Journal of Ethnopharmacology 129 (2010) 357–360

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Effects of mitragynine from *Mitragyna speciosa* Korth leaves on working memory

Evhy Apryani, M. Taufik Hidayat*, M.A.A. Moklas, S. Fakurazi, N. Farah Idayu

Department of Human Anatomy, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, 43400 Selangor, Malaysia

ARTICLE INFO

Article history:
Received 8 December 2009
Received in revised form 21 March 2010
Accepted 29 March 2010
Available online 3 April 2010

Keywords:
Mitragynine
Working memory
Mitragyna speciosa
Object location task
Psychostimulant plants

ABSTRACT

Aim of the study: Mitragyna speciosa Korth from Rubiaceae family is a tropical plant indigenous to Southeast Asia particularly in Thailand, Peninsular of Malaysia and Indonesia. The leaves have been used by natives for their opium-like effect and cocaine-like stimulant ability to combat fatigue and enhance tolerance to hard work. However there is no scientific information about the effect of mitragynine on the cognitive performances. This study is designed to examine the working memory effects of mitragynine which is extracted from *Mitragyna speciosa* mature leaves.

Materials and methods: The cognitive effect was studied using object location task and the motor activity in open-field test. Mitragynine 5, 10 and 15 mg/kg and were administered by intraperitoneal (IP) for 28 consecutive days and evaluated on day 28 after the last dose treatment. Scopolamine was used as the control positive drug.

Results: In this study there is prominent effects on horizontal locomotor activity was observed. Mitragynine significantly reduced locomotor activity in open-field test compared with vehicle. In object location task mitragynine (5, 10 and 15 mg/kg) did not showed any significances discrimination between the object that had changed position than the object that had remain in a constant position.

Conclusion: Our results suggest that chronic administration of mitragynine can altered the cognitive behavioral function in mice.

1. Introduction

The use of psychostimulant plants is a global issue that has existed since centuries ago. The effects of psychostimulant plants have been recognized and information about their uses has been passed down through generations (Spinella, 2001). *Mitragyna speciosa* is one of psychostimulant plants that has been used by natives in Southeast Asia as a herbal drug for decades. Traditionally, the *Mitragyna speciosa* was used to alleviate pain, hypertension, cough, diarrhea and as a substitute for morphine in treating addicts (Reanmongkol et al., 2007; Chee et al., 2008). Mature leaves of *Mitragyna speciosa* are recognize as a rich source of alkaloids and mitragynine was obtained as the major constituent which is 66.2% based on the crude base and followed by its analogues speciogynine, speciociliatine and paynantheine (Reanmongkol et al., 2007; Takayama, 2004; Kikura-Hanajiri et al., 2009). Phytochemical and pharmacological studies of mitragynine have been carried out due to its unique medicinal properties (Chee et al., 2008). Mitragynine possesses an antinociceptive activity through the supraspinal opioid receptors and its action dominantly mediated by μ and δ opioid receptors (Matsumoto et al., 1996a,b; Thongpradichote et al., 1998; Kumarnsit et al., 2007; Reanmongkol et al., 2007). Competitive binding study shows that mitragynine bound to 3 types of receptors with different affinity with the highest affinity in μ- followed by K- and δ-opioid receptors (Yamamoto et al., 1999).

Because of the similar effects like morphine, mitragynine is widely used as a recreational drug. Recently, the abuse of ketum has gained a lot of attention in Malaysia and Thailand (Chan et al., 2005). The study by Suwanlert (1975) showed that a chronic administration of mitragynine can lead to addiction. The users of mitragynine show some symptoms of addiction such as hostility, aggregation, inability to work, aching of muscle and bones, jerky movements of limbs, anorexia, weight loss and insomnia (Chan et al., 2005). But however the available of scientific information about mitragynine is scarce and there are no reports related to its possible effects on the cognitive behavioral function. Therefore, the aim of the present study was to investigate the effects of chronic administration of mitragynine in working and memory function using object location task and locomotor activity in open-field test.

2. Materials and methods

2.1. Plant material

Mature leaves of *Mitragyna speciosa Korth* were collected from natural sources in Malaysia. Authentication of plants was carried

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

0378-8741/$ – see front matter.
doi:10.1016/j.jep.2010.03.036

Fig. 1. Object location task. The ability of mice to remember that an object that it had experienced before had changed location was assessed in this test.

out at the Faculty of Forestry, Universiti Putra Malaysia where the herbarium vouchers (ATS 001) have been deposited. Mitragynine was extracted from leaves of *Mitragyna speciosa* according to the methods described by Houghton and Ikram (1986).

2.2. Experimental animal

Male ICR mice (25–30 g) were obtained from Animal Center Faculty of Medicine and Health Sciences (FMHS), University Putra Malaysia. Animals were housed in group of six in a temperature-controlled room. They were maintained under standard laboratory conditions with natural dark and light cycle (24 h) and fed with standard commercial food pellets and water *ad libitum*. Animals were acclimatized for 1 week prior to the experimental use. All animal procedures were performed in accordance with Animals Ethical Committee of the FMHS, UPM and associated guidelines.

