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Sedative, Cognitive Impairment and Anxiolytic Effects of Acute *Mitragyna Speciosa* in Rodents

Mohamad Aris Mohd Moklas[1], Noor Azuin Suliman[1], Che Norma Mat Taib[1], Mohamad Taufik Hidayat Baharuldin[1], Sharida Fakurazi[1], Fatin Nadzirah Zakaria[1], Muhammad Khairulasraf Muhamamd Yusof[1], Muhammad Farhan Adzhar[1], Mohd Saifuddin Md Rasul[1], Abdah Md Akim[2] and Zulkhairi Amom[3]

1. Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

2. Department Of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

3. Department of Basic Science, Faculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia

Abstract: *Mitragyna speciosa*, a plant from Rubiaceae family, was reported to have an opium-like effect and their coca-like stimulative ability to combat fatigue and enhance tolerance to hard work. There are lack of information regarding to the effect of *Mitragyna speciosa* on cognitive and behavioural performances. Therefore the project was conducted to observe the effect of *Mitragyna speciosa* on cognitive behavior of rats and mice. *Mitragyna speciosa* in methanol extract form and aqueous extract form with same dosage distributions were used; 10 mg/kg, 30 mg/kg, and 100 mg/kg. Four tests were conducted to observe the behavioural changes of the animal namely locomotor, cognitive performances, anxiety and rotarod performance. Results showed that all dosage of treatment reduced locomotor and impaired cognitive performance significantly. Study showed that *Mitragyna speciosa* induce sedative effect in dose dependant manner. Interestingly, *Mitragyna speciosa* increased the time spent in open arm of plus maze indicating low anxiety level of the rodent. As conclusion, *Mitragyna speciosa* caused sedative effect, impairment in working memory, and possess anxiolytic properties.

Key words: *Mitragyna speciosa*, Mitragynine, locomotor activity, novel-object discrimination test, anxiety.

1. Introduction

Mitragyna speciosa or Kratom/Ketum is a medicinal leaf from Rubiaceae family harvested from a large tree native to Southeast Asia. In Thailand, the leaf of this plant was known as “Kratom”, while in Malaysia, it was known as “Ketum” or “Biak”. Traditionally, *Mitragyna speciosa* leaves have been used by local populations for their opium-like effect and their coca-like stimulative ability to combat fatigue and enhance tolerance to hard work. It is reported in local media that traditional healers use *Mitragyna speciosa* to wean addicts off heroine addiction, to deworm, cure diarrhea, improve blood circulation and treat diabetic. [1]. Study also found that there are no actual studies suggesting consumption of *Mitragyna speciosa* in managing opioid withdrawal symptoms [2].

Study showed that *Mitragyna speciosa* users became addicted [3]. Because of addictive effects, Thailand, Malaysia, Myanmar and Australia did outlaw this plant. Other typical withdrawal symptoms include hostility, aggression, excessive tearing, inability to work, aching of muscles and bones and the jerky movement of limbs. It was also reported in the study that anorexia, weight loss and insomnia were common among long-term *Mitragyna speciosa* addicts.

Corresponding author: Mohamad Aris Mohd Moklas, PhD, research fields: neuroscience. E-mail: aris@medic.upm.edu.my.

There are lack of studies on effects of *Mitragyna speciosa* on behavioural and cognitive performance. Due to that scenario, this project is conducted in order to observe the effect of mitragynine, an active compound of *Mitragyna speciosa,* on cognitive behaviour scientifically. The effect is observed through locomotor and rotarod activities, novel-object discrimination and anxiety tests. It is speculated that *Mitragyna speciosa* posses a neuropharmacological properties that can be implicated in drug withdrawal management.

2. Materials and Method

2.1 Animals

64 male *Sprague Dawley* rats with age between 8 to 10 weeks with average weight 250–300 g each and 49 ICR mice with average weight 20–25 g were purchased from the animal house of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The rats were used in locomotor test, novel-object discrimination test, and anxiety test, while the mice were used in rotarod test. All the animals were allowed to acclimatize for one week before given treatments.

2.2 Treatment

Two types of *Mitragyna speciosa* extracts were used in this study, which are methanol and aqueous extracts. The dosages for each of extracts were 10 mg/kg, 30 mg/kg, and 100 mg/kg. Distilled water (vehicle) was given as negative control. Both treatments were given orally according to body weight of each rat. Meanwhile scopolamine was given intraperitoneally as positive control used in novel-object discrimination test (NOD) later on. Those treatments had been given continuously for seven days. The tests were carried out at day seven.

