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Effects of an alkaloid-rich extract from *Mitragyna speciosa* leaves and fluoxetine on sleep profiles, EEG spectral frequency and ethanol withdrawal symptoms in rats

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ABSTRACT

Background: Many antidepressants are effective in alleviating ethanol withdrawal symptoms. However, most of them suppress rapid eye movement (REM) sleep. Thus, development of antidepressants without undesirable side effects would be preferable. Previously, crude alkaloid extract from *Mitragyna speciosa* (MS) Korth was found to produce antidepressant activities. It was hypothesized that the alkaloid extract from MS may attenuate ethanol withdrawal without REM sleep disturbance.
Methods: Adult male Wistar rats implanted with electrodes over the frontal and parietal cortices were used for two separated studies. For an acute study, 10 mg/kg fluoxetine or 60 mg/kg alkaloid extract from MS were administered intragastrically. Electroencephalographic (EEG) signals were recorded for 3 h to examine sleep profiles and EEG fingerprints. Another set of animal was used for an ethanol withdrawal study. They were rendered dependent on ethanol via a modified liquid diet (MLD) containing ethanol *ad libitum* for 28 days. On day 29, fluoxetine (10 mg/kg) or alkaloid extract from MS (60 mg/kg) were administered 15 min before the ethanol-containing MLD was replaced with an isocaloric ethanol-free MLD to induced ethanol withdrawal symptoms.
Results: The sleep analysis revealed that alkaloid extract from MS did not change any REM parameters which included average duration of each REM episode, total REM time, number of REM episode and REM latency whereas fluoxetine significantly suppressed all REM parameters and delayed REM latency. However, power spectral analysis revealed similar fingerprints for fluoxetine and alkaloid extract from MS characterized by decreasing powers in the slow frequency range in frontal and parietal cortical EEG. Neither treatment affected spontaneous motor activity. Finally, both alkaloid extract from MS and fluoxetine were found to significantly attenuate ethanol withdrawal-induced hyperexcitability (increases gamma activity) in both cortices and to reduce locomotor activity.
Conclusion: The present study demonstrated that the alkaloid extract from MS alleviates ethanol withdrawal severity with no side effect on REM sleep. In addition, these data suggest that suppressive effects on slow frequency powers but not REM sleep may be hallmarks of effective antidepressants for ethanol withdrawal treatment.

Introduction

Antidepressants have been consistently reported to be useful in treatment of ethanol withdrawal and dependence (Cheaha et al. 2014; Uzbay et al. 2004). However, their most common effect is a

Abbreviations: AP, anteroposterior; CPP, conditioned place preference; EEG, electroencephalographic; EMG, electromyography; FFT, fast Fourier transform; ICLAS, International Committee on Laboratory Animal Science; MLD, modified liquid diet; MS, *Mitragyna speciosa*; NREM, non-rapid eye movement; REM, rapid eye movement; SSRI, selective serotonin reuptake inhibitor; TLC, thin layer chromatography; FLU, fluoxetine; EW, ethanol withdrawal; ANOVA, analysis of variance.

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reduction of REM sleep (Thase 1998). Consistent findings indicate that antidepressant drugs including tricyclics, tetracyclics, monoamine oxidase inhibitors or selective serotonin reuptake inhibitors (SSRIs) produce substantial effects on REM sleep. In general, they prolong REM sleep latency and suppress REM sleep time in healthy subjects (Thase 1998; Rijnbeek et al. 2003) as well as laboratory animals (Ivarsson et al. 2005). A decrease in cholinergic activity and an increase in aminergic activity are two main factors in the REM sleep suppression mechanism of antidepressant drugs (Costa e Silva 2006). In terms of etiology, sleep disturbance is positively correlated with psychiatric illness (Costa e Silva 2006). In particular, REM disturbance was related to neurodegenerative disease such as Parkinson's (Naismith et al. 2010). REM analysis also showed high predictive power for Parkinson's symptoms (Mahale et al. 2014). Any enhancement of REM disturbance is likely to aggravate neurodegeneration. Thus, antidepressants that do not disturb REM are being sought to avoid undesirable effects. Until recently, only a few antidepressants have been demonstrated with no apparent REM sleep inhibition, such as trimipramine (Riemann et al. 2002).

Previously, *Mitragyna speciosa* Korth (MS) has been used as a medicinal plant. Its antinociceptive effects have been confirmed in animal studies (Thongpradichote et al. 1998). Recently, the focus has shifted to its antidepressant-properties. Mitragynine, a major alkaloid of the MS, was found to act on noradrenergic and serotonergic descending pathways (Matsumoto et al. 1996). Alkaloid extract from MS also stimulated rat dorsal raphe nuclei, the main site for serotonin biosynthesis, and produced antidepressant-like effects in mice (Kumarnsit et al. 2007a). In a study using a nociceptive model, alkaloid extract from MS exhibited higher potency than pure mitragynine (Horie et al. 2005; Watanabe et al. 1992). Antinociceptive actions of alkaloid extract from MS are believed to also rely on 7-hydroxymitragynine, a minor constituent reported to have stronger effects than morphine when administered orally (Matsumoto et al. 2004).

