



## Activity of Ethanol extract of *Passiflora foetida* Linn leaves on Hypersensitivity Response of Immune cells in vivo

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**Abstract** : *Passiflora foetida* L leaves contain flavanoid compounds. Some studies on flavonoids show that flavonoids can modulate the immune system. The purpose of this study was to determine the difference of ethanol extract dose of *passiflora foetida* L. leaves to immunomodulator. Tests on Delayed Type Hypersensitivity using dose of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2 g/kgbw. The extract was given for 7 days. The hypersensitivity response test was performed using 25 mice (Balb/c) divided into 5 groups: control, negative control, extract groups dose of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2g/kgbw. The groups, except control group, were given antigen (SRBC) on the 3<sup>rd</sup> day intraperitoneally and on the 7<sup>th</sup> day were administered intraplantarly. The thickness of the soles of the feet was measured using a plethysmometer before and after injecting the antigen at T4, T24 and T48. The extracts dose of 0.4, 0.8, and 1.2 g/kgbw showed that swelling of the feet of mice decrease compared to control group. The results showed that the dose of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2 g/kgbw showed no significant difference ( $p > 0.05$ ).

**Keywords** : *Passiflora foetida* (L.) leaves, Delayed Type Hypersensitivity, immunomodulator.

### Introduction

One of the plants commonly used by the people of Indonesia in the treatment is *Passiflora foetida* L. The main components of the plant contain alkaloids, phenols, flavanoids glycosides, and cyanogenic components<sup>1,2</sup>. The compound that acts as an antioxidant is a phenolic group. Flavonoids have antioxidant activity and can modulate the immune system<sup>3</sup>. Variation of the Malaysian young corn ear and cornsilk affect the phenolic content and antioxidant activity<sup>4</sup>.

The slow-type hypersensitivity response is a cellular immune response involving Th cell activation that releases proinflammatory cytokines and increases macrophage activity to release inflammatory mediators. Delayed hypersensitivity is cells that play a role in the mobilization of macrophages and other inflammatory cells to the

site of slow reaction. In its function, it requires the stimulation of the Th1 cell. Hypersensitivity response test is an immunomodulatory effect test associated with a specific immune response. This reaction is relaxed by the contact of sensitized T cells with the appropriate antigen. As a result of these sensitizations, T cells release interesting cytokines and stimulate macrophages to release inflammatory mediators. This method is directly related to the cellular immune response, where T lymphocytes are sensitized then secrete lymphokines, thus attract the phagocyte cells to the site of injection and induce an inflammatory reaction<sup>5,6</sup>. At subsequent exposures of about 8-12 hours after activation, the sheep red cell (SRC) antigen induces an effector response in which the IFN- $\gamma$  secreting Th cells that activate macrophages and induce inflammation<sup>3,7</sup>. Immunomodulatory effect studies with antibody secretion parameters showed an increasing in the secretion of primary and secondary antibodies at doses of 400 and 600 mg/kgbw compared with controls<sup>8</sup>. Permot leave have antimicrobial activity<sup>9</sup> and at a dose of 400 mg/kgbw can lower glucose levels<sup>10</sup>.

## Experimental

Material used in this study were *Passiflora foetida* L from South Sulawesi Indonesia, Sheep Red Blood Cells (SRBC), distilled water, 96% ethanol, cotton, filter paper, and paper weigh ethanol.

### Delayed Type Hypersensitivity Activity

The mice were placed on free-pathogen condition and adapted into laboratory condition for two weeks. They were exposed to light cycle with twelve hours in shiny and twelve hours in dark condition. The mice fed with usual nutrition (Ethical clearance: N0: 881/H4.8.4.5.31/PP36-KOMETIK/2016).

Animals were divided into several groups. Group I (normal group), group II induced antigen Sheep Red Blood Cells (SRBC 10% v/v) without given extract. The groups: III, IV, and V induced SRBC and given permot leaf extract at doses of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2 g/kgbw. Extract given for seven days orally. 10% v/v SRBC was administered on the third day intraperitoneally for 1 mL and on the seventh day intraplantar by 0.1 mL for all treatment groups except the normal group. Measurement of the edema of the mice was performed at 4<sup>th</sup>, 24<sup>th</sup>, and 48<sup>th</sup> hours after induction of antigen by using pletismometer and slide length..

## Result and Discussion

### Delayed Type Hypersensitivity Activities

The mice were placed on free-pathogen condition and adapted into laboratory condition for two weeks. They were exposed to light cycle with twelve hours in shiny and twelve hours in dark condition. The rats fed with usual nutrition. The immune system is very important in protecting the body from infectious diseases, either because of bacteria, viruses, or other microorganisms. In addition, the immune system also plays a role in allergic diseases, autoimmune or in organ transplants. Immunomodulator is a compound capable of interacting with the immune system so that to increase or suppress the immune respons<sup>11</sup>.

The results of the extract groups showed the swelling of the feet of mice decrease at T24 than at T4. The group that was induced by SRBC without administration of the extract, the edema decreased very slowly. This shows that there is an effect of the administration of extracts on decreasing the edema of the feet. The inflammatory response is controlled by anti-inflammatory cytokine IL-4 that prevents the activation of magrophages and TGF- $\beta$  which prevents proliferation and activation of macrophages<sup>12</sup>.

The foot volume at 48<sup>th</sup> hours (T48) of extract group showed the feet volume was not significant ( $P > 0.05$ ) compared to T0. It means that in T48 the condition of the mice's feet was the same as the initial condition. The different were shown by the group that was induced by SRBC without the administration of extracts, at T48 it still showed edema in the legs of the mice.

It is possible that the antigen is almost completely neutralized from the body by the immune system of the test animal. In addition, the phagocytic effects of IL-4 and TGF- $\beta$  which drive the decrease of foot volume at 48<sup>th</sup> hours becomes faster. Based on the theory of slow type hypersensitivity reactions (type IV), increasing foot volume does not occur for 6-12 hours and reaches maximum intensity after 24-72 hours. Increased leg

volume is likely due to type I hypersensitivity reactions or arthus reactions (type III) caused by early triggering of slow-type hypersensitivity often followed by humoral immune response.

The data of measurement of foot volume change on T48 was then analyzed by using Kruskal-Wallis analysis test. The change in volume of legs for the leaf extract groups of Permot at dose of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2 g/kgbw showed significantly different results ( $p < 0.05$ ) which only induced SRC 10% v/v without administration of the extract. The experimental results can be seen in Table 1.

**Table 1. Average measurements of mice foot volume after giving antigen**

Group	Time of Mice Foot Volume			
	Initial vol ±SD	T4 ± SD	T24 ± SD	T48 ± SD
A control	0.12±0.011	0.12±0.011	0.12±0.011	0.12±0.011
B SRBC 10%v/v	0.12±0.015	0.23±0.011	0.23±0.011	0.19±0.011
C Extract 0.4g/kgbw	0.13±0.005	0.24±0.017	0.14±0.04	0.14±0.005
D Extract 0.8 g/kgbw	0.13±0.011	0.18±0.011	0.15±0.005	0.14±0.005
E Extract 1.2 g/kgbw	0.14±0.005	0.17±0.017	0.15±0.005	0.14±0.005

It shows that leaf extract at dose of 0.4 g/kgbw, 0.8 g/kgbw, and 1.2 g/kgbw can modulate the animals immune system. The results of T48 between group extract showed no significant difference ( $p > 0.05$ ), it shows that there is no statistically significant difference between each dose of extract.

## Conclusion

Extract dose of 0.4, 0.8, and 1.2 g/kgbw showed that swelling of the feet of mice decrease compared to control group.

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