2.3 Behavioural Test

At day seven, all animals were allowed to acclimatize with the new environment of behavioural room for at least one hour prior proceeded with behavioural tests.

2.3.1 Locomotor Test

The box (75 cm × 75 cm × 75 cm) used in locomotor
activity has tile with 5 × 5 small boxes. In locomotor activity, number of crossing was counted with all four paws of rats was in the same tile. Number of crossing before treatment and 30 minutes after treatment was counted. The data was transformed to $\log_{10}$ and will then be further analyzed using statistical analysis.

2.3.2 Novel-object Discrimination Test (NOD)

For novel-object discrimination test (NOD), the rat was first placed in a perspex box which was parallel to the side walls and its nose was pointed away from the objects. For first three minutes exposure, the rat was exposed to two familiar objects (E1) while at the second three minutes exposure (E2), the rat was exposed to one familiar object and one novel object. The time interval between those two exposures was one minute. The time-spent on familiar (F) and novel (N) objects was recorded for both exposures. Discrimination ratio (D1) was calculated by substracting the N to F, while discrimination index (D2) was obtained by dividing D1 to the total time spent of N and F.

2.3.3 Anxiety Test

In anxiety test, elevated plus-maze test (EPT) was used. The times of rat spent at the open and close arms were recorded in percentage (open arm to close arm) before transformed into arcsine. The total time of exploration of each rat in the apparatus was 5 minutes.

2.3.4 Rotarod

Rotarod activity was designed after locomotor activity had showed decreasing number of crossing. This test had used ICR mice and mice were given treatment 30 minutes before test. All mice were placed on the moving rotarod for 120 seconds. Time when the mouse fell from the revolving rotarod was recorded in seconds.

2.4 Analysis

All the data were analyzed using SPSS 16.0. The tests carried were one-way ANOVA and Tukey multiple comparison test.

3. Results

3.1 Locomotor Activity

Locomotor activity was counted before and after the last treatment. The differences between pre and post-treatment were converted in form of $\log_{10}$ in order to normalize the reading before proceeded to analysis using SPSS 16.0. Table 1 and Fig. 1 show antilog_{10} of the result in comparing all the dosages of each extract.

From the Table 1, all the readings of locomotor activity before treatment was given showed no comparative effect at $p > 0.05$. The count of locomotor activity after treated with 10 mg/kg of methanol extract showed significant effect as compared to control treated at $p < 0.05$, while other dosages of either methanol or aqueous extracts showed no significant effect at $p > 0.05$. In term of type of extraction, both methanol and aqueous that showed no comparable effect at $p > 0.05$ were distinguishable to control at $p < 0.001$.

Table 1 Locomotor count for each treatments and extracts with standard error of mean (S.E.M). The count for pre- and post-treatments were recorded in number. The differences were obtained by subtracting pre to post-treatment.

Extract	Dosage	Pre-treatment	Post-treatment	Differences
Methanol (M)	10 mg/kg	303 ± 32	112 ± 30	191 ± 17*
	30 mg/kg	325 ± 42	67 ± 22	258 ± 47***
	100 mg/kg	330 ± 47	6 ± 2	323 ± 47***
Aqueous (A)	10 mg/kg	304 ± 23	96 ± 32	208 ± 13**
	30 mg/kg	299 ± 27	81 ± 23	218 ± 12**
	100 mg/kg	253 ± 46	22 ± 11	230 ± 44**
Control (NC)		337 ± 18	313 ± 17	25 ± 4

Note: Table shows significant differences among the dosages of the extracts as compared to control at $p < 0.001$. The differences between the locomotor counts were obtained by subtracting the count of locomotor activiey of pre- to post-treatment. 30 and 100 mg/kg of methanol extracts show significant differences as compared to control at $p < 0.001$ (***) while 10 mg/kg gave effect at $p < 0.05$ (*). The dosages of aqueous extract showed significant differences at $p < 0.01$ as compared to control (**).

