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STUDY ON ANTI-FATIGUE AND IMMUNO-ENHANCEMENT EFFECTS OF THE EXTRACTS FROM *PARIETARIA DEBILIS* G. FORST. RHIZOME IN MICE

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Summary

Study on Anti-Fatigue and Immuno-Enhancement Effects of the Extracts from *Parietaria debilis* G. Forst. Rhizome in Mice

Parietaria debilis G. Forst. is applied to traditional medicine as a medicinal liquor or a medicinal decoction which has effects of liver detoxification, antipyretics, anti - nephritis, anti-arthritis, and so on. The aim of this research is to study the effects of anti-fatigue via Brekhman's swimming test, and immunostimulation via the immunosuppressive model by cyclophosphamide (at a single dose of 150 mg/kg, i.p) - induced in mice by the aqueous extract (at the oral dose of 0.74g/kg - 1.48g/kg) and 70% ethanol extract (at the oral dose of 0.28g/kg - 0.56g/kg) from *P. debilis* rhizome. The result showed that aqueous extract and 70% ethanol extracts from *P. debilis* rhizome at the oral doses markedly increased the mouse swimming times. Besides, the extracts from *P. debilis* rhizome enhanced the abilities of phagocytes, immune cell-mediated responses (delayed type hypersensitivity), and white blood cells.

Keywords: *Parietaria debilis* Forst. F, *Immunosuppression, Immuno - Enhancement, Anti - Fatigue, Cyclophosphamide, Mice.*

1. Introduction

Fatigue and immunity are interrelated. As we know, when people feel tired, their immunity will decrease significantly, and they will easily become infected; when the human body is infected, people also easily feel fatigued. Fatigue is a complex process involving physiological and biochemical changes, long-time or intense exercise leads to energy consumption such as glucose, muscle glycogen, HG and as well as the accumulation of metabolites including lactic acid and others, which is accompanied by immune

system damage and oxidative stress reaction, thus resulting in fatigue [1]. And, immunosenescence is also a serious problem which is a source of other dangerous diseases and opportunistic infections. Mammalian immunity plays an essential role in the body, consisting of different cells such as T cells, B cells, natural killer cells (NK cells), mast cells, macrophages, basophils, neutrophils, eosinophils, and mediators like cytokines. The immune elements have different functions, but they support each other to prevent penetrating the intracellular membrane from disadvantaged conditions and microbial, pathogens or other strange agents. Nowadays, more people increasingly get immune disorders. In developed countries, the common causes of immunosenescence are obesity, dipsomaniac, and drug addiction, while malnutrition is the most common cause leading to immunosenescence in developing countries. Besides, some organs are responsible for producing immune cells that can be cut due to surgery such as the spleen or thymus, causing a grave decline of the immune system so it is extremely easy to get bacterial infections. Indeed, the ability of immune responses against pathogens is lower in both adults and children and changes in the immune responses are not caused in people aged 40 and reduce gradually from 50 years - old due to an innate immunosenescence [2],[3].

Parietaria debilis G. Forst has been investigated for effecting illness cure and healthcare. However, the knowledge and studies of this botanical species have recently restricted so that *P. debilis* belongs to the Urticaceae family. There are fewer studies or published reports relating to *P. debilis*, Hoang Quoc Tuan, et al. (2019) isolated two chemical compositions which consist of hopane-type triterpenoids and sterols from its rhizome as the formulas of compound 1 is friedelin ($C_{30}H_{50}O$, a colorless needle crystal), compound 2 is 3-friedelanol ($C_{30}H_{52}O$, a colorless cubic crystal), compound 3 is a daucosterol with molecular weight of 576 ($C_{35}H_{60}O_6$, a white powder) in Vietnam[4]. Based on the new bioactive compounds in this herbal medicine consisting of friedelin, friedelinol, and daucosterol which was described by [5]. Friedelin, friedelinol, and daucosterol are known as immunomodulatory agents, friedelin and friedelanol strongly express analgesic and antipyretic, anti-inflammatory activities [6]. Besides, daucosterol reduces inflammation and againts bacteria or fungivia cytokine production [7]. Hence, we not only expected successful results in the improvement of health but also would prove the long-term scientific evidence about the pharmacology for folk medicine through the evaluation of anti-fatigue and immune- enhancement of 70% ethanol and aqueous extracts from *P. debilis* rhizome on cyclophosphamide-induced immunosuppression in mice.

