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Anti-Inflammation Activity of Ethyl Acetate Extract of Malacca Leaves (*Phyllanthus emblica)*

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ABSTRACT
This study aims to evaluate the anti-inflammation activity of ethyl acetate extract of Malacca leaf (*Phyllanthus emblica*) in mice. The study used 25 mice which were induced by subcutaneous injection of 1 % carrageenan. The mice were divided into 5 treatment groups: K1 was negative control, K2 (positive control) was given piroxicam suspension at dose of 20 mg/kg BW, while K3, K4, and K5 were given Malacca leaf extract at dose of 100, 200, and 300 mg/kg BW, respectively, by oesophageal intubation for 4 days. On day 5, the edema volume was measured and the differential leucocytes were counted. The data were analyzed using one way Analysis of variance (ANOVA). The lowest edema volume was observed in mice treated with different doses of Malacca leaves extract (K2, K3, K4) and significantly different ($P<0.05$) compared to mice in K1 and K2. The percentage of neutrophils was significantly lower in groups K2, K3, K4, and K5 than in groups K1. In contrast, the percentage of eosinophil, lymphocytes and monocytes were significantly higher ($P<0.05$) in K2, K3, K3, and K4 as compared to those in K1. The basophils percentage were similar among the groups, except for K4 which had the highest value ($P<0.05$) compared to other groups. It is concluded that the administration of ethyl acetate extract of Malacca leaves can reduce the edema volume and affect the number of leukocytes in carrageenan-induced mice.

Keywords: *Malacca leaf, carrageenan, ethyl acetate extract, volume edema*

1. INTRODUCTION

Inflammation is a defense reaction of the body due to physical trauma and infection that causes tissue damage [1]. The inflammatory process is characterized by the presence of five inflammation stages such as redness, swelling, heat, pain, and loss of function [2]. The inflammatory response can be reduced through the administration of non-steroidal anti-inflammatory drugs

(NSAIDs) which play a role in suppress the inflammatory mechanism [3]. Inflammation treatment is carried out with two main goals; relieve pain and stop the process of tissue damage [4].

The duration of the inflammatory process can be affected by the substances contained in the drug given. The effectiveness of a drug can be seen from the decrease in the volume of edema produced [4]. The administration of NSAIDs is known to have side effects such as kidney problems [5] so that effective anti-inflammatory drugs are developed to prevent secondary inflammation by using traditional medicinal plants. One plant that has potential as an anti-inflammatory is the Malacca plant (*Phyllanthus emblica)*.

Malacca is a plant that has many properties to cure various diseases and is easy to obtain [6]. Krishnaveni and Mirunalini [7] suggested that the Malacca plant is widely used as a diuretic, anti-inflammatory, antipyretic, antidiabetic, hypolipidemic, antiulcerogenic, hepatoprotective, gastroprotective, and chemo preventive. Furthermore, the Malacca plant also

shows analgesic and antipyretic properties [8], antioxidants [9], antimicrobial, antibacterial, and antifungal [10; 11]. Jaijov *et al*. [12] suggested that the extract of Malacca plant can cause anti-inflammatory activity by inhibiting inflammatory mediators.

The fruit, leaves, and roots of the Malacca plant are known to contain secondary metabolites, namely tannins, flavonoids, steroids, phenolics, monoterpenes, sesquiterpenes, quinolones, and saponins [13; 14]. Dhale and Mogle [15] analysed the phytochemical compounds in the fruit and leaves of Malacca and found several compounds including flavonoid, glycerol, terpenoids, benzenoid, triterpene, fats, and carbohydrates. Flavonoid possesses anti-inflammatory activity [16] and the administration of phenolic compounds from *Emblica officinalis* to rat has proven this anti-inflammatory activity [17]. This study was conducted to determine the effect of ethyl acetate extract of Malacca leaves (*Phyllanthus Emblica)* on the decreasing of edema volume and differential leukocytes in carrageenan-induced mice.

2. MATERIALS AND METHODS

2.1. Ethical Clearance Approval

The research has been approved by the Ethics Commission of Faculty of Veterinary Medicine, Universitas Syiah Kuala (No. 56/KEPH/X/2019).

2.2. Preparation of Malacca Leaves Extract

Malacca leaf samples were cleaned and dried, then blended until the powder was formed. Malacca leaf powder was macerated using ethyl acetate solvent for 72 hours. The filtrate was mixed and concentrated using a vacuum rotary evaporator until the dried extract was obtained.

2.3. Induction of inflammation

The inflammation was induced by using carrageenan according to the method described by Aria *et al*. [4]. A total of 25 mice was used and anesthetized using 0.1 ml of ketamine-xylazine. The back of the mice was clean-shaven and left for 24 hours and injected with 5 ml of air subcutaneously to form an air pocket. The 0.05 ml of 1% carrageenan was also injected. After being left for 24 hours, the air pockets formed was sucked with syringe until deflate. Then 0.1 ml of 1% carrageenan solution was injected back into the air pocket.