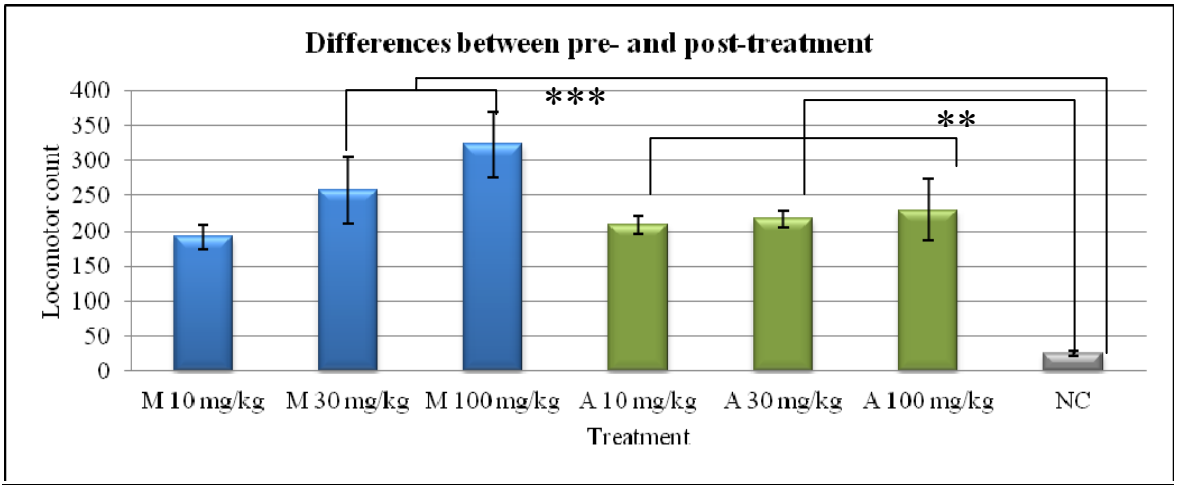

Fig. 1 Bar chart shows the differences of the locomotor count between pre- and post-treatment of each treatment. 30 and 100 mg/kg of methanol showed significant differences at $p < 0.001$ (***) in compared to control. 10 mg/kg of methanol extract showed effect as compared to control at $p < 0.05$. There are no comparative effects observed among the dosages of methanol extract. All dosages of aqueous extract showed no significant difference among themselves while it's gave significant effect as compared to control at $p < 0.01$ (**).

3.2 Novel-object Discrimination Test (NOD)

In term of differences between pre- and post-treatment, as cumulative, methanol and aqueous were not comparable at $p > 0.05$. Both extracts showed significant differences in compared to control at $p < 0.001$ regardless the dosages used. Even there are observable pattern of the same dosages of different extracts, there were no significant difference statistically observed at $p > 0.05$. As total, there are no significant differences observed comparing the methanol and aqueous extracts of *Mitragyna speciosa.*

In NOD test, the values that should be notified were the discrimination ratio (D1) and discrimination index (D2). D1 was used in predicting the differences between times spent on novel to familiar objects, while D2 was obtained in evaluating the significant time space for novel object as respect to the total time spent on both objects. Total time spent on E1 and E2 were to minimize the factors that effecting the total time spent on both objects. The following Tables 2 and 3 show the readings for E1, E2, D1 and D2, specifically to each treatment, respectively.

From the Table 2, there is no significant effect observed in term of total time spent on the objects. With the same ratio time spent, the only different is the time spent on each objects exposed expressed through the discrimination ratio (D1) and discrimination index (D2).

Table 2 The mean of total time spent on novel and familiar object during E1 and E2 was recorded in seconds with standard error of mean (S.E.M).

Extract	Dosage	E1	E2
Methanol (M)	10 mg/kg	17.93 ± 2.50	15.07 ± 1.22
	30 mg/kg	23.20 ± 3.82	15.74 ± 1.64
	100 mg/kg	19.37 ± 2.18	11.87 ± 1.17
Aqueous (A)	10 mg/kg	16.78 ± 3.09	8.85 ± 1.72
	30 mg/kg	19.73 ± 2.31	14.58 ± 2.39
	100 mg/kg	14.35 ± 1.15	10.69 ± 0.93
Positive control (PC)		20.37 ± 2.74	13.88 ± 1.81
Negative control (NC)		19.74 ± 1.98	18.59 ± 2.52

Note: Table shows the means for total spent during E1 and E2 with no significant differences at $p > 0.05$.

Table 3 The following D1 and D2 were recorded as value yielded from the formulations.

Extract	Dosage	D1	D2
Methanol (M)	10 mg/kg	-8.01 ± 1.86***	-0.50 ± 0.08***
	30 mg/kg	-10.51 ± 1.06***	-0.67 ± 0.02***
	100 mg/kg	-10.45 ± 0.96***	-0.88 ± 0.02***
Aqueous (A)	10 mg/kg	-3.59 ± 0.46***	-0.38 ± 0.04***
	30 mg/kg	-7.97 ± 1.57***	-0.56 ± -0.10***
	100 mg/kg	-8.28 ± 0.89***	-0.78 ± 0.07***
Positive control (PC)		-12.51 ± 1.84***	-0.90 ± 0.05***
Negative control (NC)		10.08 ± 1.37	0.55 ± 0.06

For D1 and D2, there are significant differences at $p < 0.001$ by comparing the treatments and controls.

