INVOLVEMENT ARACHIDONIC ACID CASCADE IN MEMORY DEFICIT BY KRATOM (*Mitragyna speciosa*) IN MALE WISTAR RATS

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Abstract

Kratom (*Mitragyna speciosa*) is a plant that is widely found in Southeast Asia particularly, in Indonesia. Mitragynine, which was the main alkaloid of kratom, has been reported associated with addictiveness and memory impairment in animal studies. However, the mechanism of memory impairment is still unclear. In the present study, we investigated the involvement of the arachidonic acid cascade in memory impairment caused by kratom. Male Wistar rats were divided into seven groups, namely vehicle (CMC Na 0.5%; oral), kratom ethanolic extract (50, 100 and 200 mg/Kg; oral (p.o)), and the group given Diclofenac Sodium (5 mg /Kg; Intraperitonial (i.p)) 30 mins before administration of kratom ethanolic extract (50, 100 and 200 mg/Kg; oral) for 14 days. Memory impairment was carried out using a spatial memory test on days 10-16 using the Morris Water Maze and a working memory test using the Y Maze on the 17th day. Kratom administration was shown to impair spatial memory and working memory when compared with the vehicle group ($P<0.05$). Diclofenac sodium prevents spatial memory and working memory impairment due to Kratom when compared to the group administered by kratom monotherapy at the equivalent dose ($P<0.05$). In this study, it was found that there was involvement of the arachidonic acid cascade in memory impairment by kratom.

Keywords: Mitragyna speciosa, Memory Impairment, Arachidonic Acid, Wistar Rat, Y Maze, Morris water maze.

KETERLIBATAN KASKADE ASAM ARAKHIDONAT DALAM GANGGUAN MEMORI YG DISEBABKAN OLEH KRATOM (*MYRTAGINA SPECIOSA*) PADA TIKUS WISTAR JANTAN

Abstrak

Kratom (*Mitragyna speciosa*) adalah salah satu tanaman yang banyak terdapat di Asia Tenggara, termasuk Indonesia Belakangan ini mitraginin yang merupakan alkaloid utama dari kratom dilaporkan menyebabkan kecanduan dan menurunkan memori pada hewan. Namun, mekanismenya dalam menurunkan memori belum sepenuhnya diketahui. Penelitian ini bertujuan untuk menginvestigasi keterlibatan jalur asam arakhidonat pada gangguan memori akibat kratom. Tikus wistar jantan yang dibagi kedalam tujuh kelompok yaitu pembawa (CMC Na 0,5%; oral), Ektrak etanol daun Kratom (50, 100 dan 200 mg/KgBB; oral), dan kelompok yang diberi Natrium diklofenak (5 mg/KgBB; Intraperitonial) 30 menit sebelum pemberian Ektrak etanol daun Kratom selama 14 hari. Pada hari ke 10-16 dilakukan uji memori spasial menggunakan *Morris water maze*. Dan pada hari ke 17 dilakukan evaluasi pada working memori menggunakan Y *Maze*. Pemberian kratom terbukti menurunkan memori spasial dan memori kerja spasial ketika dibandingkan dengan grup pembawa ($P<0,05$). Natrium diklofenak mencegah perburukan memori spasial dan memori kerja spasial akibat kratom jika dibandingkan dengan kelompok yang diberi monoterapi kratom pada dosis setara ($P<0,05$). Pada penelitian ini ditemukan adanya keterlibatan kaskade asam arakhidonat terhadap perburukan memori oleh kratom.

Kata kunci: *Mitragyna speciosa*, Gangguan Memori, Asam arakidonat, Tikus Wistar, *Y-maze*, Morris water maze.

INTRODUCTION

Kratom (*Mytragina speciosa*) belongs to the Rubiaceae family, which is numerous found in tropical regions, especially Southeast Asia including Indonesia (Rech 2015, Mukhlisi et al. 2018). The main compound of kratom is mitragyninee (Meireles et al, 2019). According to Raini (2017), Kratom from Indonesia, especially those found in Kapuas Hulu, contains mitragynine of 54% of its total alkaloids.

Reported by Swogger et al. (2018), kratom has effects resembling morphine but with less severe side effects. Nowadays, kratom was reported as a less expensive substitute for opioids, and demand for exports of this plant has increased, especially from Western countries (Cinosi et al. 2015). The opioid activity of kratom could pose a risk, including addiction. Chronic use of kratom could trigger withdrawal symptoms (Swanlert 1975). Mitragynine observation in animals also shows effects such as addiction (Yussof et al. 2016). Drug addiction was a chronic relapsing disorder that affects brain alternations associated with cognitive, motivational, and emotional alternations (Goldstein and Volkow 2002; Fernández-Serrano et al. 2010). It has been also reported that acute or chronic administration of mitragynine causes significant cognitive deficits and emotional disturbances in animals (Apryani et al. 2010, Iman et al. 2021, Hassan et al. 2019).