2. Materials and methods

2.1. Plant materials

P. debilis G. Forst rhizome was harvested in March 2023 on Con Dao Island located in Ba Ria - Vung Tau province. It was conserved in the Research Center of Ginseng and Medicinal Materials in Ho Chi Minh City (botanical sample and gene conservation). The material was extracted with 70% ethanol by percolation and aqueous by the decoction method (ratio 1 g herbal power: 15 ml solvent). The 70% ethanol extracts were collected at a rate of 2 ml/min and concentrated using a rotary evaporator at 60°C under reduced pressure to obtain crude 70% ethanol extracts (PDE). The aqueous extract collected solution was heated in a water bath at a temperature of 70°C until it transferred into the condensed extract (PDA). The yields of PDE and PDA viscous extracts were 59.32% and 22.42%, respectively. The humidity of PDE and PDA semi-solid extracts were 10.03% and 16.15%, respectively.

Based on the folk experience and guidelines for Preclinical and Clinical Study of Traditional and Natural Medicines" approved by the Ministry of Healthy- Vietnam (attached with Decision No.141/QĐ-K2ĐT dated 27/10/2015)), which referred to the experimental dose used for human based on the extraction efficiency of PDE and PDA (51.50%, 23.57%, respectively). According to the decision of 141/QĐ-K2ĐT of safe dosages on experimental animals, oral safe dosages were calculated based on the above results of absolute yields of extracts and the conversion of oral dosage between humans and mice equivalents to 2.5 g (herbal materials)/kg (b.w) and 5 g (herbal materials)/kg (b.w). However, the pharmacology experimental doses of PDE and PDA are 0.28 g/kg - 0.56 g/kg body weight mice, 0.74 g/kg - 1.48 g/kg body weight mice (equivalent to 1.25 g and 2.5 g materials per kilogram body weight mice), respectively.

2.2. Animals

Swiss albino male mice (18 ± 2 g, aged 5-6 weeks) were used for the experiments. All of the mice were purchased from the Institute of Vaccines and Medical Biological Products in

Nha Trang City (IVAC). The manipulation of animals was conducted according to "Guidelines for Preclinical and Clinical Study of Traditional and Natural Medicines" approved by the Ministry of Health in Vietnam (attached with Decision No.141/QĐ-K2ĐT dated 27/10/2015). Tap water and standard laboratory food were made available ad libitum. The mice were allowed to acclimatize for a week prior to the experiment. The oral or injection volume was 10 mL/kg b.w.

2.3. Chemicals- Reagents

Cyclophosphamide, zymosan, levamisole, ovalbumin (Sigma, USA). All other chemicals were of analytical grade.

2.4. Survey of the anti-fatigue effect

Before performing the swimming test, mice were adapted to the laboratory housing for one week and then habituated to swim for 15 min/twice a week. Each mouse was forced to individually swim with weights attached to the mouse's tail to correspond to 5% of the mouse's body weight. An acrylic plastic pool (20 x 30 cm) filled with tap water (25 cm in depth) and maintained at a temperature of 29 ± 1°C was used for the swimming test.

At the 1st swimming time, mice were forced to swim. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 20 sec. Then, the mice were immediately picked up and the basal exhaustive swimming test (T_0) was recorded. These mice would have a five-minute break and be randomly divided into five groups (n = 8) showed as Table 1.

After 60 minutes of oral administration, the mice kept swimming again and the result of the 2nd swimming time ($T_{60\ minutes}$) was recorded (single dose). Continuously, mice groups were orally the samples for 7 subsequent days. On the 7th day, an hour after the last oral administration, the mice were forced to swim in the 3rd swimming time (T_{7days}) and repeated doses were recorded.

Table 1. Experimental groups of survey of the effect on physical endurance

Groups (n= 8)	Sample
Physiology	Distilled water
PDE	0.28 (g/kg)
	0.56 (g/kg)
PDA	0.74 (g/kg)
	1.48 (g/kg)

The results of swimming tests after oral administration of T_{60mins} and T_{7days} were generally called T_t compared to the first swimming time (T_0):

-$T_{60\ mins}$ / T_0 (%) of the experimental samples were higher than $T_{60\ mins}$ / T_0 of the control group: the samples are instantaneously effective on endurance capacity.