Serotonergic hypofunction has been found to underlie ethanol withdrawal symptoms. Various substances with serotonergic enhancing properties have been successfully used to ease ethanol withdrawal symptoms (Uzbay et al. 2004). In addition, the MS aqueous extract was effective in alleviating ethanol withdrawal in mice (Kumarnsit et al. 2007b). Hence, the alkaloid extract from MS was proposed to be a potential candidate for ethanol withdrawal therapy. However, its effects on sleep pattern were still uninvestigated.

The initial phase of ethanol withdrawal studies relied mainly on animal behavior observation. It was simple to detect obvious changes that reflect mild or severe symptoms. However, some discrepancies frequently arose depending on the symptoms or behaviors being assessed (Slawecki et al. 2006). Moreover, subjective methods of analysis were sometimes used for behavioral studies. To improve drug discovery, more scientific methods with objective biomarkers were utilized for bias-free analysis. Recently, Fast Fourier transform has been extensively applied for neurophysiological signal analysis, especially in brain research. This algorithm allows for scientifically qualitative (sleep–wake) and quantitative (electroencephalography, EEG power) analysis of electrical brain activity.

This study was performed in a rat model. Administration of pure mitragynine produced significant reward response in a conditioned place preference (CPP) test, whereas the alkaloid extract from MS did not (Sufka et al. 2014). This suggested a higher risk of possible substance dependence for mitragynine. Thus, alkaloid extract from MS was used. First, acute effects of the fluoxetine and alkaloid extract from MS were examined in order to characterize their sleep parameters and EEG fingerprints. Additionally, effects of these substances on ethanol withdrawal-induced physical and cortical hyperexcitabilities were examined. Changes in fast frequency oscillation, especially gamma activity, have been previously recognized as an index of cortical hyperexcitability induced by ethanol withdrawal (Cheaha et al. 2014; Slawecki et al. 2006). Fluoxetine, a standard antidepressant SSRI, was used as a reference compound (Cheaha et al. 2014; Uzbay et al. 2004).

Material and methods

Plant materials

The *Mitragyna speciosa* (MS), found mainly in Thailand and Malaysia, belongs to the family Rubiaceae, genus Mitragyna. It is well-known as 'kratom' in Thai. Young leaves of MS were collected from natural sources in Songkhla and Satun provinces, Thailand during 2004–2005. Authentication of the plant material was carried out at 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.

Extraction and analysis of alkaloid extract from MS

An MS alkaloid-rich extract was prepared as previously described (Kumarnsit et al. 2007a). In brief, dried MS leaves were powdered and macerated with methanol (repeated three times). The methanol filtrates were combined and evaporated under reduced pressure. The crude methanol extract dissolved in 10% acetic acid solution was well shaken and left to stand overnight. The acidic filtrate was washed with petroleum ether, then brought to pH 9 with 25% ammonia solution and extracted with chloroform. The chloroform extract was washed with distilled water, dried over anhydrous sodium sulfate and evaporated to yield a dry alkaloid extract from MS (with an approximately 0.25% yield based on the fresh leaf weight). An aliquot (2.5 g) of alkaloid extract was subjected to silica gel column chromatography, eluted with 5% methanol in chloroform to obtain a major alkaloid (1.27 g), which appeared as a single spot on thin layer chromatography (TLC) analysis (four solvent systems).

Identification of alkaloid extract from MS

The extract was analyzed by using high performance liquid chromatography (HPLC). Mitragynine was used as a standard. Firstly, 150 ppm pure mitragynine was prepared in methanol. Then, the sample was injected into the HPLC column. Retention time of the only dominant peak was 7.582 (Fig. 1A). After that, the alkaloid extract from MS (25.62 mg) was prepared in methanol (2 ml) and diluted 10 fold. The sample of alkaloid extract from MS (10 μl) was run under the same conditions. The identical retention time of the dominant peak was obtained with 80.453 percent area (Fig. 1B). Finally, the dominant peak was confirmed by adding 500 ppm pure mitragynine into the sample of alkaloid extract from MS. The result showed that the dominant peak appeared with the same retention time and additional height (86.861 percent area) (Fig. 1C).

Reagents and chemicals

The alkaloid extract from MS was dissolved in co-solvent (Tween 80: propylene glycol: H_2O at a 1:4:4 ratio). A concentration at 60 mg/kg was chosen for alkaloid extract from MS according to its antidepressant effects (Kumarnsit et al. 2007a). Fluoxetine (Flutine®, Merck, Thailand), a standard drug of SSRI, was dissolved in sterile distilled water to a desired concentration. A 5-cm curved stainless steel gavage needle with 3-mm ball tip was used for intragastric administration with a volume of 1 ml/kg body weight. Animals in the control group were treated with the co-solvent as vehicle group.