According to Zul Aznal (2022), one of the metabolites that caused memory impairment by kratom is arachidonic acid. Changes in arachidonic acid caused by drug metabolism have also been linked to neuroplasticity, signal transduction, and cognitive impairment (Sambra et al. 2021, Thomas et al. 2017). Arachidonic acid plays an important role in inflammation which produces prostaglandins through cyclooxygenase pathway. Many studies reported that prostaglandin promotes the production of cytokine that is harmful to neurons (Tzeng et al. 2005).

This study aimed to determine the involvement arachidonic acid cascade of kratom administration in memory impairment with behavioral measurement.

MATERIALS AND METHODS

Plant Material

Powder leaves of kratom were purchased from Pontianak, Kalimantan, Indonesia. Taxonomic identification was confirmed by Herbarium Jatinangor, Taxonomical Laboratory, Padjadjaran University with the number 36/HB/09/2023. Powdered leaves (400g) were extracted with ethanol 96% using the soxhlet apparatus followed by evaporation with Rotavapor (Buchi R-215) and water bath to a give crude ethanolic extract.

Animals

Six to eight weeks old male Wistar rats (weighing 180-250 grams) were purchased from CV. Kencana. Before the study was conducted, the rats were acclimatized for seven days with a constant temperature of 25±2 ℃ under a 12-hrs light-dark cycle. The animals were allowed to free access to standard commercial food pellets food and water *ad libitum* except during behavioral observations. The experimental protocols for the care and use of laboratory animals described in this study were guided and approved by the ethical committee from Bandung Institute of Technology (KEP/I/2023/VIII/H190723CA/EKTA)

Grouping of Animal Subjects

Animal subjects were divided into seven groups as follows:
Group 1 was given Vehicle (Na-CMC 0. 5%)
Group 2 was given kratom ethanolic extract (KEE) 50 mg/Kg
Group 3 was given KEE 100 mg/Kg
Group 4 was given KEE 200 mg/Kg
Group 5 was given Na Diclofenac (5 mg/Kg) + KEE 50 mg/Kg
Group 6 was given Na Diclofenac (5 mg/Kg) + KEE 100 mg/Kg
Group 7 was given Na Diclofenac (5 mg/Kg) + KEE 200 mg/Kg

Drug Administration

Vehicle Group (Na CMC 0, 5 %) was administered orally (p.o). KEE was dissolved in Na-CMC 0.5 % and administered orally (p.o) for 14 consecutive days using an intragastric gavage needle. Diclofenac sodium 5mg/kg (Novell, Indonesia) was dissolved in normal saline and administered

intraperitoneally (i.p) 30 mins before administering KEE.

Morris Water Maze

The Morris water maze (MWM) test followed procedures described by Arslan and Uygur (2010) with a few modifications. A circular pool was used with specifications 135 cm diameter and 60 cm high. The powdered milk added to make it opaque. The escape platform constructed from plastic cylinder (18 cm high, 13 diameter) was used. The water level was 1.5 cm – 2 cm above the platform, to making it invisible. The additional poster and furniture around the maze were constant throughout experimental days as extra-maze visual cues.

Rats were tested in the MWM in 3 phases: acquisition, probe, and sensorimotor trials. Acquisition trials were measured on days 10-14 (4 trials/days). In all trials, rats were trained to swim to find an invisible platform that was located in the northeast quadrant. Every trial started from random quadrant (east, north, south, or west). If the rat did not find the platform within 60 sec, it was guided to the platform and allowed to stay on it for approximately 15 sec. The escape latencies were recorded in every trial. Spatial memory was measured in a probe trial (no drug) on day 15. The rat was allowed free to explore the pool in 60 sec with no platform present. The time and frequency of rats in the target quadrant were assessed. Sensorimotor was assessed on day 16. The platform was visible above the surface and had a flag above the platform as additional cues. The latency to find the platform was measured and recorded.

