Analgesic Activity of Myricetin Isolated from *Myrica rubra* Sieb. et Zucc. Leaves

Yan Tong¹, Xiao-Mian Zhou¹, Shu-Jun Wang², Yang Yang¹, and Ying-Lin Cao¹

¹Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang, PR China and ²Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, PR China

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Myrica rubra Sieb. et Zucc. leaves are commonly used as an astringent, antidiarrheic, and analgesics in folk medicine in China. In the present study, the analgesic activity of myricetin, a major compound in *Myrica rubra* Sieb. et Zucc. leaves was evaluated in vivo. The analgesic effect of myricetin was tested by a serial of models, such as acetic acid-induced writhing response, formalin-induced paw licking and hot plate test. The sedative activity was evaluated by pentobarbital-induced sleep time. Platelet aggregation induced by collagen and arachidonic acid was also performed in vitro. Myricetin showed a significant inhibition on chemical nociceptive models such as the acetic acid-induced writhing response and the licking time on the late phase in the formalin test in a dose-dependent manner, but did not manifest a significant effect in hot plate test. Myricetin was also not able to increase the sleeping time induced by pentobarbital, which further indicated that the analgesic effect of myricetin was unrelated to sedation. In addition, myricetin inhibited the content of PGE2 in the peritoneal fluid and platelet aggregation induced by collagen and arachidonic acid in vitro. These results collectively demonstrated that myricetin possessed potent analgesic activity, which was related with peripheral analgesia, but, not with the opioid system. Myricetin may be a potent COX-1 inhibitor with anti-platelet activity.

Key words: *Myrica rubra* Sieb. et Zucc., Myricetin, Analgesic activity

INTRODUCTION

*Myrica rubra* Sieb. et Zucc. belongs to the Myricaceae family, which is native to eastern Asia, especially China and also widespread in tropical, subtropical, and temperate areas of the world (Chen et al., 2004). In China, it is cultivated mainly in the south of Yangtze River. Zhejiang province is the top area producing bayberry fruit, with an annual production over 300,000 tons (data provided by Zhejiang Agricultural Office). The barks and leaves of *Myrica rubra* Sieb. et Zucc. have been used as an astringent, antidiarrheic, and analgesics in Chinese traditional medicine since ancient times (Matsuda et al., 2002).

Several flavonoids, tannins, triterpenes and diarylheptanoids have been isolated from the barks and leaves of *Myrica rubra* Sieb. et Zucc. (Nonaka et al., 1983; Sakurai et al., 1986, 1991). Cushnie et al. also isolate and identify that *Myrica rubra* Sieb. et Zucc. kernels and leaves contain myricetin (Cushnie and Lamb, 2005; Zou, 1995). In fact, myricetin in nature is widespread among plants including tea, berries, fruits, vegetables and medicinal herbs (Hertog et al., 1993; Ong and Khoo, 1997). It has been reported that quercetin, kaempferol, and myricetin are the three most common flavonols that are also the most widely distributed flavonoids in plants (Lee et al., 1995). Tang et al. also have reported one of main active component in *Myrica rubra* Sieb. et Zucc. bark and leaves is myricetin (Tang et al., 2006). Myricetin (Fig. 1), a flavonoid compound with hydroxyl substitutions at the 3,5,7,3',4' and 5' positions (Subramanian and Nair, 1972), has been re-
ported to possess antioxidative, antiproliferative and anticarcinogenic effects as major biological features (Gordon and Roedig-Penman, 1998). Myricetin also possesses neuroprotective effect on the Parkinson models both \textit{in vivo} and \textit{in vitro} through its anti-oxidation and anti-apoptosis activity (Yang et al., 2001). In addition, some preliminary work has shown that myricetin may be of benefit as an antithrombotic agent (Willoughby et al., 2002).

Although evidence is accumulating for the role of \textit{Myrica rubra} Sieb. et Zucc. leaves in analgesic activity, there are few stringent pharmacologic tests for this purpose thus far. In the present study, we evaluated the \textit{in vivo} analgesic profile of myricetin in several conventional nociceptive models and possible mechanism of action.

\section*{MATERIALS AND METHODS}

\subsection*{Plant material}

The leaves of \textit{Myrica rubra} Sieb. et Zucc. were purchased from Ningbo Sino-Taipio Herbal Science Co., Ltd., Zhejiang, China and identified by Prof. Jiu-Zhi Yuan (Shenyang Pharmaceutical University).

\subsection*{Extraction and isolation}

Crude powder of \textit{Myrica rubra} Sieb. et Zucc. was first extracted with 70\% (v/v) ethanol twice under reflux and concentrated under reduced pressure. The concentrated extracts were applied onto polyamide column, which was eluded with distilled water first to remove high polar material, then eluded with 50\%(v/v) ethanol. The collected eluate was dried with a rotavapor under reduced pressure and then was hydrolyzed with 1\% (v/v) H$_2$SO$_4$ for 40 min at 100°C. The hydrolyzate was dried once again and recrystallized twice with 100\% ethanol to get the final purified material.

\subsection*{Animals}

Male Swiss mice (18-22 g) were used for the analgesic study. Female mice (18-22 g) were used only in the hot plate test. Rabbits (2,000-2,500 g) were used for \textit{in vitro} platelet aggregation. The animals were maintained at room temperature (25 ± 3°C) in 12 h light/dark cycle, with both food and water ad libitum. All animals were used only once in all pain models. The research was conducted in accordance with the Regulations of Experimental Animal Administration issued by State Committee of Science and Technology of the People’s Republic of China on 14 November 1988.

\subsection*{Drugs}

Myricetin was suspended in 0.5\% CMC-Na in distilled water. Aspirin (100 mg/kg), morphine (10 mg/kg, i.p.) and diazepam (4 mg/kg) were used as a positive control, respectively. Blank control animals were administered with the corresponding vehicles. Test drugs were orally administered daily for 7 days at doses of 50, 100 and 200 mg/kg before the experiments in a volume of 20 mL/kg. All drugs were prepared just before use.

\subsection*{Acetic acid-induced writhing test in mice}

The acetic acid-induced writhing model in mice was carried out as described by Duarte et al. (1988). The writhings were induced by injection of an aqueous solution of 0.6\% acetic acid in a volume of 10 mL/kg body weight into the peritoneal cavity at 1 h after the final administration and the animals were then placed in a transparent plexiglass chamber. The number of writhings were observed for 20 min following the injection of acetic acid and the writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body. Aspirin (100 mg/kg) was used as a positive control.

\subsection*{Prostaglandin production in mice peritoneal cavity}

Acetic acid-induced prostaglandin formation in mice peritoneal cavity based on the method as previously described (Huang et al., 1993). At 5 min after injection of 0.6\% acetic acid, the mice were killed by cervical dislocation. The peritoneal cavity was exposed through a 15 mm incision and the lavage fluid was pipetted off and transferred to a polypropylene tube. The samples were centrifuged at 2,500 rpm for 10 min at 4°C and the supernatant (0.5 mL) was transferred to a polypropylene tube containing 2 mL of 0.5 mol/L KOH-methanol. The mixture was incubated at 50°C for 20 min, which was then dissolved in 5 mL methanol and centrifuged at 2,500 rpm for 10 min. The solution was read with UV-260 spectrophotometer at 278 nm.

![Fig. 1. Structure of myricetin](image)