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Short communication

Antidepressant-like effect of mitragynine isolated from *Mitragyna speciosa* Korth in mice model of depression

N. Farah Idayu[a], M. Taufik Hidayat[a,b,*], M.A.M. Moklas[a], F. Sharida[a], A.R. Nurul Raudzah[a], A.R. Shamima[a], Evhy Apryani[a]

[a] *Human Anatomy Laboratory, Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400 Selangor, Malaysia*
[b] *Laboratory of Physical Performance and Skills Analysis, Sports Academy, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia*

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ABSTRACT

Mitragyna speciosa Korth. leaves have been used for decades as a traditional medicine to treat diarrhea, diabetes and to improve blood circulation by natives of Malaysia, Thailand and other regions of Southeast Asia. Mitragynine is the major active alkaloid in the plant. To date, the role of mitragynine in psychological disorders such as depression is not scientifically evaluated. Hence, the present investigation evaluates the antidepressant effect of mitragynine in the mouse forced swim test (FST) and tail suspension test (TST), two models predictive of antidepressant activity and the effect of mitragynine towards neuroendocrine system of hypothalamic-pituitary-adrenal (HPA) axis by measuring the corticosterone concentration of mice exposed to FST and TST. An open-field test (OFT) was used to detect any association of immobility in the FST and TST with changes in motor activity of mice treated with mitragynine. In the present study, mitragynine at dose of 10 mg/kg and 30 mg/kg i.p. injected significantly reduced the immobility time of mice in both FST and TST without any significant effect on locomotor activity in OFT. Moreover, mitragynine significantly reduced the released of corticosterone in mice exposed to FST and TST at dose of 10 mg/kg and 30 mg/kg. Overall, the present study clearly demonstrated that mitragynine exerts an antidepressant effect in animal behavioral model of depression (FST and TST) and the effect appears to be mediated by an interaction with neuroendocrine HPA axis systems.

Introduction

Mental disorder particularly major depression causes a significant burden on health worldwide. The main symptom of depression is characterized by a pervasive low mood, feeling of helplessness, loss of interest and loss of pleasure in most of the usual activities (Bhutani et al. 2009). Stressful environment, adverse life-event and lack of supporting relationship are some of the causal factor that contributes to development of depression in human.

Interactions between monoamine neurotransmitters system including 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) in the brain along with their specific reuptake and receptor protein has gain so much interest in the spectrum of antidepressant studies (Yi et al. 2008). As far, antidepressant drugs available in the market claimed to be effective in treatment of depression. However, the main significant drawbacks of that synthetic antidepressant are due to their high incidence of dangerous side effects and inadequate for number of individuals (Binfaré et al. 2009). Therefore, other mechanism should be considered, as they might provide potential effective target with higher efficacy for treatment of depression, and perhaps with fewer drawbacks.

The hypothalamic-pituitary-adrenal (HPA) axis in the neuroendocrine system is one of the complicated neurobiological mechanisms which play important roles as similar as monoamine neurotransmitters system in the new antidepressant development. Dysfunction or hyperactivity of HPA axis system provides significant indicator of depression together in the response to stressors reflected by overproduction of glucocorticoid hormones mainly corticosterone in rodent and cortisol in human (Xu et al. 2008). Hence, the normalization of the HPA axis system as another prime mechanism of antidepressant actions appears to be one of the special interest.

Recently, more herbal medicine has being used as alternative therapy for depression (Kessler et al. 2001). Due to its natural constituent and availability, natural herbs which obtained from natural sources are believed to provide less untoward effect profiles and provide greater effectiveness as compared to synthetic drug available over the market. *Mitragyna speciosa* Korth. is a tropical plant endemic to Southeast Asia particularly in northern peninsula of Malaysia, central and southern part of Thailand, and Indone-

* Corresponding author. Tel.: +603 89472356.
E-mail address: taufik@medic.upm.edu.my (M. Taufik Hidayat).

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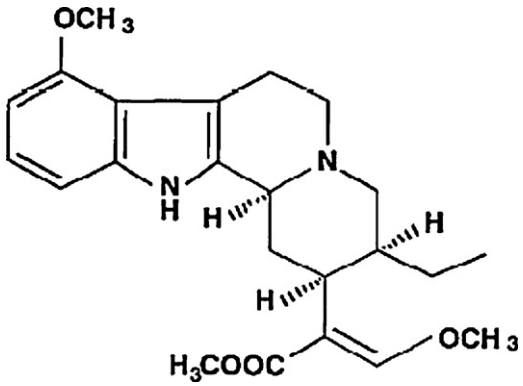

Fig. 1. Chemical structure of mitragynine.

sia (Jansen and Prast 1988; Mossadeq et al. 2009). It is popularly known by local as 'ketum' in Malaysia and 'kratom' in Thailand. The plant was classified under the coffee family of Rubiaceae. *Mitragyna speciosa* leaves have been used by natives for its opium-like effect and cocaine-like stimulant ability as anti-fatigue, anti-pain and as tonic to increase endurances or performances of work under hot sunlight (Reanmongkol et al. 2007). It is traditionally used by villagers as an alternative treatment for fever, malaria, cough, hypertension, diarrhea, to prolong sexual intercourse as well as substitution for treatment of opiate addiction such as morphine (Chan et al. 2005; Assanangkornchai et al. 2004).