-T_{7days} / T_0 (%) of the experimental samples were higher than $T_{60\ mins}$ / T_0 of the control group: the samples are effective on endurance after 7 days [3],[8].

2.5. Survey of the effect on immune - enhancement

Table 2. Experimental groups of survey of the effect on immune-enhancing in mice

Groups (n= 8)		Sample
CY (-)	Physiology	Distilled water
CY (+)	Pathology	Distilled water
	PDE	0.28 (g/kg)
		0.56 (g/kg)
	PDA	0.74 (g/kg)
		1.48 (g/kg)
	Positive control	Zymosan/Levamisole

CY (-): injecting with cyclophosphamide; CY (+): injecting without cyclophosphamide.

Determine the index of phagocytes by carbon clearance

Mice were weighed and intraperitoneally injected 150 mg/kg of CY(+), then orally administered distilled water and different experimental samples. After that, mice were intraperitoneally injected with 10 mg/kg of zymosan (i.p.). On the 5th oral administration after an hour, mice were intravenously injected with Parker Quink ink with its quantitative concentration corresponding to the amount of 750 mg/kg carbon (i.v.). Finally, mice were taken 20 µl blood from their orbits by capillary tubes (containing EDTA) at 0 minute and 5 minutes. These blood samples were mixed into 2ml of 0.1% $NaHCO_3$ buffer and measured OD at 640

nm[2,3]. The results of the index of phagocytes were calculated following the formula:

$$K = \frac{InOD1 - InOD2}{T2 - T1}$$

In there:

- K: the constant of the expression in the carbon clearance
- OD_1 and OD_2: OD measurements at T_1 (0 min) and T_2 (5 mins), respectively.

Survey of the immune cells (antigen-antibody response in using OVA)

Mice were weighed and intraperitoneally injected with 150 mg/kg of CY(+), then mice were continued orally administered with distilled water, different experimental samples, and the 25 mg/kg of levamisole. On the 14th day, mice were intravenously injected with 0.5 mg/kg of ovalbumin (OVA) for the first injection. On the 18th day, mice were subcutaneously injected for the right hind paw of mice with 25 mg/kg of OVA for the second time injection (the injection volume of 50 µl/the right hind paw). The left hind raw was used to be a control (V_0). The paw edema mice were measured after 4 hours of OVA injection (V_1) and after 24 hours (V_2)[2,3].

The calculation of the swell of mice's feet is the following formula:

$$\%V(After\ 4hours) = \frac{V1 - V0}{V0} x100$$

$$\%V(After\ 24hours) = \frac{V2 - V0}{V0} x100$$

Evaluation of the amount of white blood cells (WBC)

Mice were weighed and intraperitoneally injected with 150 mg/kg of cyclophosphamide once (CY(+)). After that, mice were orally administered with distilled water, different experimental samples, and 25 mg/kg of levamisole as Table 1. Finally, blood samples of the experimental mice were collected after 5 days. The samples were analyzed by counting the numerous WBCs and the proportion of types of WBCs [2],[3].

2.7. Data analysis

The results were expressed in terms of mean ± SEM (Standard error of the mean) using MS Excel 2016 software. Data were analyzed by Systat statistical software (version 3.5, Inc. SigmaStat) using a t-test and One-way ANOVA followed by the Student-Newman-Keuls test.

3. Results

3.1. The anti-fatigue effect of the samples

As shown in Fig 1., there was not any significant difference in exhaustive swimming time (T60) of all experimental groups as compared to the physiological group. So, the PDE and PDA did not have an instant effect.

The PDE groups (0.28 g/kg - 0.56 g/kg) and PDA groups (0.74 g/kg - 1.48 g/kg) orally administered in repeated doses for 7 days showed a significant increase in percentages of relative swimming time as compared to respect the physiological group.

