

## Antiinflammatory Effects of Ethanolic Extract of Purple Passion Fruit (*Passiflora edulis* Sims.) Peel Against Inflammation on White Male Rats Foot

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**Abstract :** Purple passion fruit (*Passiflora edulis* Sims.), Passifloraceae. Previous studies of purple passion fruit peel is to know the safety limits as a drug. The purpose of this research is to determine the anti-inflammatory effects of ethanolic extract of purple passion fruit peel (EEPPFP) using a paw edema method with pletismometer. As the animal experiment 25 rats were divided into 5 groups, each group numbered 5 rats. As used inducer of  $\lambda$ -carrageenan 1% given intraplantar. Na-CMC 0.50 % as a negative control, diklofenac sodium dose of 4.50 mg / kg bw as a positive control and extract EEPPFP dose of 200, 300 and 400 mg / kg bw, as the test material is administered orally. Observations carried out for 6 hours. The test data were statistically analyzed using one-way ANOVA followed by Duncan test.

The test results of antiinflammatory activity of EEPPFP extract dose of 200 mg / kg bw, EEPPFP dose of 300 mg / kg bw, EEPPFP dose of 400 mg / kg bw have anti-inflammatory effects as against artificial inflammation in the rat foot induced with  $\lambda$ -carrageenan 1% in intraplantar. EEPPFP 400 mg / kg bw has the greatest effect of average inflammatory inhibition compared to EEPPFP dose of 300 mg / kg bw and 200 mg / kg bw. Statistical analysis showed that there was no significant difference between EEPPFP dose of 400 mg / kg bw with diclofenac sodium 4.50 mg / kg bw at 95% confidence level.

**Key Words :** Ethanolic Extract, Purple Passion Fruit (*Passiflora edulis* Sims.), Antiinflammatory Effects, Carrageenan.

### Background

Purple passion fruit (*Passiflora edulis* Sims.), Passifloraceae, used as a refreshing drink. The purple passion fruit skin of the fruit has antihypertensive, antibacterial and anti-inflammatory activity. Study of the purple passion fruit peel has a lot to do, such as: methanol extract of passion fruit rind efficacious as antihypertensives<sup>1,2</sup>. Ekstrak (etanol dan air = 10:1) kulit buah markisah ungu bersifat antioksidan<sup>3</sup>. Water extract of passion fruit leaves have anti-inflammatory activity<sup>1</sup>.

Inflammation occurs due to injury or damage to the tissue that is characterized by redness on the location of the pain or swelling and sometimes accompanied by a burning sensation<sup>4</sup>. Treatment with non-steroidal anti-inflammatory drugs generally have side effects on the gastrointestinal, therefore, sought a new discovery as an alternative medicine that has side effects that are smaller or completely safe<sup>5</sup>.

Continuous research has been conducted by researchers of the passion fruit peel that studies the toxicity of ethanol extract of the purple passion fruit rind of the liver of mice<sup>6</sup>. EEPPFP compounds containing glycosides, flavonoids, steroids / triterpenoids and saponins and the acute toxicity effects of ethanol extract of

purple passion fruit peel (EEPPFP) in mice has been investigated. The results indicate that EEPPFP at a dose of 5 g / kg bw mice were practically non-toxic<sup>7</sup>. Based on the above matters, the researchers wanted to test the effect of anti-inflammatory EEPPFP against male rats.

## Materials and Methods

### Apparatus

Pletismometer (Ugo Basile cat No. 7140), Analytical Balance (Vibra AJ), Animal Balance (GW-1500), Incubator (Gallenkamp) and Rotary Evaporator (Stuart).

### Materials

Materials used in this study EEPPFP (Ginting, et al., 2016) diclofenac sodium, Na-CMC,  $\lambda$ -carrageenan 1%, NaCl physiological solution 0.9% and ethanol 96%.