Table 3 showed the different effect of *Mitragyna speciosa* on different dosages and extracts for both D1 and D2. The extracts showed no significant different as compared to negative control used and showed obvious observable differences as compared to positive control used, scopolamine (***). Figs. 2 and 3 are showing the graphical data of each D1 and D2, respectively.

As cumulative data in D1, aqueous extract shows explainable differences as compared to positive control and other treatments at $p < 0.05$ and to negative control at $p < 0.001$. There are no significant differences between positive control and methanol extract of *Mitragyna sp.*. As for D2, positive control was observed show distinguishes statistically by comparing to aqueous and methanol extracts at $p < 0.001$ and $p < 0.05$, respectively. Methanol and aqueous extract were non-significantly difference at $p > 0.05$.

3.3 Anxiety

Data collected from the EPT was in second which later was converted to percentage. For normalization, the percentage was transformed to arcsine before proceed to analysis procedure. The following data was

in percentage of time spent on open- and close-arms, which is more explainable.

Percentage of differences was obtained through the differences between percentages of close-arm to open-arm. From the table, there are obvious differences observed among the treatments. 30 mg/kg methanol extract and 10 mg/kg aqueous extract showed significant differences at $p < 0.05$ (*) and $P < 0.01$ (**), respectively, as compared to negative

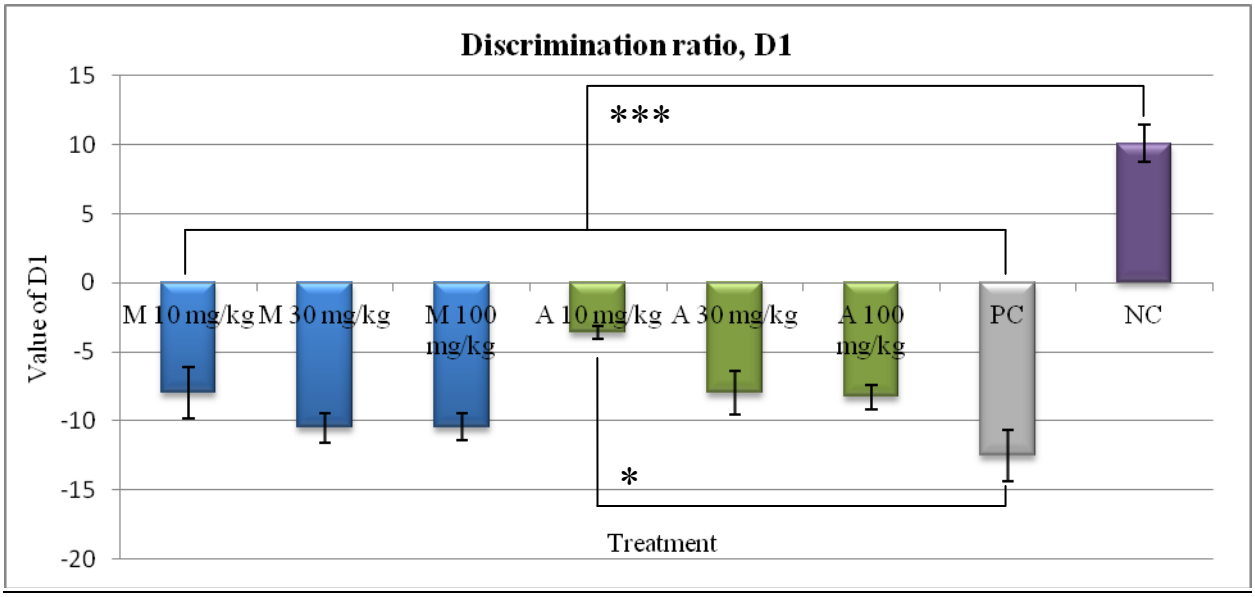

Fig. 2 The above bar chart show the discrimination ratio, D1, of each treatment. All the *Mitragyna speciosa* dosages and positive control treatments showed significant differences statistically at $p < 0.001$ (***) as compared to negative control. As compared among the mentioned treatments, there are no significant differences observed to positive control at $p > 0.05$ except for 10 mg/kg of aqueous extract of *Mitragyna speciosa* at $p < 0.05$ (*).