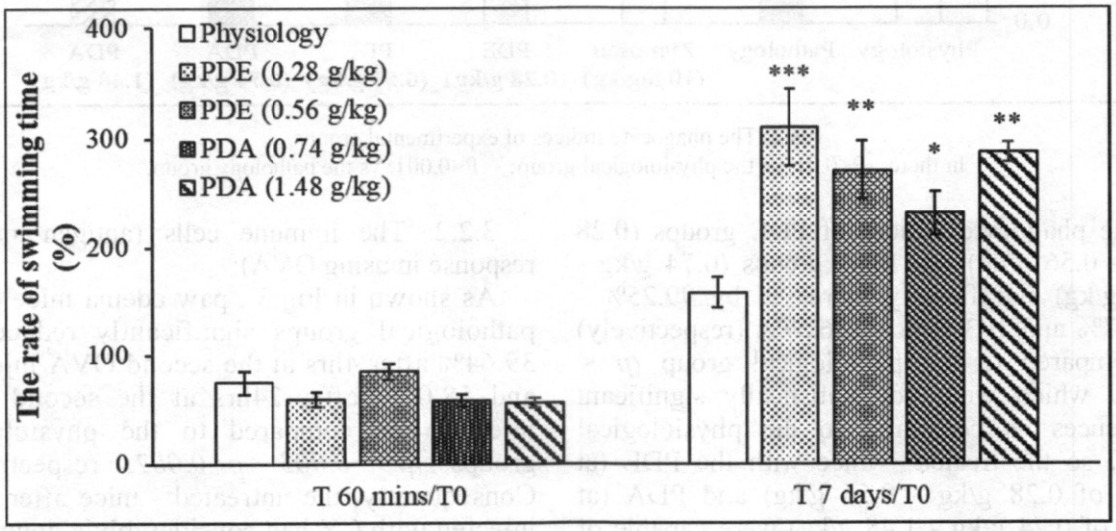

Fig 1. The rate of swimming time in experimental samples.
In there: *P<0.05: vs the physiological group at the same time; **P<0.01: vs the physiological group at the same time; ***P<0.001: vs the physiological group at the same time.

In particular, the PDE group (0.28 g/kg - 0.56 g/kg) exerted percentages of swimming time which significantly increased by 72.09% - 96.74% after 7 days as compared to the physiological group *($p = 0.004$; $p < 0.001$;* respectively*)*. The PDA groups (0.74 g/kg - 1.48 g/kg) exerted percentages of swimming time which significantly increased by 47.00% - 82.73% after 7 days as compared to the physiological group *($p = 0.028$; $p = 0.002$;* respectively*)*. Hence, the PDA (at doses of 0.74 g/kg - 1.48 g/kg) affected intensifying the capacity of endurance after seven - day treatment as well as PDE (at doses of 0.28 g/kg - 0.56 g/kg).

3.2. The effect on immune - enhancement

3.2.1. The index of phagocytes by carbon clearance:

As shown in Fig 2., the phagocyte index of the pathological group significantly reduced by 44.40% as compared to the physiological group *($p = 0.003$)*, so the untreated - mice after five days of being injected with CY had a downtrend of phagocyte index. The phagocyte index of the zymosan group significantly increased by 133.58% as compared to the pathological group *($p < 0.001$)* which was returned to normal value as compared to the physiological group *($p=0.235$)*. Zymosan is used at 10 mg/kg by intraperitoneal injection in mice which enhances the phagocyte activity. Zymosan can stimulate particle engulfment by binding to phagocytic receptors on macrophages, and then induce Toll-like receptors TLR2 and TLR6 to activate inflammatory signals in macrophages that responding inflammation by the activation of NF-kB and TNF-a production as well as cytokine production through zymosan in mouse macrophage [9].