2.4. The Administration of Malacca Leaf Extract

After inflammation induction, the mice were divided into the following treatment groups: K1 was negative control, K2 (positive control) was given piroxicam suspension at dose of 20 mg/kg BW, while K3, K4, and K5 were given Malacca leaf extract at dose of 100, 200, and 300 mg/kg BW, respectively, by oesophageal intubation for 4 days.

2.5. Parameters analyzed

On day 5**,** edema volume was measured and differential leukocytes was analyzed using haemocytometer. A thin blood smear was prepared, dried, fixed in methanol and stained with *methylene blue* solution. The samples were viewed under a microscope at a magnification of 100x.

2.6. Data analysis

Data were analyzed using one way ANOVA using SPSS for Windows 24.0.

3. RESULTS AND DISCUSSION

3.1 Edema Volume

The edema volume of mice from different treatment groups are presented in Table 1.

Table 1. Volume of edema (mean ± SD) in mice

Group	Mean ± SD (ml)
K1 (negative control)	0.10 ± 0.02^{a}
K2 (positive control)	0.07 ± 0.03^{c}
K3 (dose 100 mg / kg BW)	0.03 ± 0.01^{b}
K4 (dose 200 mg / kg BW)	0.03 ± 0.02^{b}
K5 (dose 300 mg / kg BW)	0.02 ± 0.01^{b}

$^{a, b, c}$ Different superscripts in the same column show significant differences ($P < 0.05$).

Table 1 showed that the highest edema volume was observed in K1 (negative control) and statistically different from those in other treatment groups. Carrageenan administration induced acute inflammation by stimulating mast cell lysis, releasing inflammatory mediators, and causing vasodilation, all of which resulting in exudation of the capillary walls that causes edema [18, 19]. Carrageenan triggers edema reactions but do not cause tissue damage, leaves no scars, and is more sensitive to anti-inflammatory drugs [20, 21]. The edema volume in K2 (given 20

mg/kg BW piroxicam) decreased. Pyroxicam is one of non-steroidal anti-inflammatory drug (NSAIDs) which has anti-inflammatory, antipyretic, and analgesic activities by inhibiting prostaglandin synthesis [22].

The therapeutic effect of NSAIDs is related to the inhibition mechanism of the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes that are needed in the biosynthesis of prostaglandins [23]. Prostaglandins are eicosanoid compounds synthesized from arachidonic acid derived from cell membrane phospholipids. Arachidonic acid metabolism occurred through two pathways: cyclooxygenase to produce prostaglandins and thromboxane, and lipoxygenase to produce leukotriene [24]. Prostaglandins increase sensory nerve sensitivity as well as vascular permeability and act as vasodilators [25]. Leukotriene increases capillary permeability and increases leukocyte adhesion to capillaries during inflammation [26].

Anti-inflammatory efficacy was assessed based on the volume of exudate formed after treatment. The effectiveness of the ethyl acetate extract of Malacca leaves as an anti-inflammatory agent was showed by reduced edema volume in the K3, K4, and K5. This effect might be related to the presence of secondary metabolite compounds such as flavonoids, steroids, tannins, saponins, alkaloids, phenols, and glycosides [27].

Flavonoids are the largest group of phenolic compounds found in plant tissues [28]. Several flavonoids such as quercetin, kaempferol, myricetin, apigenin, luteolin, vitexin, and isovitexin have antioxidant, antipyretic, analgesic, and anti-inflammatory properties [29]. Flavonoids inhibit the release of arachidonic acid by neutrophils [30]. The metabolic action of flavonoid is related to its ability to block cyclooxygenase pathway which is required for eicosanoid biosynthesis, thereby reduce the rate of prostaglandin synthesis [31].Edema is produced through the action of inflammatory mediators such as histamine, bradykinin, serotonin, and prostaglandins in local inflammation [32].

3.2 Differential Leukocytes Counts

Leukocytes are white blood cells that play a role in the body's defence against disease. Differential leukocytes are a unit of white blood cells that are grouped into two parts: granulocytes consisting of neutrophils, eosinophils, basophils, and agranulocytes consisting of lymphocytes and monocytes (Handayani and Haribowo, 2008).

Data on the differential leukocyte counts in carrageenan-induced edema in mice is shown in Table 2.

Table 2. Average percentage of Differential leukocyte counts in all treatment groups

Treatment Group	Differential Leukocyte%				
	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
K1	66.8 ± 4.3^{a}	8.4 ± 3.1^{a}	2.4 ± 0.5^{a}	18.4 ± 4.6^{a}	4.0 ± 2.1^{a}
K2	37.6 ± 2.1^{bc}	11.6 ± 1.8^{b}	1.8 ± 0.8^{ab}	39.0 ± 1.9^{b}	10.0 ± 2.2^{b}
K3	34.6 ± 2.5^{b}	12.6 ± 2.9^{b}	2.4 ± 0.9^{a}	38.6 ± 1.1^{b}	11.8 ± 1.8^{b}
K4	38.4 ± 1.5^{c}	14.0 ± 2.0^{b}	3.0 ± 0.7^{b}	33.4 ± 3.5^{c}	11.2 ± 1.8^{b}
K5	27.0 ± 1.6^{d}	14.0 ± 2.0^{b}	2.4 ± 0.9^{a}	51.0 ± 4.8^{d}	9.6 ± 2.1^{b}

$^{a, b, c, d}$ Different superscript in the same column indicate significant differences ($P<0.05$).