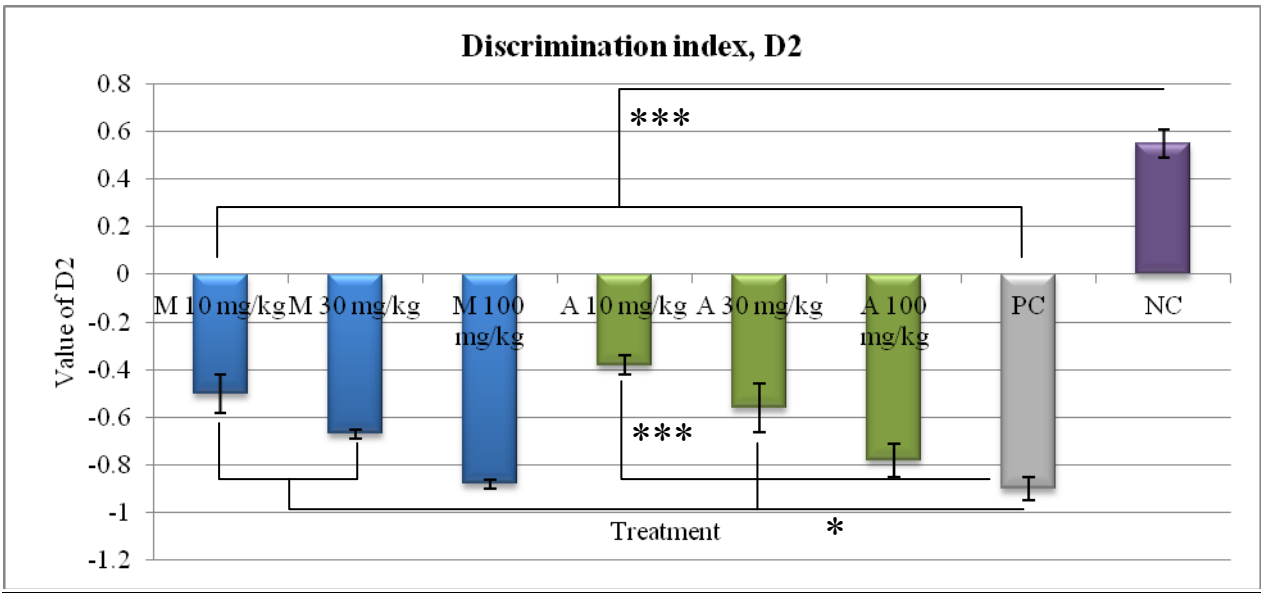

Fig. 3 The bar chart above shows the discrimination index, D2, for all treatments. There are observable different statistically showed by all *Mitragyna speciosa* dosages and positive control as compared to negative control at $p < 0.001$ (***). As among the Mitragyna speciosa, there are difference showed by 10, 30 mg/kg of methanol extracts and 30 mg/kg of aqueous extract at $p < 0.05$, while 10 mg/kg of aqueous extract showed statistically observable different at $p < 0.001$ (***) as compared to positive control.

Table 4 The percentage of arms spent by the rat in five minutes exposure after analytical procedure.

Extract	Dosage	% open-arm	% close-arm	% differences
Methanol (M)	10 mg/kg	10.85±1.26	89.15±1.27	78.29 ±2.53
	30 mg/kg	14.40±1.17	85.60±1.17	71.20±2.34*
	100 mg/kg	24.30±1.92	75.70±1.92	61.68±3.84***
Aqueous (A)	10 mg/kg	16.07±0.30	83.93±0.30	67.85 ± 0.60**
	30 mg/kg	21.85±2.76	78.15±2.76	56.31±5.53***
	100 mg/kg	24.1±1.75	75.90±1.75	51.80±1.73***
Negative control (NC)		7.68 ± 2.29	92.32± 2.29	84.63 ± 4.58

The differences between close- arm and open-arm showed significant differences at $p < 0.001$ for all the treatments.

control. 100 mg/kg methanol extract, 30 and 100 mg/kg aqueous extracts showed observable differences at $p < 0.001$ (***) as compared to negative control. There is no significant observed between 10 mg/kg methanol extract as compared to negative control at $p > 0.05$.

As total view, methanol extract showed no significant different to aqueous extract at $p > 0.05$ while both extracts showed explainable differences as compared to negative control at $p < 0.001$.