Mitragyna speciosa contains abundant of indole alkaloids (Matsumoto et al. 2008). Mitragynine (Fig. 1) was found to be the primary active alkaloid compound of the entire plant which might hold the key for the effects of *Mitragyna speciosa* (Takayama 2004). It has a molecule formula of 9-methoxy-corynantheidine ($C_{23}H_{30}N_2O_4$) with the molecular weight of 398.50 (Chee et al. 2008). Previously, in two different studies, alkaloid extract and aqueous extract of *Mitragyna speciosa* has been shown to have antidepressant-like effects in mouse models of behavioral despair tests (Kumarnsit et al. 2007a, 2007b). However, antidepressant mechanism of the plant in both studies is not clearly demonstrated. In consideration of previous findings, mitragynine could be a beneficial option in prevention and treatment for stress-induced disorder such as depression.

In the present study, we evaluated the antidepressant-like effect of mitragynine using two classical behavioral models of antidepressants screening known as forced swimming test (FST) and tail suspension test (TST) together with open-field test (OFT). In accordance to the tests, the effect of mitragynine on serum corticosterone concentrations (an index of HPA axis status) was also simultaneously investigated in the present study.

Materials and methods

Preparation of mitragynine from Mitragyna speciosa leaves

The fresh leaves of *Mitragyna speciosa* Korth. were collected from its natural sources around Peninsula Malaysia particularly in Perlis and Kedah. The plant was authenticated by botanist from Department of Botany, Faculty of Forestry, Universiti Putra Malaysia (UPM), Serdang, Malaysia, based on their microscopic and macroscopic characteristics. A voucher specimen (ATS 001) was deposited in the Herbarium of the Department of Botany, Faculty of Forestry, Universiti Putra Malaysia (UPM), Serdang, Malaysia. Mitragynine was purified from the fresh leaves of *Mitragyna speciosa* Korth according to the method described by previous study (Houghton and Ikram 1986; Ponglux et al. 1994; Reanmongkol et al. 2007).

The fresh leaves of *Mitragyna speciosa* (1 kg) were dried at 45–50 °C, powdered and macerated with absolute methanol for 72 hours. The extracts were mixed, filtered and evaporated using rotary evaporator (Eyela N-1000, Tokyo Rikakikai CO., LTD., Tokyo, Japan) to yield 14.5% (w/w) of crude methanol extract. The methanol extract was dissolved in 10% acetic acid solution, well shaken, left to stand for 24 hours and filtered to give acidic filtrate. The acidic filtrate was washed with petroleum ether, made into alkaline (pH 9) with 25% ammonia solution and extracted with chloroform. The combined chloroform extracts were washed with distilled water, dried over anhydrous sodium sulphate and evaporated to yield 0.73% (w/w) of crude alkaloid extract. The major alkaloid was isolated by silica gel column chromatography eluting with diethyl ether was identified as mitragynine with standard spectroscopic methods (^{1}H NMR, ^{13}C NMR). Over all, the yield of mitragynine was approximately 0.087% (w/w) of the fresh leaves weight.

Reagent and chemicals

Mitragynine was dissolved in 20% (v/v) Tween-80 (polyoxyethylene sorbitan monooleate, Sigma–Aldrich Co.). Fluoxetine hydrochloride, amitriptyline hydrochloride and amphetamine hydrochloride were purchased from Sigma Chemical Co. (USA) and were used as reference drugs. All drugs were dissolved in physiological saline (NaCl 0.9%). All other reagents used in the study were of analytical grade.

Animals

Male mice from the ICR strain purchased from Northern RK Supplier, Sri Kembangan, Selangor, weighing 25–35 g were used. Animals were placed at Animal house, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia Serdang, Selangor, Malaysia, housed 8 per cage under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m and had free access to water and food pellets. They were allowed at least 7 days to adapt to the laboratory prior to the administration. Experiments were carried out between 9:00 a.m. and 3:00 p.m. All studies were conducted in accordance with Animal Care Committee, Faculty of Medicines and Health Sciences, Universiti Putra Malaysia, Serdang. The minimum number of animals and duration of observations required to obtain consistent data were employed. All efforts were made to minimize animal suffering and to reduce the number of animal used.

Drugs administration

The animals were randomly assigned into control and six experimental groups (8 mice per group) as follows: vehicle-treated; mitragynine 5 mg/kg; mitragynine 10 mg/kg; mitragynine 30 mg/kg; fluoxetine 20 mg/kg; amitriptyline 10 mg/kg and amphetamine (1 mg/kg). Mitragynine and all drugs were intraperitoneally (i.p.) administered once. Mitragynine was dissolved in 20% Tween-80 and diluted to the desired concentration on the day of testing while fluoxetine, amitriptyline and amphetamine were dissolved in normal saline (0.9% NaCl). Vehicle and mitragynine were injected i.p., 30 min before testing. Fluoxetine was administered i.p., 60 min before testing, amitriptyline was administered i.p., 30 min before testing and amphetamine was administered i.p., 15 min before undertaking the test.

Forced swimming test (FST)

The test was conducted according to the reported methodology originally described by Porsolt with minor modification (Porsolt et al. 1977). Briefly, mice were individually forced to swim in an