Y Maze

In the present study, on day 17 spatial working memory was measured by using the Y maze spontaneous alternation test, as previously described with a few modifications (Sarter et al. 1988, Kim et al. 2007). Each rat was gently placed at the end of one arm and was allowed to move freely for 8 mins. The apparatus was constructed with three identical arms (50 cm x 10 cm x 10 cm x 10 cm) in which the arms were symmetrically separated at 120˚ and were made from black acrylic. Between sessions of each animal, the maze was cleaned thoroughly with a 70% ethanol solution to remove residual odors. For working memory measurement, the percentage of alternations that the rat made was calculated [% alternation = number of alternation/ (total arm entry-2) *100]. An actual alternation was defined as entries into all three arms sequentially without repetition (ABC, CAB, BCA, not BAB).

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 8.4 software, using one-way or two-way ANOVA and followed by an LSD test for multiple comparisons. Differences between groups were considered significant if $p<0.05$.

RESULT AND DISCUSSION

Involvement of arachidonic acid cascade in spatial memory by kratom

The MWM test is a tool that has been extensively used to investigate spatial memory performances (Zhao et al, 2019). We reported that repeated exposure of KEE for 14 days (one hour before the acquisition trial) reduce spatial learning according to the result of the MWM test. During repeated learning, analysis of the escape latencies showed that animals treated with KEE 200 mg/Kg were significantly prolonged ($p<0.05$) compared with the vehicle group in all trials. Animals treated with 50 and 100 mg/Kg of KEE showed deficit learning ($p<0.05$) but not in all trials (Figure 1).

Twenty-four hours after the last acquisition trial, probe trial (drug free) was measured (without platform). The time and the frequency in the target quadrant were significantly reduced for animals that had received KEE dose-dependence compared with vehicle groups ($p<0.05$; Figure 2 (I) and (II)).

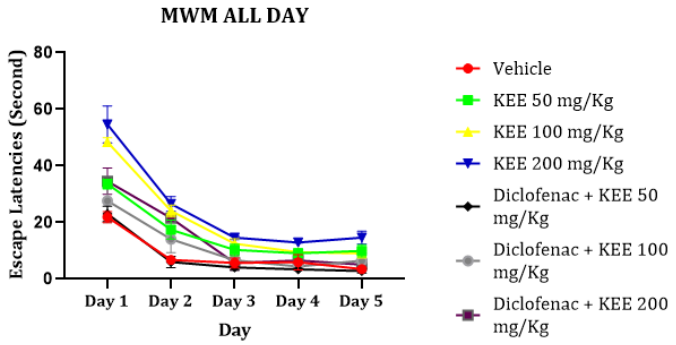

Figure 1. Chart of Acquisition Trial in Morris Water Maze Task

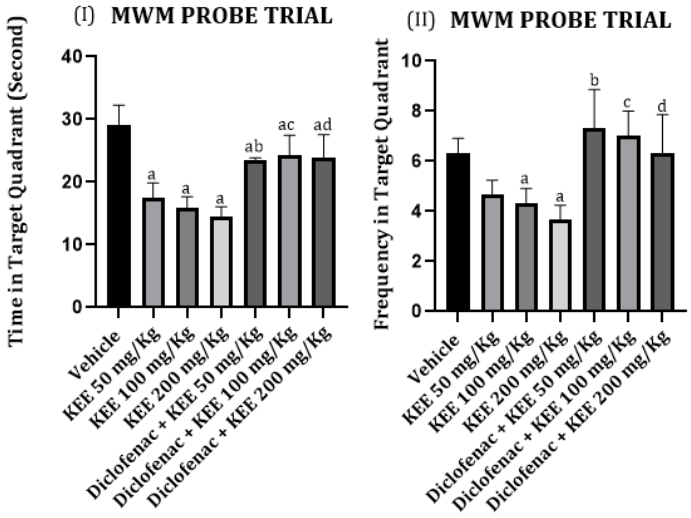

Figure 2. Chart of Probe Trial in Morris Water Maze Task
a: significant compared with vehicle group; b: significant compared with KEE 50mg/Kg; c: significant compared with KEE 100 mg/Kg; d: significant compared with KEE 200mg/Kg.

Sensorimotor trial (visible platform and additional cues) was measured one day after the probe trial. The performances of all groups showed no significant escape latencies compared with vehicle group ($p<0.05$, Figure 3). The results indicate that reduction of memory was not caused by sensory, motor and motivation from the subject (Craig et. al., 2009; Uygur and Arslan, 2010).