3.4 Rotarod

Data collected from rotarod test was recorded as the rat was exposed to revolving rotarod for 120 seconds. The potential of the rat to hold on the revolving rotarod was expressed in seconds. The data then was used in statistical procedure.

As total, methanol extract has no explainable differences statistically to aqueous extract at $p > 0.05$. Both extracts instead showed observable effect as compared to negative control.

4. Discussion

The locomotor activity is useful and less robust than other behavioral tests. Locomotor activity is often used in primary evaluation of drugs [4]. Based on that reason, locomotor activity is used in this project to evaluate the effectiveness of the *Mitragyna speciosa* as potential psychostimulant by assessing the behaviour of the rats. Reduction in number of crossing in locomotor activity might due to sedative properties of mitragynine of *Mitragyna speciosa*. Lower dose of *Mitragyna speciosa* is believed to cause stimulant effect, the *Mitragyna speciosa* will stimulate the level of dopamine in brain [5]. Higher dose of *Mitragyna speciosa* cause release of dopamine in mesolimbic system and thus inhibit locomotor activity. Previous research had found that these locomotor suppressive effects of *Mitragyna sp.* may due to opioid receptor in opioid pathway. Suppression of locomotor activity observed through reducing number of crossing may regulated by the dopamine in mesolimbic system. Increasing level of dopamine is stimulate by activation of μ-receptors then leads to disinhibition of A10 dopaminergic neuron [6–8].

The rotarod unit was automated and interfaced to a personal computer allowing automatic recording of the time that each rat was able to stay on the rod at different rotational speeds. Research done had related sedative effects on mice to accumulation of dopamine in the striatum [9, 10]. Accumulation of dopamine is probably due to a selective interruption of the flow of the nerve impulses in the dopaminergic nigrostriatal tract [11]. Reductions of time-spent on revolving rotarod can be concluded to the sedative properties of *Mitragyna speciosa*. Since both locomotor and rotarod tests showed decreasing activity to the increasing dosages, we can postulate that the lower dosages of *Mitragyna speciosa* used can show no significant difference in the motor activity. In further usage of this leaves, low dose should be use to prevent the withdrawal to motor activity.

In the novel-object discrimination test, the result was analyzed by discrimination index (D2) which takes into account individual differences in overall levels of exploration [12]. The physiological evidence for working memory in animals has typically come from studies in which animals were given a brief cue to hold in memory during a delay period of a few seconds and then required to make some choice or

response based on this cue [13]. The D1 receptor, selectively distributed on prefrontal neurons in a way has a specific role in regulating neuronal activity associated with the mnemonic process. Increasing dopamine D1 receptor stimulation with a D1 receptor agonist infusion into the prefrontal cortex is sufficient to induce working memory deficits [14]. Thus, the normal action of dopamine is inhibitory, constraining neuronal activation during performance of a working memory task. Usage of *Mitragyna speciosa* seems to give negative effect to memory performance. For further study, lower dosages of *Mitragyna speciosa* should be recommended to give no significant difference to control, so that this plant can have more positive effects.

The elevated plus-maze test (EPT) is based on the fact that exposure to an elevated and open maze alley leads to an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an enclosed maze alley. The percentage of open arm entries provides a measure of fear-induced inhibition of exploratory activity. This ratio of percentage of open arm and close arm is increased by anxiolytic and reduced by anxiogenic compounds [15]. Following this test, *Mitragyna speciosa* possess an anxiolytic property. This property was clearly observed by increasing time-spent in open arm of elevated plus-maze test. The mechanism of action of anxiety was usually related to serotonergic (5-HT) pathways. In general, decreasing 5-HT neurotransmission produces an anxiolytic effect whereas increasing 5-HT stimulation tends to increase anxiety. These effects are relatively weak and confined to a narrow dose range [16]. Compared to other tests, anxiety test showed positive effect of *Mitragyna speciosa*. *Mitragyna speciosa* can be used in treating anxiety since increasing dosages of *Mitragyna speciosa* can significantly possess anxiolytic property.

5. Conclusion

As a whole, it is postulated that *Mitragyna speciosa* possess sedative and anxiolytic properties. Sedative property of *Mitragyna speciosa* may lead to cognitive impairment. *Mitragyna speciosa* can be used in low dosages to prevent the sedative and cognitive impairment. Through this study, *Mitragyna speciosa* was an antianxiety agent that can be used in treating anxiety. In contrast, *Mitragyna speciosa* impaired cognitive and motor performance. However, further study in needed to delineate the pharmacological properties of the plant.

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