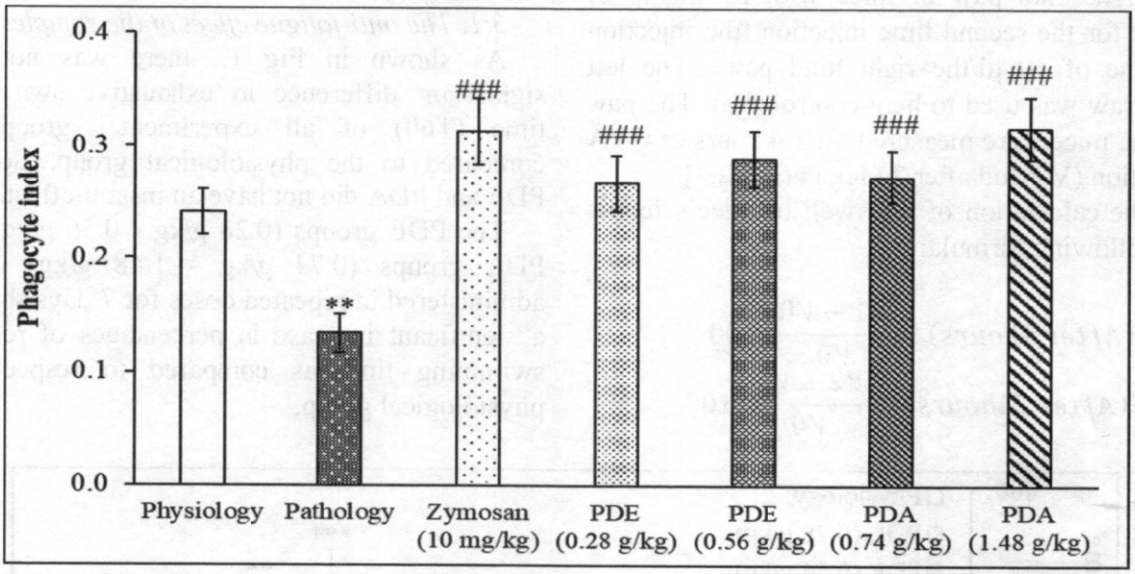

Fig 2. The phagocyte indices of experimental groups.
In there: $^{*}P<0.01$: vs the physiological group; $^{###}P<0.001$: vs the pathology group.

The phagocyte indices of PDE groups (0.28 g/kg - 0.56 g/kg) and PDA groups (0.74 g/kg - 1.48 g/kg) significantly increased by 99.25% - 115.67% and 103.73% - 136.57% (respectively) as compared to the pathological group *($p < 0.001$)* which were not statistically significant differences as compared to the physiological group, so the treated - mice with the PDE (at doses of 0.28 g/kg - 0.56 g/kg) and PDA (at doses of 0.74 g/kg - 1.48 g/kg) were capable of phagocyte stimulation as well as zymosan (10 mg/kg) and all these returned phagocyte index to normal values.

3.2.2. The immune cells (antigen-antibody response in using OVA):

As shown in Fig 3., paw edema mice of the pathological groups significantly reduced by 39.64% after 4hrs at the second OVA injection and 58.07% after 24hrs at the second OVA injection as compared to the physiological groups *($p =0.003$; $p=0.002$;* respectively*)*. Consequently, the untreated - mice after being injected with CY had considerably a downtrend of immune response ability leading to a decrease in paw edema mice after 4 hours and 24 hours.

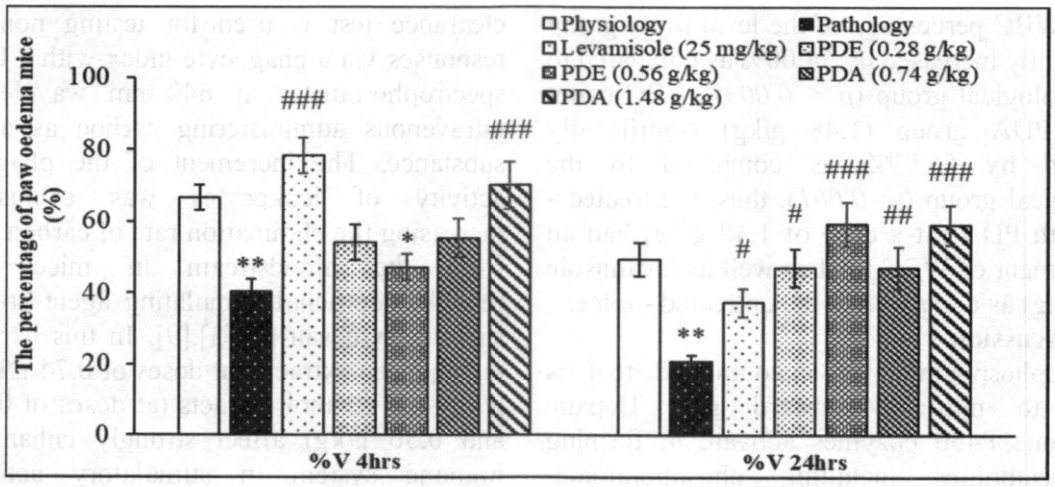

Fig 3. The percentage of paw edema mice after 4 hours and 24 hours
In there:. *P<0.01: vs the physiological group at the same time; #P<0.05: vs the pathological group at the same time; ##P<0.01: vs the pathological group at the same time; ###P<0.001: vs the pathological group at the same time