The main component of kratom (mitragynine) was reported to decrease reference memory in the water maze task (Zul Aznal et. al. 2022, Meireles et. al. 2019). Mitragynine was also reported to have activity closely resembles morphine due to their opioid activity that caused dependence and memory impairment (Swogger et al. 2018, Kitanaka et al. 2015). One of the several pathways caused by mitragynine that affect memory is arachidonic acid cascade (Zul Aznal et. al. 2022). Free arachidonic acid plays a crucial role in neuroinflammation related to neurodegenerative disease. An arachidonic acid enriched diet was also reported to induce short-term memory impairment in adult male BALB/c mice (Thomas et al. 2017). This study showed that there was involvement of arachidonic acid path way due to memory deficit caused by kratom (Figure 1).

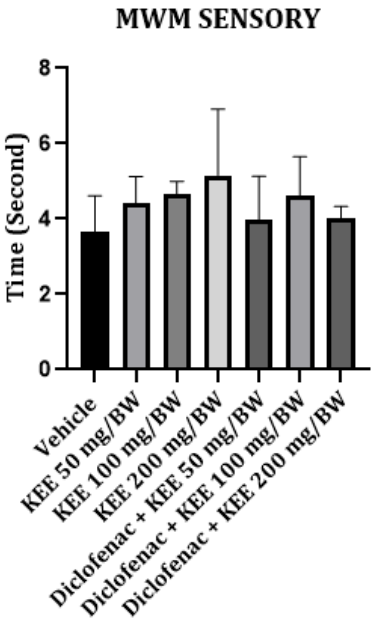

Figure 3. Chart of Sensorimotor Trial in Morris Water Maze Task

The performances of the groups that were treated with diclofenac before KEE was administered were significantly increased in escape latencies, times, and frequencies in the target quadrant compared with the KEE group in the equivalent dosage ($p<0.05$; Figure 1; Figure 2).

Involvement of arachidonic acid cascade in working memory by Kratom

The Y maze test is regarded as measurement of short-term spatial working memory and learning (Hritcu et al. 2012, Muhamad 2019, Kraeuter et al. 2019). The measurement is based on the natural curiosity of animals to explore the new environment (Luszczki et al, 2005). Spatial working memory monitoring using the results of spontaneous alternation. A high Spontaneous alternation percentage showed good working memory (Kraeuter et al., 2019). Figure 4 shows the percent of spontaneous alternation in this study indicating that administration of KEE (5, 100, and 200 mg/Kg) during 14 days lower the spontaneous alternation compared with the vehicle group significantly ($P<0.05$).

This is related with a study in the morphine experiment that reflected working memory deficits (Kitanaka et al. 2015, Galizio et al. 2003, Zhu et al. 2011). The study reported by Kinataka (2015) suggested that morphine induced spatial memory impairment was mediated by μ opioid receptors. Mitragynine was reported to have competitive binding to 3 types of receptor with different affinities with the highest affinity at μ followed by κ and δ opioid receptors (Yamamoto et al. 1999). The effects of kratom in this experiment to induce working memory deficits was consistent with some other reports using other procedures (Apryani et al. 2010, Compton et al. 2014).

In line with the spatial memory tests, pretreated diclofenac enhanced the spontaneous alternation in the Y-maze test more than the group that received KEE in the same dose ($p<0.05$). Diclofenac sodium acts as a potent inhibitor of cyclooxygenase and reduces arachidonic acid release (Capone et al. 2007). Diclofenac is also reported to prevent repeated stress-induced memory deficits in rats (Emad et al. 2017).

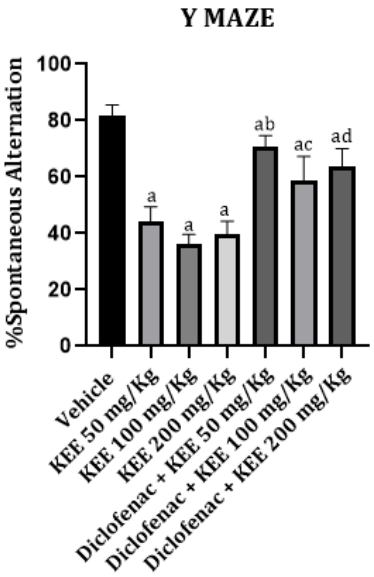

Figure 4. Chart of Spontaneous Alternation in Y-Maze
a: significant compared with vehicle group; b: significant compared with KEE 50mg/Kg; c: significant compared with KEE 100 mg/Kg; d: significant compared with KEE 200mg/Kg.

CONCLUSION

Kratom induced spatial and working memory deficit significantly more than the vehicle group. One of the mechanisms that caused memory deficit was caused by arachidonic acid cascade. Kratom is indicated to trigger the arachidonic acid cascade, leading to memory impairment.

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