*. To answer this question phytochemical research on this plant should be done and it is beyond the scope of this work.

We further performed pentobarbital-induced sleeping test to evaluate the activity of MLO on the central nervous system (CNS). Although MLO did not induce sleep in itself, the mice treated with MLO exhibited relaxed condition. MLO also prolonged pentobarbital-induced hypnosis in a dose dependent manner. The MLO-mediated decreased sleep latency and increased sleeping time may be closely related to regulation of sleep mechanism in CNS or inhibition of pentobarbital metabolism (Gouemo *et al*., 1994; Kaul and Kulkarni, 1978). Since potentiation of pentobarbital-induced hypnosis is an indicator of sedation resulting from depression of CNS (Ozturk *et al*., 1996), our observation reflects that MLO has CNS depressant activity.

In summary, it was obviously demonstrated that MLO had potent antinociceptive activities on both central and peripheral mechanism as a partial opioid receptor agonist.

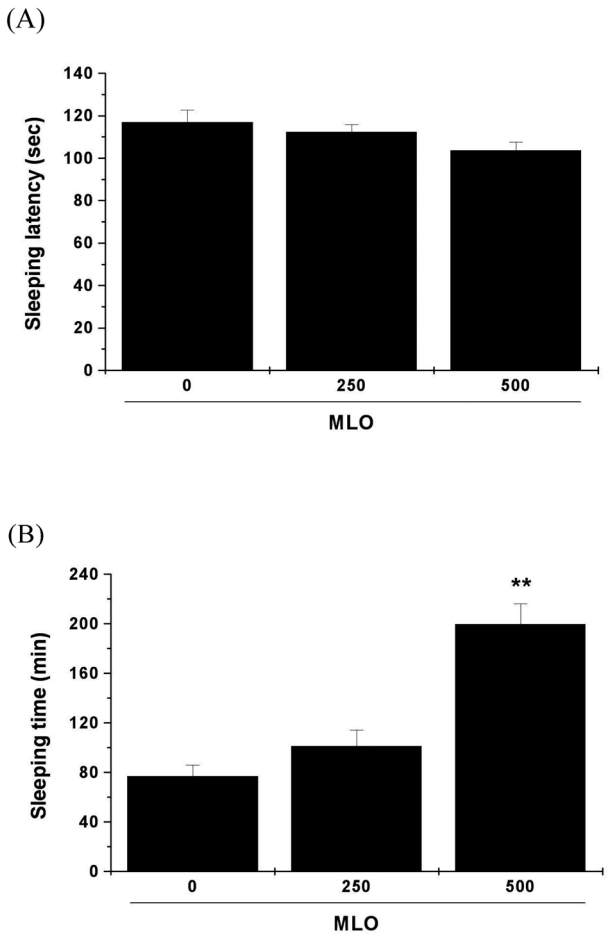

Fig. 1. Effect of MLO on sleeping latency and sleeping time in pentobarbital-treated mice. Mice were fasted for 24 h before the experiment. Administration of MLO and D.W, a vehicle was conducted 1 h prior to pentobarbital (50 mg/kg, i.p.) injection. The sleeping latency (A) and sleeping time (B) were recorded. All the present data represents mean ± S.E.M. Differences between groups were statistically analysed by Student-*t* test. $^{**}p < 0.001$ compared to vehicle-treated group.

MLO also showed sedative activity in the pentobarbital-induced sleeping test. Based on these results, MLO would have a great promise for use in many diseases as an antinociceptive and sedative agent.

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