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Anti-inflammatory effects and potential mechanisms of Mitragyna speciosa methanol extract on λ-karagenan-induced inflammation model

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Anti-inflammatory effects and potential mechanisms of *Mitragyna speciosa* methanol extract on λ-karagenan-induced inflammation model

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ABSTRACT

Introduction: Mitragyna speciosa is known to have beneficial effects, such as antinociceptive and anti-inflammatory effects. There are some components that involve in inflammatory, such as Inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2) enzymes, and also cytokines. This study aimed to investigate the anti-inflammatory effect and its mechanism of Mitragyna speciosa leaf.
Methods: Carrageenan-induced rat paw edema model was used for inflammation induction. Rats were given Mitragyna speciosa leaf methanol extract at a dose of 75, 150, and 200 mg/kg.
Results: Intraplantar injection of carrageenan led to the development of time-dependent peripheral inflammation, which, in turn, was a significant increase in the swelling process as measured by a plethysmometer. However, administration of EMS (75,150 and 200mg/kg) can reduce edema in a dose-dependent manner. We also demonstrated that skin histology on EMS administration significantly preserved skin thickness at 4 h after carrageenan injection.
Conclusion: In conclusion, we reveal the role of Mytragyna speciosa methanol extract as anti-inflammatory and maintain skin thickness in the inflammatory process.

Keywords: *Carrageenan, inflammation, skin-thickness.*
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INTRODUCTION

Inflammation is the basic pathological process of a disease, but it is also a response of the body as a localized protector. Several factors that can cause inflammation are the proliferation of microbes, such as viruses, bacteria, infectious parasites, and direct hypersensitivity reactions that cause inflammation.[1] However, there are certain factors, such as physical factors, chemicals that have irritanting and corrosive aspect, tissue infarction and injury are also important factors in inducing inflammation.[2] Inflammation is commonly treated with non-steroidal anti-inflammatory drugs (NSAIDs) or steroid anti-inflammatory drugs (SAIDs). NSAIDs, that are most commonly used such as aspirin, naproxen, or ibuprofen are considered antipyretic, analgesic, and anti-inflammatory drugs which has mechanism to inhibit the cyclooxygenase (COX) enzyme in the arachidonic acid metabolic pathway.[3] SAIDs consist primarily of glucocorticoids (GC) anti-inflammatory drugs, commonly used as prednisone and dexamethasone acetate. These drugs have its anti-inflammatory effect, specifically in inhibit inflammation genes by binding mechanism in glucocorticoid receptors. Furthermore, it can exhibits anti-inflammatory activity by non-genetic-mediated organizations.[4] Regarding its efficacy in certain diseases, this medication also has side effects, such as NSAIDs may affect gastrointestinal and lead to injury and hepatotoxicity, while GCs may cause sodium retention, obesity, and osteoporosis which can lead to any serious health problems.[5] Therefore, there is still need a development regarding the appropriate therapy that can provides safety and effective anti-inflammatory effect in patients.

Mitragyna speciosa Korth, is a member of the Rubiaceae family, which is a tropical plant that is commonly found in the rainforests of Malaysia, Borneo, and Thailand. The leaves of the M. speciosa Korth tree are known as "Ketom" in Malaysia and as 'kratom' in Kalimantan and Thailand. Kratom plants have been used in the treatment of minor ailments such as fever, diarrhea, diabetes, and pain, as a wound dressing, as well as to reduce tension and physical fatigue. In addition, it is also used for treatment in suppressing opiate withdrawal symptoms.[6] The specific mechanism of Kratom as an anti-inflammatory has not been widely studied, so this study aims to determine the effect of Kratom Extract (Mytragina Speciosa) as an anti-inflammatory in animal models with carrageenan induction.

METHODS

Plant extraction procedure

The extraction method used in this research is maceration. A total of 3 kg of

dry Mitragyna speciosa Korth simplicia powder was extracted using 96% methanol as solvent. Replacement of solvent every 1x24 hours and macerated for 7x24 hours. The maserate was concentrated with a rotary evaporator and a water bath to obtain a thick extract. Then dissolved according to the treatment group, namely 75 mg/kg, 150 mg/kg, and 200 mg/kg.

Animals

Eight-week-old male Rats with weights between 20 to 30 g, were obtained from PUSVEPMA laboratory in Surabaya. Furthermore, these animals were acclimatized for one week at room temperature (25 until 30 °C), and had access to nutritional support (feed abd water) ad libitum under a 12-h light/12-h dark.

