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Kratom *(Mitragyna speciosa)* Leaf Ethanol Extract Showed In Vivo Analgesic Activity

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Abstract

Analgesic drugs like morphine and other opioids exhibit several harmful effects. Thus, the exploration of a new and safer analgesic drug is necessary. Utilizing Indonesia's biodiversity richness, medicinal plants can serve as an alternative source of novel analgesic drugs. Here, we show the analgesic activity of Kratom (*Mitragyna speciosa*) leaf ethanol extract (KE) in formaldehyde-induced rat (*Rattus norvegicus*) models. The effect of KE was examined by observing the duration of spontaneous nociceptive behavior such as paw licking or limp leg. The result showed that rats treated with 70 mg/kg KE demonstrated significantly ($p<0.01$) decreased nociceptive behavior compared to those receiving vehicles. However, a higher KE dose (210 mg/kg) failed to increase the analgesic effect and showed a slight reduction (not significant) compared to the control group. These findings proved that kratom leaf ethanol extract has the potential to alleviate painful conditions.

Keywords: *Mitragyna speciosa*, kratom leaves, ethanol extract, opioid analgesic

Introduction

Pain is a distressing sensory and emotional experience related to actual or potential tissue damage or mimicking that experience. Pain is a multidimensional sensory experience and can be distinguished in intensity (mild, moderate, severe), quality (blunt, burning, sharp), duration (transient, intermittent, persistent), and spread (superficial or deep, localized or diffuse).[1]

Based on the 2013 Baseline Health Research (Riskesdas) data, the citizen of East Java Province, Indonesia, was the most users of analgesic drugs in all provinces in Indonesia. This shows the high prevalence of pain in Indonesia, especially in East Java.[2] Regarding the treatment of mild pain, non-steroidal anti-inflammatory drugs (NSAIDs) are still effective in blocking pain sensations. However, for moderate to severe pain due to cancer or post-surgery, more potent opioid analgesics are needed, such as codeine, tramadol, oxycodone, morphine, or fentanyl. This class of drugs has proven effective in treating severe pain by blocking opioid receptors in the central nervous system (CNS).

On the other hand, these opioid analgesics also possess concerning side effects, such as constipation, respiratory depression, addiction, and the potential for drug abuse.[3] Therefore, it necessitates developing a new and safer opioid analgesic drug. Utilizing

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Indonesia's biodiversity richness, medicinal plants are a viable option since they contain various chemicals from which new analgesic medications might be developed.

Mitragyna speciosa, known as kratom, has several properties such as analgesic, antinociceptive, sedative, antiobesity, anticancer, anti-inflammatory, antioxidant, and antibacterial.[4–8] Specifically for its analgesic activity, several studies have reported that the content of kratom leaves can bind to opioid receptors in the CNS, resulting in severe pain cessation.[4,9] This effect is mediated by two major constituents, mitragynine and 7-hydromitragynine, which are isolated from the methanol extract of kratom. Whereas withdrawal symptoms and addiction were evident in both animal models and regular kratom users, indicating that mitragynine possesses the risk which closely mimics those of morphine.[10,11] Therefore, the present study aimed to explore antinociceptive activity of kratom leaf ethanol extract in rats (*Rattus norvegicus*).

Methods

Collection and preparation of plant extract

Fresh leaves of Kratom (*Mitragyna speciosa*) were bought from a supplier in West Kalimantan, Indonesia, and authenticated by Tri Puji Lestari Sudarwati (Pharmacy Academy of Surabaya, Indonesia). Leaves (around 30 g) were then washed, shade dried, and crushed into powder. Subsequently, dry leaves powder (20 g) was macerated with 150 mL of 96% ethanol for 24 hours at ambient temperature. The mixture was stirred occasionally to maintain homogeneity and then filtered.[5] The crude extract (3.38 g) was then concentrated using a rotary evaporator and coded as KE (Kratom Extract). The yield of the extract was found to be 16.9% w/w.

Animals

The preclinical study protocol was approved by the Institutional Ethical Committee of the University of Surabaya, decree number: 97A/KE/VII/2022. Male Wistar rats used in this study were purchased from the animal house of Drh Rachmad Priyadi farm weighing 80–150 g. The animals were placed in plastic cages in a room maintained at a room temperature (21°C) and 12 h light: dark cycle, with unlimited access to standard chow and water, then acclimatized for seven days before the study started.

