Journal of Ethnopharmacology 208 (2017) 129–137

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Effects of alkaloid-rich extract from *Mitragyna speciosa* (Korth.) Havil. on naloxone-precipitated morphine withdrawal symptoms and local field potential in the nucleus accumbens of mice

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ARTICLE INFO

Keywords:
Mitragyna speciose
Morphine withdrawal
Opioid
Naloxone
Reward
Nucleus accumbens

ABSTRACT

Ethnopharmacological relevance: *Mitragyna speciosa* (Korth.) Havil. (*M. speciosa*) is among the most well-known plants used in ethnic practice of Southeast Asia. It has gained increasing attention as a plant with potential to substitute morphine in addiction treatment program. However, its action on the central nervous system is controversial.

Aim of the study: This study investigated the effects of *M. speciosa* alkaloid extract on naloxone-precipitated morphine withdrawal and neural signaling in the nucleus accumbens (NAc, brain reward center) of mice.

Materials and methods: The effects of *M. speciosa* alkaloid extract and mitragynine, a pure major constituent, on naloxone-precipitated morphine withdrawal were examined. Male Swiss Albino (ICR) mice were rendered dependent on morphine before injection with naloxone, a nonspecific opioid antagonist, to induce morphine withdrawal symptoms. The intensity of naloxone-precipitated morphine withdrawal was assessed from jumping behavior and diarrhea induced during a period of morphine withdrawal. To test possible addictive effect of *M. speciosa* alkaloid extract, mice were implanted with intracranial electrode into the NAc for local field potential (LFP) recording. Following *M. speciosa* alkaloid extract (80 mg/kg) and morphine (15 mg/kg) treatment, LFP power spectra and spontaneous motor activity were analyzed in comparison to control levels.

Results: One-way ANOVA and multiple comparisons revealed that *M. speciosa* alkaloid extract (80 and 100 mg/kg) significantly decreased the number of jumping behavior induced by morphine withdrawal whereas mitragynine did not. Additionally, *M. speciosa* alkaloid extract significantly decreased dry and wet fecal excretions induced by morphine withdrawal. LFP analysis revealed that morphine significantly decreased alpha (9.7–12 Hz) and increased low gamma (30.3–44.9 Hz) and high gamma (60.5–95.7 Hz) powers in the NAc whereas *M. speciosa* alkaloid extract did not. Spontaneous motor activity was significantly increased by morphine but not *M. speciosa* alkaloid extract.

Abbreviations: AP, Anteroposterior; ANOVA, Analysis of variance; BW, Body weight; CNS, Central nervous system; DV, Dorso-Ventral; FFT, Fast Fourier transform; HPLC, High performance liquid chromatography; ICLAS, International Committee on Laboratory Animal Science; LAAM, levo-alpha-acetylmethadol; LC-MS, liquid chromatographic mass spectrometry; LFP, Local field potential; ML, Medio-Lateral; NAc, Nucleus accumbens; PSD, Power spectral density; TLC, Thin layer chromatography; S.E.M., Standard error of the mean

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http://dx.doi.org/10.1016/j.jep.2017.07.008
Received 8 March 2017; Received in revised form 4 July 2017; Accepted 4 July 2017
Available online 05 July 2017

Conclusions: Taken together, *M. speciosa* alkaloid extract, but not mitragynine, attenuated the severity of naloxone-precipitated morphine withdrawal symptoms. Neural signaling in the NAc and spontaneous motor activity were sensitive to morphine but not *M. speciosa* alkaloid extract. Therefore, treatment with the *M. speciosa* alkaloid extract may be useful for opiate addiction treatment program.

1. Introduction

Mitragyna speciosa (Korth.) Havil. (*M. speciosa*), locally called Kratom in Thailand, is a member of the Rubiacaea plant family. It has been used as a medicinal plant for centuries (Jansen and Prast, 1988; Suwanlert, 1975). In modern studies, four alkaloids namely mitragynine, 7-hydroxymitragynine, corynantheidine and speciociliatine appeared to act on opioid receptor (Takayama et al., 2002). However, kratom consumption for opium-like effect was previously reported (Suwanlert, 1975). In Malaysia, there is a record that kratom was used as an affordable opium substitute (Burkill, 1935). However, the efficacy of kratom use as an opium substitute has not been clearly elucidated. There were still many questions needed to be answered in scientific ways.

The antinociceptive effects of this plant are well known and have been confirmed by many studies. The main component of the *M. speciosa* extract, mitragynine, has consistently exhibited antinociception centrally *via* the descending noradrenergic and serotonergic systems (Matsumoto et al., 1996) by acting on opioid receptors (Thongpradichote et al., 1998). However, the selectivity of mitragynine for opioid receptor subtypes is different from that of morphine (Matsumoto et al., 1996; Thongpradichote et al., 1998). In addition, the action of mitragynine on other physiological systems such as gastric acid secretion is also mediated *via* opioid receptors (Tsuchiya et al., 2002). These consistent data confirmed morphine-like actions of the plant and seemed to support an original idea that this plant could replace morphine in treatment programs (Jansen and Prast, 1988). Mitragynine was demonstrated to inhibit morphine withdrawal successfully *in vitro* (Watanabe et al., 1997). In particular, mitragynine was found to attenuate withdrawal syndrome in morphine-withdrawn zebrafish (Khor et al., 2011). These findings appeared to suggest that among many *M. speciosa* alkaloid components, mitragynine has gained the most attention as a potential morphine substitute. However, it remained to be examined whether mitragynine or crude *M. speciosa* alkaloid extract would show therapeutic effect for opiate withdrawal treatment.