Paw edema mice of the levamisole groups significantly increased by 94.54% after 4hrs at the second OVA injection and 80.14% after 24hrs at the second OVA injection as compared to the pathological groups (*p<0.001; p=0.023*; respectively). Paw edema mice of the levamisole group did not a statistically significant difference after 4 hours and 24 hours as compared to the physiological group (*p=0.212; p=0.331*; respectively).

PDE groups (0.28 g/kg - 0.56 g/kg) and PDA groups (0.74 g/kg) were no statistically significant differences after 4 hours at the second OVA injection as compared to the pathological group while PDA groups (1.48 g/kg significantly rose by 70.06% after 24hrs at the second OVA injection as compared to the pathological groups (*p<0.001*). After 24 hours, paw edema mice of PDE group (0.28 g/kg-0.56 g/kg) and PDA group (0.74-1.48 g/kg) significantly increased as compared to the pathological group *(p=0,003; p<0.001;p=0.002; p<0.001;* respectively*)*, so all these had strong effect on the immune response enhancement after 24 hours at the second OVA injection as well as levamisole (25 mg/kg) which returned to normal values of paw edema mice as compared to the physiological group.

3.2.3. The total white blood cells count:

As shown in Fig 4., the WBC percentage of the pathological group significantly reduced by 83.20% as compared to the physiological group (*p < 0.001*). Thus, the untreated - mice after being injected with CY had a downtrend of total WBC.

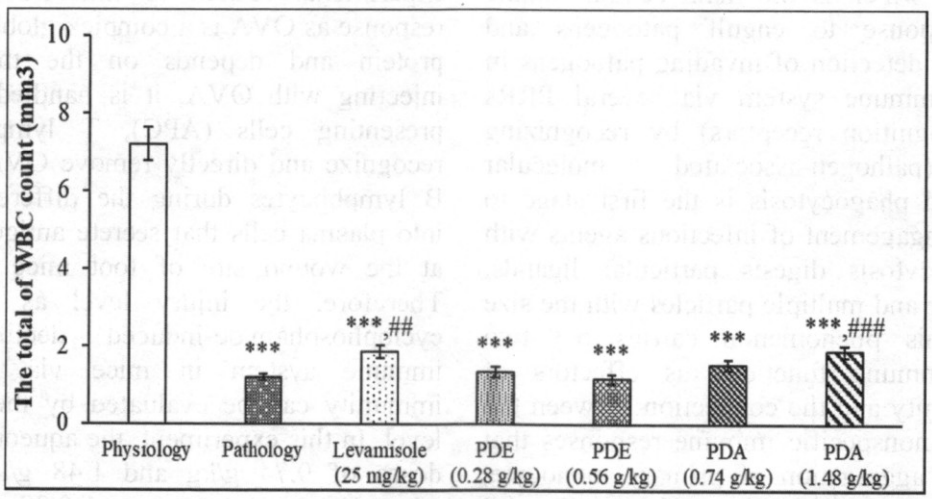

Fig 4. The total WBC count of the groups.
In there: ***P<0.001: vs the physiological group; ##P<0.01: vs the pathological group; ###P<0.001: vs the pathological group

The WBC percentage of the levamisole group significantly increased by 54.00% as compared to the pathological group ($p < 0.003$). WBC count of the PDA group (1.48 g/kg) significantly increased by 55.99% as compared to the pathological group ($p=0.001$), thus, the treated - mice with PDA (at a dose of 1.48 g/kg) had an improvement of WBC total as well as levamisole (25 mg/kg) as compared to the untreated - mice.