Carrageenan-induced paw edema

Induction of acute inflammation by intraplantar injection with carrageenan 10mg/kgBW. Inflammatory activity was measured using a plethysmometer (Ugo Basile, Varese, Italy). The paw volume was measured before (0 h) the stimulus and at selected time intervals (1, 2, 3, and 4 h) after the stimulus using a plethysmometer (Panlab, Spain). The results were expressed as the variation in paw volume (mL), calculated as the difference from the basal volume (0).

Histological analyses

The skin samples were processed from each animal removed after sacrificing, fixed in 10% formalin solution, and processed by the paraffin technique.[7] Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E) for histological examination.[8]

Data analysis

The data are analyzed using SPSS and presented as the mean ± standard error of six animals per group. ANOVA test was conducted and followed by Bonferroni's test. The histopathological scores were analyzed to Kruskal-Wallis and Dunn's multiple comparisons tests. In addition, the normality distribution of each parameter was determined using the Lilliefors [Kolmogorov–Smirnov (K–S)] test for the pooled data. A $P < 0.05$ was taken to indicate a statistically significant difference.

RESULTS

The degree of inflammation was evaluated by inflammation scores from 0 to 5 which were determined based on the parameter edema (table 1). Based on the results of this study, it was found that carrageenan-induced leg edema with maximum edema occurring at 4 hours (0.05±0.002 mL) after injection. Giving Mitragyna speciosa extract significantly ($p<0.001$) reduced edema, especially in the fourth hour at a dose of 75mg/kg (0.011±0.001), at a dose of 100mg/kg and 200 mg/Kg with the same value (0.013±0.002) with the group control.

DISCUSSION

Effect of *Mitragyna Speciosa* Extract on carrageenan-induced foot edema

Previous research has shown that carrageenan-induced rat paw edema can be considered a test to analyze the effectiveness of anti-inflammatory agents.[9] These phlogistic agents produce biphasic edema, the first phase of which is mediated by the release of histamine and serotonin from mast cells, and t second phase involves neutrophil infiltration and the release of prostaglandin E2, the cytokine (mainly IL-1β), and NO. Other studies also explain the Effects of carrageenan on cell permeability, cytotoxicity, and cytokine gene expression in human cells such as hepatic cell lines, Huvecs, and Breast cancer cells.[10,11]

In this study, we confirmed that the oral administration of *Mitragyna speciosa* extract produces a pronounced anti-inflammatory effect on carrageenan-induced leg edema in rats. Concerning the first phase, the release of histamine and other mediators may increased vascular permeability surrounding the damaged tissue and furthermore resulting in edema. Therefore, the increase of vascular permeability inhibition and subsequent exudation will implicate the extent of inflammatory reaction produced at the site of injury. In this model, the sub-plantar injection of carrageenan in control animals produced local edema, which increased progressively to reach maximal intensity 4 h after the injection, after which the effect gradually declined with time. However, EMS (75,150,200 mg/kg) inhibited the development of paw edema. According to this study, the extract may have capability yo suppress the early phase of edema, the possible mechanism that may occur by inhibiting the synthesis, release, or actions of the various hyperalgesic mediators which are

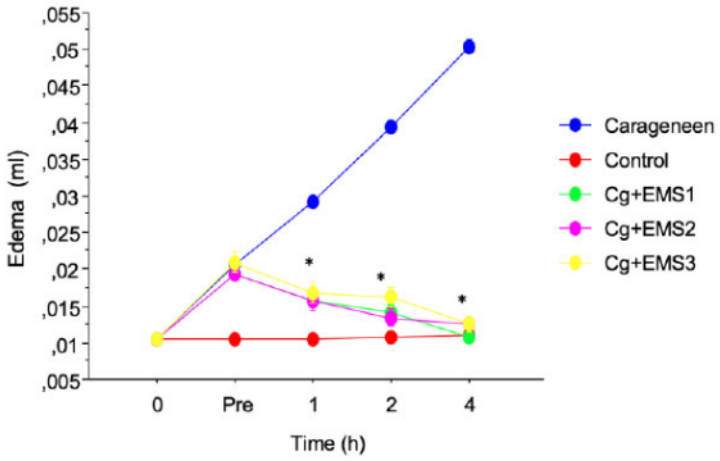

Figure 1. Effect of *Mitragyna Speciosa* Extract on carrageenan-induced foot edema. . Mean ± S.E.M. (%, n = 6) (ANOVA, Bonferroni tests).* $p < 0.05$ compared to the carrageenan (Cg) group.