All feasible measures were taken to minimize animal suffering and limit the number of animals utilized in research. On day one, they were randomly placed in a group of three. Later on day eight, each group was assigned to receive different treatments as follows: Group I was the negative control (vehicle-treated), Group II was the positive control tramadol 0.9 mg/kg p.o, Group III received KE 70 mg/kg p.o., and Group IV received KE 210 mg/kg p.o. All animals were sacrificed after the treatment and measurement to avoid further pain.

Formaldehyde-induced pain model

The test substances and controls were administered to the subject animals according to the previously outlined protocol. After administering the treatments for one hour, 50 µL of a solution containing 2% formaldehyde was injected subcutaneously into the left/right hind paw of the Wistar rats and immediately transferred to a transparent plastic cage for better observation. The spontaneous nociceptive behavior was determined instantaneously by looking at the animal behavior and measuring the duration every time they were licking paws and limping injected-leg.

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The paw licking and limping injected-leg duration was examined from 0 to 5 minutes (first phase, neurogenic) to 15 to 30 minutes (second-phase, inflammatory).[12] The inhibition percentage (%) of nociceptive behavior was also calculated following this formula:

$$\text{Inhibition}(\%) = \frac{\text{Duration of nociceptive behavior (control)} - \text{Duration of nociceptive behavior (test)}}{\text{Duration of nociceptive behavior (control)}} x\ 100\% \quad (1)$$

Statistical Analysis

The results were reported as mean ± standard error of the mean (SEM). The statistical analysis was determined by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests and performed using GraphPad Prism version 8.0.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com; $P<0.05$ was considered as significant.

Results and Discussion

In the past decade, kratom's popularity has soared in Western nations; recent estimates suggest that about five million Americans regularly take kratom. This is supported by the easy use of kratom leaves such as chewing, brewing like tea, smoking as cigarettes, and swallowing directly as compressed tablets or capsules.[13] In Indonesia, kratom obtained from the West Kalimantan region has not been widely studied for antinociceptive activity. Only two articles have been published, the first examines the effect of the dichloromethane fraction in mice, and the second examines the water fraction in male mice.[14,15] In the present study, we opted to determine the antinociception properties of kratom leaves ethanol extract in Wistar albino rats.

Following the oral administration of test substances, animals were induced with formaldehyde to examine the analgesic activity of kratom leaves ethanol extract and compare it with positive (tramadol) and negative (vehicle) control. Figure 1 depicted the duration of paw licking or limping leg after formaldehyde induction, while Table 1 showed the inhibition percentage of rats' nociceptive behaviors. Compared to the control group, KE

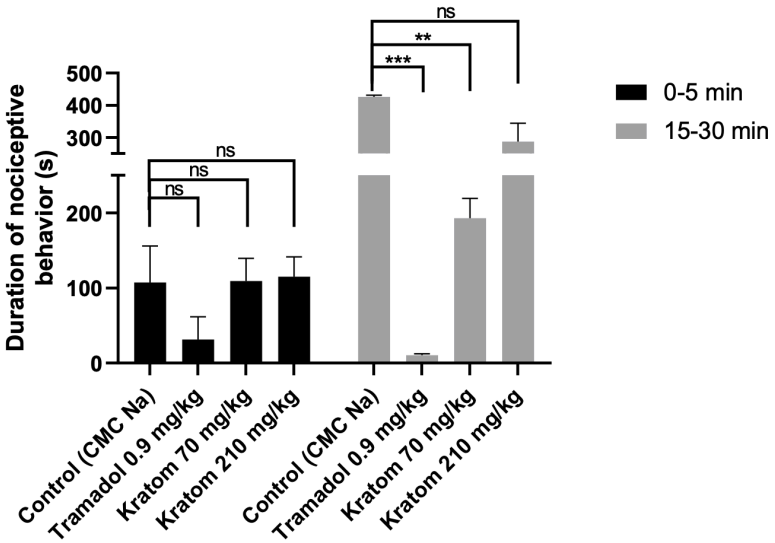

Figure 1. Effect of KE at all the tested doses in both early neurogenic (0-5 min) and late inflammatory phases (15-30 min) estimated by formalin-induced pain models. The duration of nociceptive behavior is presented as mean ± SEM (n = 3). *** P < 0.001, ** P < 0.01 versus negative control using one-way ANOVA followed by Tukey's post hoc multiple-comparison test