Mitragynine is a major indole alkaloid of *M. speciosa* shown to have an antinociceptive action. However, 7-hydroxymitragynine, a minor constituent of this plant, also exhibits an even higher potency than mitragynine both *in vitro* (Takayama et al., 2002) and *in vivo* (Matsumoto et al., 2004a). Moreover, other alkaloid constituents of the plant may also exert potent therapeutic effects. Previously, *M. speciosa* alkaloid extract was found to act in the central nervous system (CNS) and produce antidepressant-like action *in vivo* models (Kumarnsit et al., 2007). Therefore, mode of CNS action of *M. speciosa* alkaloid extract has been tested extensively. Ultimately, its actions in addition to opioidergic mechanisms were also evidenced. The *M. speciosa* alkaloid extract was found to produce electroencephalographic patterns in the frontal and parietal cortices similarly to that of fluoxetine, a standard antidepressant drug (Cheaha et al., 2015b). These findings suggest other mode of *M. speciosa* action and possibility to apply this extract as an antidepressant-like compound.

From some qualitative studies in human, kratom is considered addictive with its patterns of use and symptoms (Saingam et al., 2013; Ahmad and Aziz, 2012). This was found as a major concern. However, classical drugs of addiction are known to act on the mesolimbic dopamine pathway as a reward system (Seiden et al., 1993; Koob, 1992). It remained to be examined whether the *M. speciosa* extract also has actions on the brain reward areas.

Opiate substances are known to produce dependence and the abrupt cessation causes withdrawal syndrome (Gold et al., 1978). Acute morphine withdrawal can also be induced in animal models. It emerges in morphine-dependent animals immediately after the administration of naloxone, a nonspecific opiate antagonist. Opioid receptor agonists including methadone have been used to relieve withdrawal symptoms (McMillan et al., 1976) but methadone also has side effects and can cause withdrawal by itself (Beswick et al., 2003; Gossop et al., 1989, 1987). Later, levo-alpha-acetylmethadol (LAAM), a synthetic μ opioid receptor agonist, was also found to have some potential advantages with better preference (Trueblood et al., 1978). However, use of LAAM was believed to be linked with arrhythmia (Clark et al., 2002). For more effective treatment of the opiate withdrawal syndrome, partial or non-opiate substances have also been sought for treatment of opiate addiction.

The *M. speciosa* alkaloid extract was hypothesized to reduce the intensity of opioid withdrawal. The effects of *M. speciosa* alkaloid extract on morphine withdrawal symptoms were tested. Jumping behavior and diarrhea during withdrawal period were measured as indicative data to represent the intensity of morphine withdrawal symptoms in mice. In this study, pure mitragynine and *M. speciosa* alkaloid extract were used for treatment of morphine withdrawal symptoms. In addition, addictive potential of *M. speciosa* alkaloid extract was examined. Following treatment with *M. speciosa* alkaloid extract, patterns of local field potential (LFP) in the nucleus accumbens, a brain reward center, and spontaneous motor activity were evaluated.

2. Materials and methods

2.1. Plant materials

Young leaves of *M. speciosa* Korth (Rubiaceae) were collected from natural sources in Songkla Province, Thailand. Plant materials were identified by Dr. Niwat Keawpradub, the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand where the herbarium voucher specimens (no. PCOG/MS001-002) have been deposited. The use of plant materials was approved by the Ministry of Agriculture of Thailand and was restricted to research purposes only.

2.2. Extraction and analysis of alkaloid extract from M. speciosa and mitragynine

An *M. speciosa* alkaloid-rich extract and mitragynine were prepared as described in previous studies (Cheaha et al., 2015a). The same batch of *M. speciosa* alkaloid-rich extract was also used for the present study within the same time period. For *M. speciosa* alkaloid-rich extract, young leaves were dried at 45–50 °C, powdered and macerated with methanol. The filtrate was evaporated *in vacuo*. The residue was dissolved in 10% aqueous acetic acid, filtrated and washed with petroleum ether, then made into alkaline (pH 9) with 25% ammonia solution and extracted with chloroform. The combined chloroform extracts that were washed, dried over anhydrous sodium sulphate and evaporated gave 0.25% of dry crude alkaloid extract. The extract was analyzed by using high performance liquid chromatography (HPLC). Mitragynine was used as a standard. HPLC analyses revealed the dominant peak of *M. speciosa* alkaloid-rich extract with the same retention time to mitragynine (Supp. Figs. 1 and 2, inset).