### Animals

Experimental animals used were male rats weighing 150-200 g as many as 25 animals. Animal house from the Faculty of Pharmacy USU Medan. Rats were divided into 5 groups, each group consisted of 5 male rats. As a positive control group was given diclofenac sodium, negative control by Na-CMC and the test group was given EEPPFP (dose of 200 mg / kg bw, 300 mg / kg bw and 400 mg / kg bw)

### Anti-inflammatory activity

On the day of testing each mouse was given a mark on the right foot and mice, and then weighed. The right foot of rats put in a cell that contains a special liquid on the appliance pletismometer ( $V_0$ ). Each rat was given orally EEPPFP suspension in accordance with the group. One hour later, each foot of mice induced with 0.1 ml carrageenan 1% intraplantar. After 30 minutes, right foot of rats was measured by dipping the feet of mice on a pletismometer cell<sup>8</sup>, changes in volume ( $V_t$ ). Measurements were made every 30 minutes during 360 minutes<sup>9</sup>. At the time of measurement of the last rat foot edema, 6 hours later the rat blood was drawn for calculating the number of leukocytes of mice. 1 mL of blood was added politube containing EDTA, then calculated the total leukocytes. The results obtained from the calculation multiplied by  $10^9$  to get the total leukocytes in 1 L.

### Results Analysis

Data obtained from the research results were analyzed by the Kruskal Wallis test followed by a Mann Whitney test.

## Results and Discussions

### Anti-inflammatory activity

From the numbers on the Pletismometer, changes in fluid volume were noted as the rat foot volume is measured every 30 minutes for 360 minutes. The results of measurements of volume foot rat are shown in the graph. Figure 1 shows the inflammatory changes in percent on the average rat foot. The treatment groups show the decrease of inflammation percent than the control group and it means inflammation suppressing caused by carrageenan.

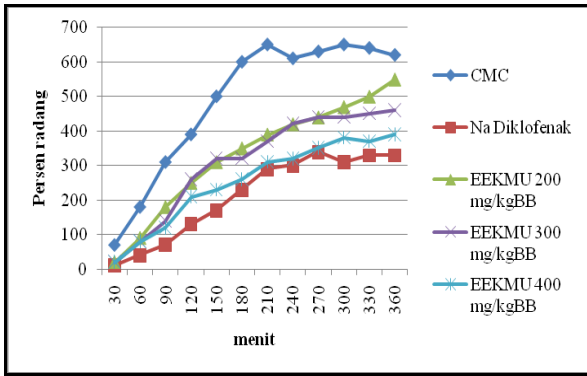


Figure 1. Average rat foot inflammation percent graph each time of observation

The formation of inflammation in the rat foot by carrageenan produces acute inflammation, but does not cause tissue damage, although inflammation can last for 360 minutes and gradually diminished during the day<sup>10</sup>. Rat foot inflammation percent smaller than the control indicates that diclofenac sodium suspension and the suspension EEPPFP ability inhibit inflammation in the foot caused carrageenan rat but significantly different. This capability is called inhibition of inflammation (Figure 2). Flavonoid, alkaloid dan terpenoid<sup>11</sup> juga saponin yang bersifat busa mampu menghambat peradangan<sup>12</sup>. Sifat antiinflamasi EEPPFP didasarkan kepada senyawa flavonoid yang mempunyai sifat antioksidan<sup>13</sup>.

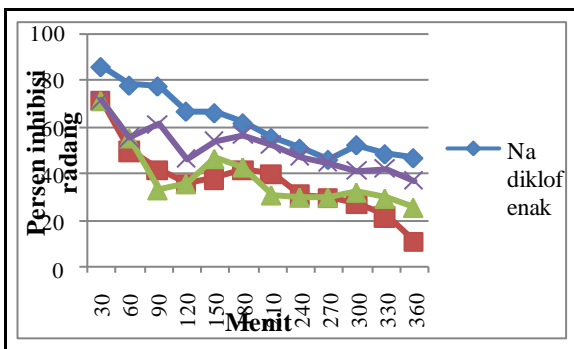


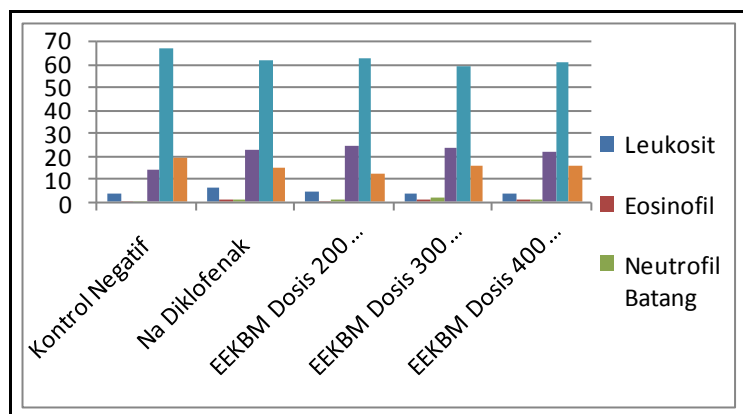
Figure 2. Average rat foot inflammation inhibition percent graph each time of observation

**Total leukocytes and differential leukocytes**

The calculation of the number of leukocytes in the blood cells of male mice induced by carrageenan 1%, after the ethanol extract of purple passion fruit peel each one encompassing a total leukocyte, neutrophil cell segment, neutrophils cell stem, monocytes, lymphocytes and eosinophils.