Mitragynine was dissolved in 0.5% (v/v) Tween 80 (Sigma–Aldrich, USA) and administered to the mice at doses of 5, 10 and 15 mg/kg in 28 days consecutive days. All administration was performed intraperitoneally (IP) at volume of 0.5 ml/kg bodyweight. Negative control received 0.5% (v/v) Tween 80, the positive control animals were administrated with scopolamine (Sigma–Aldrich, USA) which is well known as a reference drug for inducing amnesia (Isomae et al., 2003; Hoon Oh et al., 2009; Saraf et al., 2010). Mitragynine were administrated 30 min before the open-field test and object location task.

2.3. Object location task

The object location task was conducted according to Barker et al. (2007) with some modification. One day before object location task, all mice were exposed for 60 min to the empty test box for habituation. The object location task consisted of 2 trials which is sample phase (T1) and test phase (T2) with a 30 min interval between the 2 trials. In sample phase, mice were exposed to object A1 and A2, which were placed in the far corner of the area (Fig. 1). The animal was allowed to explore both objects during a sample phase for 3 min and the amount of exploration of each object was recorded. After a delay of 30 min, the test phase was started. In the test phase, object A3 was placed in the same position as object A1 in the sample test while object A4 was placed in the corner adjacent to the original position of A2, so that object A3 and A4 were in diagonal corners. Thus, both objects in the test phase were equally familiar, but one was in a new location. The position of the moved object was counterbalanced between mice. All measures experiments were made with the experimenter blind to the treatment status of each animal. The basic measure was the total time spent by rats exploring each object during T1 and T2 trials. Exploratory was defined as the animal directing the nose toward the object at a distance of <2 cm. Looking around while sitting or resting against the object was not considered as exploration. On object location task the amount of time exploring each object (object in the new location versus object in familiar position) is reported as an object discrimination ratio (ODR) and calculated using the following formula:

$$\frac{\text{Exploration time of object in the new location} - \text{Exploration time of object in familiar location}}{\text{Total exploration of both object}}$$

Table 1
Chronic effects of vehicle and drugs on object location task.

Groups	Time exploring object A3(s) (Mean ± SEM)	Time exploring object A4 (s) (Mean ± SEM)
Saline	15.17 ± 4.35*	41.83 ± 5.17*
Scopolamine 0.5 mg/kg	31.83 ± 5.17	31.33 ± 4.98
Tween80	15.00 ± 5.47*	39.40 ± 7.26*
Mitragynine 5 mg/kg	24.00 ± 5.50	24.60 ± 4.92
Mitragynine 10 mg/kg	20.00 ± 6.14	19.00 ± 7.23
Mitragynine 15 mg/kg	23.67 ± 5.81	21.00 ± 6.60

Vehicle treated but not scopolamine and mitragynine (5, 10 and 15 mg/kg) significantly sustained novelty recognition.
* $p < 0.05$ new location object versus. familiar location object, Student's paired *t*-test two tailed distribution.

2.4. Open-field test

Mice were initially accustomed with the experimental room for approximately 30 min before the experiment. Horizontal activity was assessed with a camera recording mounted on the ceiling. Mice were placed in the centre of the box (45 × 45 × 45 cm) with Plexiglas front and the floor was divided into nine squares (15 × 15 cm) for 30 min observation of locomotor activity. The number of squares entered with all four paws was counted. Before the next assessment the mice were removed and the box was cleaned with a damp cloth. The video recording was saved to provide a permanent record (Moklas et al., 2008).

2.5. Statistical analyses

The analysis of data was performed by means of one-way Anova for group comparison followed by *post hoc* Turkey's test. Additional analyses examined whether individual group has discriminated between the objects using a within-subjects *t*-test (two-tailed). For locomotor activity, data were analysed using one-way Anova followed by Dunnet's test. Group differences were considered statistically significant at $p < 0.05$.

3. Result

On the trial phase, all animals spent an equal amount of time exploring each of two identical objects (A1 and A2) ($F = 1.367$, $p > 0.05$, significant level between groups, data not shown). Nevertheless, when the test phase was conducted 30 min after the trial phase, scopolamine and mitragynine treated mice cannot show significant discrimination between the object that had changed position than the object that had remained in a constant position (A3 and A4) (Table 1). The results showed there are significant differences on discrimination ratio among the groups ($F = 13.531$, $p < 0.05$, Fig. 2).

The performance of the 4 groups (Tween 80, mitragynine 5 mg/kg, mitragynine 10 mg/kg, and mitragynine 15 mg/kg) in the test phase is shown in Fig. 2. Additional analysis using *t*-test found that the Tween 80 group and mitragynine dose 5 mg/kg groups did showed significant preference for the two objects that had changed position compared with objects that had remained in the same position ($p < 0.05$). There are also statistically significant differences