4. Discussion

Cyclophosphamide is a compound that is similar to nitrogen mustard gas. Hepatic cytochrome P450 enzymes activate it, forming two metabolites including phosphoramide-mustard and acrolein. In this way, acrolein will block the tissue's antioxidant defense system and manufacture reactive oxygen species (ROS) that interfere with protein amino acids resulting in changes in structure and functions. The half-life of CY is between 17 minutes to 25 minutes in mice [10]. Consequently, CY causes the decline of the whole immune system in mice and the inhibition of cellular immunity. It damages the liver, thymus, spleen, adrenal glands, and kidneys in untreated - mice, and decreases reduces white blood cells, B or T lymphocytes, monocytes, and neutrophils at the high dose (a single dose from 150 mg/kg - 250 mg/kg by i.v or i.p) as compared to normal mice after five - day injection [11]. Moreover, the humoral immunity and cell-mediated immunity in the immune response's ability also reduce as compared to normal mice after 5 days [2],[3].

Phagocytes are a kind of WBC and use phagocytosis which is the main cellular innate immune response to engulf pathogens and bacteria. The detection of invading pathogens in the innate immune system via several PRRs (pattern recognition receptors) by recognizing PAMPs (pathogen-associated molecular patterns), and phagocytosis is the first stage to initiate the engagement of infectious agents with PRR. Phagocytosis digests particular ligands, usually larger and multiple particles with the size of 1μm. This phenomenon carries out two important immune functions as effectors of innate immunity and the connection between the specific and nonspecific immune responses that promote the aggregation of primary monocyte, neutrophil, macrophage, and eosinophil populations to create an arrest barrier to round up strange structures. Therefore, the carbon clearance test is used for testing non-specific responses via a phagocyte index with a UV - Vis spectrophotometer at 640 nm wavelength by intravenous administering carbon as a foreign substance. The increment of the phagocytosis activity of leukocytes was evaluated by increasing the elimination rate of carbon particles from the bloodstream in mice by the reticuloendothelial stimulating agent stimulators such as zymosan [2],[3],[9]. In this experiment, the aqueous extracts (at doses of 0.74 g/kg - 1.48 g/kg) and ethanol extracts (at doses of 0.28 g/kg and 0.56 g/kg) affect strongly enhancing the immune system in stimulatory activity on cytokine release from macrophages into the bloodstream and return phagocyte index to a normal level as compared to healthy mice after 5 days of treatment.

The cell-mediated immune reaction is assessed by delayed-type hypersensitivity response via the excessive reaction causes organizational disorder and injury when the interaction between the T lymphocytes that have been previously sensitized, and the same antigen transform into lymphoblasts and lymphokine secretion attracting more cells to the reaction site [3],[12]. Macrophages are attracted by lymphokine along with T lymphocytes, and then it is activated to release cytokines that increase the permeability of capillaries and cause swelling because these cells arrive at the reaction site and are immobilized in here leading to the enhancement of inflammatory response. Ovalbumin (OVA) is used commonly for experiments related to immune cell-mediated response as OVA is a complex globular antigenic protein and depends on the thymus. After injecting with OVA, it is handled by antigen-presenting cells (APC), T lymphocytes can recognize and directly remove OVA, or support B lymphocytes during the differentiated stage into plasma cells that secrete antigens inflaming at the wound site of foot mice [2],[13],[14]. Therefore, the injury level as well as the cyclophosphamide-induced decline of the immune system in mice via cell-mediated immunity can be evaluated by the foot's swell level. In this experiment, the aqueous extracts (at doses of 0.74 g/kg and 1.48 g/kg) and 70% ethanol extracts (at doses of 0.28 g/kg and 0.56 g/kg) do not express the immune response obviously after 4hrs at the first OVA injection,

but they strongly stimulate the capacity of the immune response via the level of paw edema mice after 24 hours at the second OVA injection and return to a normal level as compared to healthy mice after 18 days of treatment.