Based on Table 2, the percentage of neutrophils in K1 significantly higher ($P<0.05$) over other treatments. The normal value of neutrophils in the blood of mice ranges from 6-40% [33], this indicated that percentage of neutrophil cells in K2, K3, K4, and was in the normal range.

Mice in K2 (positive control) had a lower number of neutrophils than K1 negative control. According to Wirawan [34], a decrease in the number of neutrophils (neutropenia) might be occurred due to the influence of anti-inflammatory drugs. Piroxicam is a non-steroidal anti-inflammatory drug derived from oxicam [35} that can inhibit prostaglandin synthesis through inhibition of the cyclooxygenase enzyme [36]. Among the group treated with Malacca leaves extract, the data showed that the greater the dose given, the greater the anti-inflammatory activity obtained. The decrease in neutrophils value indicated the effectiveness of Malacca leaves as anti-inflammatory in reducing neutrophil infiltration to the inflammation areas.

The percentage of eosinophil in K2, K3, K4, and K5 groups did not significantly different (P>0.05) among treatments but significantly higher compared to negative control. However, all the values were within the normal range for mice which ranged from 0-15% [33]. Eosinophils play a role in the allergic process, parasite infestations, and the occurrence of inflammation. The conditions in which the number of eosinophils is above the normal range are called eosinophilia [37] and usually due to parasitic infection [38]. Thus, this result indicated that the mice in each treatment group did not suffer allergies and parasitic infections. The lower percentage of eosinophil in K1 might be due acute inflammation [37].

Basophils values in this study were fluctuated but were within the normal range 0-3% for mice [33]. Basophils play a role in systemic allergy by releasing histamine and heparin [39], and increased number of basophils, called basophilia, can usually occur during inflammation, drug influence, and infection. Saponin compounds have anti-inflammatory activity by inhibiting inflammatory mediators such as histamine, bradykinin, and serotonin, while apigenin and luteolin compounds can inhibit histamine release and have anti-hygienic activity [40].

Lymphocytes are cells that appear at the end of the acute inflammatory process which divided into two: T lymphocytes that play a role in response to cellular immunity and B lymphocytes that play a role in the response of the immune humoral, [41, 42]. In Table 1, it can be seen that the number of lymphocytes increased significantly in K2, K3, K4, and K5 compared to those in K1, but the values is still in the normal range (36-90%) for mice [33]. The increase in lymphocytes number in K3, k4, and K5 is due to the administration of ethyl acetate extract from Malacca leaves which can stimulate the formation of immunity in mice. Flavonoid compounds can modulate the immune system, improve lymphocyte function, and initiate specific immune responses.

A condition in which the number of lymphocytes increases is called lymphocytosis [37], usually due to stress, trauma, and the influence of drugs. An increase in the number of lymphocytes is usually accompanied by a decrease in the number of neutrophils [34]. The decrease of lymphocytes as shown in K1 might be due to most of the lymphocytes are withdrawn from the circulation and moved to the tissue where the inflammation occurs [43].

Similar to lymphocyte, the number of monocytes K1 (4.0 ± 2.1%) showed the lower value (P<0.05) than the other treatment groups. Monocytes play a role in digesting injured and dead cells, engulfing bacteria [44], immunological resistance to infectious organisms, and are activated together with neutrophils during inflammation [45]. Asti [46] stated that the administration of high doses of extracts can increase the number of lysed monocyte cells, thereby reducing monocyte phagocytosis. Monocytes in each treatment group experienced fluctuating changes but were still within the normal range. The percentage of normal monocytes in mice is 0.7-14% [33].

When inflammation occurs, monocytes move rapidly leaving blood vessels to the inflamed area to carry out phagocytosis. Monocytes can penetrate the walls of the capillaries into the tissues and differentiate into macrophages. The condition in which the number of monocytes increases is called monocytosis. Monocytosis occurs during macromolecular phagocytosis process and infection healing process [37].

The results in this study proved that the ethyl acetate extract of Malacca leaves with 3 different doses had anti-inflammatory activity. However, the dose of 300 mg/kg BW showed the least fluid accumulation, thus this dose was assumed to pose the better anti-inflammatory activity. This statement is supported by the results of research by Asmilia *et al*., [47] who observed that the ethanol extract of Malacca leaves at dose of 300 mg/kg BW effectively decrease the edema volume of mice.

4. CONCLUSION

The administration of ethyl acetate extract of Malacca leaves can reduce the edema volume and affect the differential leukocytes count of carrageenan-induced inflammation in mice.

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript.

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