Table 1. **Effect of Mytragina Speciosa Extract on Carrageenen-induced foot edema in mice.**

Kelompok	Paw Edema (mL)			
	Pre	1h	2h	4h
Kontrol	0.01±0.001	0.01±0.001*	0.011±0.001*	0.011±0.001*
Cg	0.021±0.001	0.029±0.001*	0.039±0.002*	0.05±0.002*
Cg+EMS1	0.019±0.001	0.016±0.003*	0.014±0.002*	0.011±0.001*
Cg+EMS2	0.019±0.001	0.016±0.003*	0.013±0.003*	0.013±0.002*
Cg+EMS3	0.021±0.003	0.017±0.003*	0.016±0.003*	0.013±0.002*

Mean ± S.E.M. (%, n = 6). (ANOVA, Bonferroni tests)
* $p < 0.001$ compared to Cg (Carragenen)

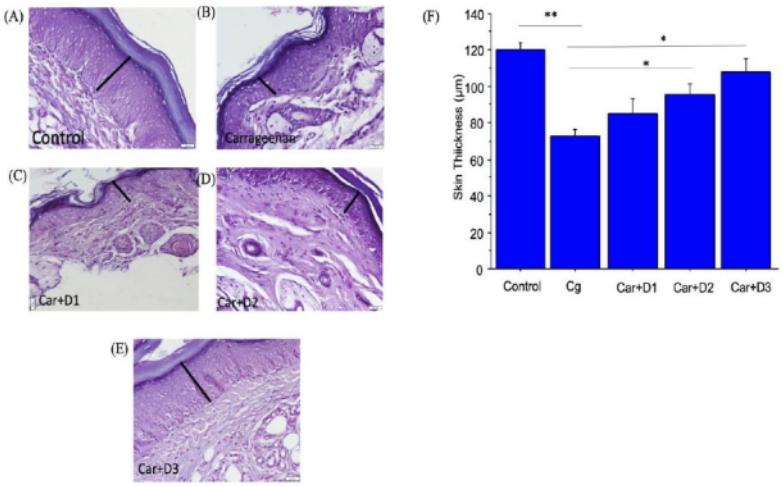

Figure 2. Histopathologic examination of paw tissue of rats treated with EMS, 4 h after injection of carrageenan (Car). (a) Normal appearance of epidermis and dermis without any lesion and skin thickness was found normal in normal rats. (b) Carrageenan-injected paw tissue showed vasodilatation with edema, and decreasing skin thickness. (C-E) Carrageenan-injected paws of rats treated with EMS (75, 150, and 200 mg/kg) show the edema reduced and increased the skin-thickness. Sections were stained with H and E, x40.

known to mediate acute inflammation induced by phlogistic agents and then sensitivity to pain receptors is reduced. In addition, the previous study found that *Mytragyna speciosa* has analgesic and anti-inflammatory properties through inhibition of the COX 2 pathway and prostaglandin E2 mRNA expression.[12]

Effect of Mytragina Speciosa Extract on changes in inflammation of skin thicknesses on HE staining

In this study, the control group showed histology of sub-plantar tissue with normal skin thickness (Fig. 2). On the other hand, injection of carrageenan into the feet of mice caused the accumulation of inflammatory cells and edema so that the skin thicknesses decreased (Fig. 2B). The type of cells that experienced infiltration in the carrageenan induction group was inflammation dominated by polymorphonuclear leukocytes (neutrophils), which indicated the occurrence of acute inflammation. However, edema seen from skin thickness (skin-thickness) significantly increased after administration of *Mitragyna Speciosa* extract at doses of 150mg/kg and 200mg/kg (Fig. 2 D-E) significantly ($p < 0.05$) (Fig. 2F).

The results of this study suggest that differences in skin thickness in induced mice explain the acute anti-inflammatory activity of *mytragina speciosa* extract against the inflammatory process induced by carrageenan.

CONCLUSION

This study suggested that Methanol extract of Mitragyna (EMS) Speciosa possesses anti-inflammatory effects in carrageenan-induced rat paw edema. The anti-inflammatory mechanism of EMS may be related to protecting the skin thickness. Our study findings provide new perspectives on the therapeutic use of EMS in the management of inflammatory diseases.

DISCLOSURE

Author Contribution

All authors have contributed to this research process, including conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, collection and assembly of data.

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Conflict of Interest

There is no conflict of interest for this manuscript.

Ethical Consideration

This research was approved by Ethics Commission of Nahdlatul Ulama

University Surabaya, Indonesia No 1325/EC/KEPK/UNUSA/2021.

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