Nowadays, the species of *P. debilis* G. Forst. have few studies have isolated three main bioactive compounds from its roots including friedelin, 3-friedelanol, and daucosterol, they depend on hopane-type triterpenoids and sterols, whereas friedelin, 3-friedelanol, and daucosterol are known as immunostimulatory compounds from plants [4]. Hence, the aqueous extracts (0.74 g/kg and 1.48 g/kg) and 70% ethanol extracts (0.28 g/kg - 0.56 g/kg) have strong stimulatory abilities of the anti-inflammation and immune cell-mediated response in mice which is completely reasonable. This enhancement results in daucosterol compounds. Daucosterol is the glucoside of β-sitosterol depending on a natural phytosterol-like compound. β-sitosterol can strongly suppress IL - 6 to stimulate macrophages by reducing NO production from lipopolysaccharide (LSP) - induced RAW 264.7 mice as well as decreasing secretions of TNF - α and IL - 1β, while TNF - α is known as a main mediator in inflammatory diseases. Besides, the more macrophages and neutrophils generated, the more NO is produced. When macrophages are attacked, leading to a large amount of NO production from oxygen and L-arginine by various nitric oxide synthetases and inorganic nitrate reductions. Thus, LPS of inflammatory cytokines and bacteria can regulate the activity of inducible NOS in macrophages. On the other hand, friedelin has been demonstrated to have antipyretic, anti-inflammatory, and analgesic effects. It can inhibit some mediators during inflammatory responses such as serotonin and histamine are responsible for the response of the immediate inflammation, meanwhile, PGs and kinins regulate prolonged response. Friedelin may play a crucial role in the suppression of PGs synthesis [15],[16].

Blood components in the peripheral blood consist of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. When the spleen and thymus were destroyed by cyclophosphamide causing significant changes in the ratios of the weights of thymus and spleen to body - weight as well as in bone marrow and in the peripheral blood in mice such as decreasing the number of red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) after 2, 7 and 14 days at two doses (25 and 50 mg/kg). Furthermore, the change in WBC count in the peripheral blood also reflected the effect of drugs on stem cells in bone marrow. Thus, white blood cell count in the peripheral blood was a quantitative index to reflect both the innate and specific immune responses that were hematological indices to be tightly observed on clinical CY treatment [17]. In this experiment, the aqueous extracts (at doses of 0.74 g/kg and 1.48 g/kg) and 70% ethanol extracts (at doses of 0.28 g/kg and 0.56 g/kg) can improve the WBC count, but all these did not return to a normal level as compared to healthy mice after 7 days of treatment which corresponded to these previous studies [11]. Recently, researchers have divided into friedelin and friedelanol depend on pentacyclic triterpenoids, while daucosterol (β-sitosterol) belongs to phytosterols which are relative to the proliferation of WBC's blood component [14]. According to the previous study, β -sitosterol stimulates various blood components of WBC, especially, when it enhances the production of more lymphocytes in mice. Conversely, some recent studies have also recognized that friedelin, 3-friedelanol, and β-sitosterol have decreased inflammatory responses by highly inhibiting T lymphocyte proliferations and elements in the peripheral blood, decreasing IL - 2Rα expression (CD25) and IL - 2 secretions and activate procyanidin B2 to reduce inflammatory cytokines production by increasing IL - 1 receptor expression associated with protein kinase, so that can be a reason to explain WBC count which does not recover to a normal index (IRAK-M) [18],[19]. Improving immunity to keep the body in the best condition can enhance the human body's exercise endurance, delay the appearance of fatigue, and accelerate the recovery of fatigue. Phagocytes, which can recognize, phagocytize, and destroy foreign bodies entering the living body, are the major defense line of the human immune system. sIgA is a major antibody in exocrine fluid and the first immune defense line against pathogens and harmful substances in the respiratory tract, digestive tract, and urogenital tract. It is the most important antibody in mucosal immunity. Thus phagocyte activity and level of sIgA can directly reflect the immune activity of the body [20]. This

research also demonstrated that *P. debilis* Forst. f. extracts have the effect of anti-fatigue via Brekhman's swimming test. This result suggests that it may act as an energy source that will support health and enhance immunity.

In the limitation of studying this topic and information of the species of *P. debilis* Forst.f., the topic has not found appropriate compounds to explain the results of changes in the relative weights of the thymus, spleen and adrenal gland, and WBC count by the aqueous and 70% ethanol extracts at all experimental doses in detail. To solve this problem, we need to determine more different compounds as well as marker compounds of this species which are responsible for their main bioactive effects.

5. Conclusion

The 70% ethanol extracts (at doses of 0.28 g/kg and 0.56 g/kg) and aqueous extracts (at doses of 0.74 g/kg and 1.48 g/kg) from *P. debilis* G. Forst. had the anti-fatigue effect after 7 days of taking the sample and the immune-enhancement effects through increasing the strong production and stimulation of phagocytes, the immune response via expression on paw edema. In there, aqueous extracts (at doses of 1.48 g/kg) from *P. debilis* G. Forst. had an improvement in total WBCs.

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