Table 1. Average Percentage Inhibition of Nociceptive Behavior

Groups	Neurogenic phase (0-5 min)	Inflammatory phase (15-30 min)
Control (CMC Na)	Ref	Ref
Tramadol 0.9 mg/kg	70.1%	97.5%
Kratom 70 mg/kg	-1.6%	54.7%
Kratom 210 mg/kg	-7.1%	32.5%

with the oral dose of 70 mg/kg significantly ($P<0.01$) reduced rats' nociceptive behavior in the late phase (54.7% reduction). Although, the higher dose did not show a significant reduction, and tramadol (0.9 mg/kg) had more significant ($P<0.001$) reductions than tested extracts. Furthermore, in the first neurogenic phase, kratom leaf ethanol extract did not exhibit blockage to formaldehyde-induced pain stimulation. This model is a biphasic pain reaction.

The induced pain is mediated by glutamate during the neurogenic phase of pain transmission. While the second phase (15 to 30 minutes) of the inflammatory pain response is characterized by releasing inflammatory mediators such as prostaglandins, excitatory amino acids, and histamine. Bradykinin has the unusual ability to influence both stages simultaneously.[16]

Interestingly, another study reported that methanol extract of kratom leaves with a dose of 200 mg/kg could show significant reductions in both phases of the formalin test, indicating that extract active compounds can act in central and peripheral pain.[8] In contrast, our findings suggest that active compounds from 70 mg/kg ethanol extract tend to act as antiinflammatory pain, which inhibits peripheral pain pathway. Meanwhile, a study by Goh et al. (2021) revealed that 200 mg/kg of kratom leaf ethanol extract possessed a similar antinociceptive effect as morphine (5 mg/kg) in the tail-flick test. This means ethanol extract of kratom leaves also possesses central pain blockage. Yet, Goh and co-workers used an accelerated solvent extraction technique that increases the interfacial interaction with the analyte by driving the solvent into the sample matrix's pores, resulting in enhanced analyte recovery and dry yield of extract (29.1% w/w).[17] Taken together, kratom leaves ethanol extract might show antinociceptive and anti-inflammatory properties.

Its antinociceptive activity is influenced by the alkaloid content of mitragynine and the active metabolite of 7-hidroxymitragynine (7-HMG), which can bind to brain opioid receptors.[9,18] Within ethanol-dried extract of kratom, it contains approximately 6.5% of mitragynine, slightly lower than methanol extract, which has more than 7%.[17] Regarding the pharmacokinetics profile, mitragynine is a lipophilic, weak base that passively crosses the intestinal and blood-brain barrier; thus, it quickly permeates and is dispersed in the brain.

The bioavailability was calculated to be 21%, and 85-95% of the drug is bound to plasma proteins.[19] Therefore, kratom and its mitragynine demonstrate potential utility for managing severe pain; however, abuse potential and addiction risk hurdle their clinical usage. According to Hemby et al. (2018), 7-HMG possesses more abuse potential and induces withdrawal than mitragynine,[20] whereas 7-HMG is the product of phase I metabolism

of mitragynine.[21] Structural modification to avoid the formation of 7-HMG during metabolism might ease further development of mitragynine as an opioid analgesic.

This study has limitations, such as the number of animals and a single parameter for antinociceptive activity measurement. Furthermore, the formaldehyde-induced pain model measurement relies on rats' behavior might result in observation bias. However, the chosen method was beneficial in exploring the possibility of both antinociceptive and antiinflammatory activity of a certain compound.

Conclusion

To conclude, we reported the analgesic activity of kratom leaves ethanol extract based on Wistar rats' behavior following formaldehyde induction. Prior to a human clinical study, future researchers should pave the way to examine the exact mechanism of kratom alleviating pain and its safety profile.

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Conflict of Interest

The authors declared no conflict of interest in the manuscript.

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