Table 1. Total leukocytes and differential leukocyte

Group	Cell Numbers					
	Leukocytes (10 <sup>9</sup> / L)	Eosinophils	Neutrophils Stem	Neutrophils Segment	Lymphocytes	Monocytes
Control Na-CMC 0,5 %	3,18 ± 1,49	0,2 ± 0,44	0,2 ± 0,44	14,2 ± 3,27	66,20 ± 7,79	19,4 ± 4,82
Diklofenac Sodium (4.50 mg/kg BW)	5,96 ± 1,13	0,8 ± 0,44	0,8 ± 0,83	22,2 ± 6,53	61,20 ± 10,75	15,00 ± 5,04
EEPPFP (200 mg/kg BW)	4,06 ± 1,64	0,6 ± 0,54	1,0 ± 1,0	24,2 ± 13,42	62,00 ± 17,69	12,20 ± 4,32
EEPPFP (300 mg/kg BW)	3,10 ± 1,43	1,0 ± 0,71	1,6 ± 0,54	23,2 ± 6,83	59,00 ± 9,35	15,2 ± 5,16
EEPPFP (400 mg/kg BW)	3,80 ± 0,68	1,0 ± 0,71	1,20 ± 0,44	22,0 ± 3,53	60,20 ± 6,01	15,6 ± 2,61



**Figure 3. Total leukocytes and differential leukocyte graph**

According to the results, the relationships of the number of leukocytes from the blood of rat on EEPPFP capable of lowering the value of total leukocytes, monocytes and lymphocytes, but an increase in the number of neutrophils stem, neutrofil segment and eosinophils.

Statistical test results using Kruskal Wallis, that the total number of leukocytes, monocytes and lymphocytes decreased insignificantly ( $p > 0.05$ ). The number of eosinophils, neutrophils stem increased significantly ( $p < 0.05$ ) and neutrophils segment lymphocytes increased insignificantly ( $p > 0.05$ ). The number of leukocytes in the blood after administration EEPPFP decreased because one of the signs of recovery inflammation is reduced number of leukocytes migrating in tissues that are exposed by a foreign object<sup>14</sup>. It is seen that the total number of leukocytes, monocytes and lymphocytes decreased from varied dosing EEPPFP. In the leukocyte cells that decreased the lowest is dose 400 mg / kg bw, for the monocyte cells that decreased the lowest is dose 200 mg / kg bw and for lymphocyte cells that decreased the lowest is dose 300 mg / kg bw.

The eosinophil cell count does not become the standard in assessing the effectiveness of anti-inflammatory because of the number of eosinophils is not influenced by carrageenan, but is influenced by multicellular parasites such as worms and allergic reactions<sup>14</sup>. Value monocytes increased in chronic inflammatory phase because monocytes are the second line of defense against infection from bacteria and foreign object<sup>15</sup>.

Secondary metabolites in plants containing phenol and flavonoid compounds have pharmacological activities such as: antioxidant, antiinflammatory<sup>16</sup>, antidiabetic<sup>17</sup>, antimalarial<sup>18</sup>, anticancer<sup>18-20</sup>, antibacterial<sup>21,22</sup> and antiaging<sup>23,24</sup>. Anti-inflammatory activity of the flavonoids have been proven both in vitro and in vivo. The mechanism of flavonoids in inhibiting the inflammation that inhibits the release of arachidonic acid and enzyme secretions lysosomes of cells, neutrophils and endothelial cells, and inhibits the proliferative phase and exudation phase of inflammatory processes<sup>17</sup>.

## Conclusions

The EEPPFP dose of 400 mg / kg bw have anti-inflammatory activity but differ significantly with diklofenac sodium. The number of leukocytes from the rat blood with treatment of EEPPFP at a dose of 400 mg / kg bw able to lower the value of total leukocytes, monocytes and lymphocytes, but an increase in the number of neutrophils stem, neutrophils segment and eosinophils.

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