The Immunomodulatory Activity of Pirdot Leaf Extract (*Sauraia Vulcani* korth.) on the Immune System of Male Rats

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Abstract: Immune-mediated diseases are a significant problem in developing countries such as infectious diseases, cancer, and autoimmune disorders. The Sauraia vulcani Korth was studied has flavonoid compounds that have the potential as immunostimulants. The purpose of this study was to prove the immunomodulatory effect on phagocytic activity of phagocytic cells in male rats. The treatment group for carbon clearance test was divided into 6 groups with each consisting of 5 male rats including 0.5% CMC-Na group, Imboost® 32.5 mg / kgBB, ethanol extract of Sauraia vulcani Korth. doses of 50, 100, 200 and 400 mg / kgBB orally for 7 days and 8th day given carbon suspension i.v. Taking blood was carried out at a certain time and then measured the absorbance using a UV-Vis spectrophotometer. The rat's liver and spleen were taken and weighed.The results showed that the administration with doses of 50, 100, 200 and 400 mg / kgBB of ethanol extract of Sauraia vulcani Korthsignificantly increased phagocytic activity compared to 0.5% CMC-Na ($p < 0.05$).This study proves that the ethanol extract of Sauraia vulcani Korth has an immunostimulatory effect on phagocytic activity which can be seen through phagocytic index parameters, stimulation index, and carbon clearance value.

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1 INTRODUCTION

The environment around us has various types of pathogenic microorganisms, such as bacteria, viruses, fungus, protozoa and parasites that can cause infections in humans. Infections that occur in normal people are generally brief and rarely leave permanent damage. Human immunodeficiency virus (HIV) and Mycobacterium TB (Mtb) have a synergistic relationship. In coinfected people, tuberculosis (TB) causes cell activation and excessive production of cytokines and chemokines. Later stimulates (Carla et al., 2017).

Human immunodeficiency virus infection results in early disruption of anthropometric indicators in children and adolescents. However, combination antiretroviral therapy has increased safety with the immune response and viral infection, the severity and the increase in the severity of the infection in previously infected (Golluci et al., 2019). The maintenance of immune homeostasis is essential for regulation of healthy physiological function and prevention of disease. Immune dysregulation can lead to opportunistic infection or, on the other extreme, self-targeted autoimmune diseases. Secreted proteins such as cytokines and antibodies serve as essential messengers and regulators of the immune system, which has motivated extensive efforts to use them as therapeutics Despite significant advancements in protein drug development, the field is still hampered by challenges in expression, stability, specificity, and activity. New platforms and methodologies in protein engineering have revolutionized drug development by pioneering novel strategies to enhance the behavior of natural proteins and engineer new molecules customized for specific disease applications (Kureshi et al., 2018).

Oxidative stress is considered to be critically involved in the normal aging process but also in the develop-ment and progression of various human pathologies like cardiovascular and neurodegenerative diseases,as well as of infections and malignant tumors. These pathological conditions involve an overwhelm-ing production of reactive oxygen species (ROS), which are released as part of an anti-proliferativestrategy during pro-

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inflammatory immune responses. Moreover, ROS themselves are autocrine forwardregulators of the immune response. Most of the beneficial effects of antioxidants are considered to derive from their influence on theimmune system. Due to their antioxidant and/or radical scavenging nature, phytochemicals, botanicalsand herbal preparations can be of great importance to prevent oxidation processes and to counteractthe activation of redox-regulated signaling pathways. Antioxidants can antagonize the activation ofT-cells and macrophages during the immune response and this anti-inflammatory activity could beof utmost importance for the treatment of above- mentioned disorders and for the development of immunotolerance (Becker et al., 2014).

An additional immune system is needed to maintain a balanced immune system. Artificial immunity can be given by immunization. The immune response will increase with the administration of immunostimulant compounds (Trivedi et al., 2016).

Diseases mediated by the immune system are significant problems. Such as: treatment for HIV and Ebola Virus which requires an aggressive and innovative approach to the development of new treatments. Therefore, it is necessary to add immune enhancers Among the many herbs used one of them is Sauraia vulcani Korth L. which is a family of Sauraia plants (Sharma et al., 2015).

Natural herbal products are used in the prevention or treatment of cancer in many countries. Althoughless antitumor effect than chemotherapy drugs, it can producemore immune enhancement and less toxicity (Jeong, Koh, Kim, &Kim, 2011; Kim, Moon, Choi, Kim, & Lee, 2013; Li et al., 2012; Wang et al., 2012).

Along with the development of increasingly sophisticated technology, the use and utilization of traditional medicines in Indonesia has increased dramatically. Traditional medicines are re-used by people who believe in the principle of back to nature as an alternative treatment, in addition to synthetic medicines that are developing in the market. Traditional medicines derived from plants and pure natural ingredients have side effects, danger levels and risks that are much lower compared to chemical drugs (Sharma et al., 2015).

Medicinal plants have the potential to be used as natural fungicides. This is because medicinal plants contain secondary metabolites which can act as antifungal agents. Secondary metabolites such as saponins, alkaloids, coumarin, xanton, flavonoids, fatty acids, phenol compounds, terpenes, essential oils, lectins and polypeptides have been reported to have antifungal activity.

Synthetic drugs for infections have been widely marketed. However, resistance related to synthetic drugs has been widely discussed.Treatment of infections with multidrug shows the effects of HIV treatment too early and the delay in co-infected patients receiving treatment for TB. This study shows that the burden of coinfection is highly dependent on when antiretroviral therapy (ART) is started (Mallela et al., 2016; Spangler et al., 2015).

Previous research (Sitorus, 2015), pirdot leaves (Saurauia vulcani Korth.) Had an antidiabetic effect on mice. Previous research related to the genus Sauraia has potential as an antioxidant and antidiabetic. Previous research on the leaves of the pirdot plant, said that this plant has been used by the people of North Sumatra for a long time to treat wounds and prevent bacterial infections (Farid et al, 2012).

Pirdot leaves contain compounds in the form of steroids, flavonoids, saponins, tannins, triterpenes, and also have antioxidant power. Secondary metabolites found in pirdot leaves are thought to have potential as antifungal so testing is needed to update the potential of these pirdot leaves.

Based on previous research reports, pirdot leaves (Sauraia vulcani Korth.) contain essential oils, saponins, flavonoids, tannins and triterpenoids. Previous research also stated that the pirdot leaf (Sauraia vulcani Korth.) Contains constituents such as saponins, flavonoids, tannins and triterpenoids (Setyowati dkk., 2014). The ethanol extract of the pirdot leaf (Saurauia vulcani Korth) studied has potential as an antibacterial and high antioxidant activity.

Immunotherapy strategies to date have used antibodies to block inhibitory receptors (also called "checkpoints") that are regulated on T cells capable of entering tumor cells (Golluci et al., 2019; Adel et al., 2016; Moon et al., 2017). Immune system activity testing can be done by various methods, namely hemagglutination antibody titer, slow type hypersensitivity response, and phagocytosis activity test using carbon cleansing method (Carbon Clearance) (Shukla et al., 2009).

Phagocytosis activity test uses the carbon clearance method to determine the picture of a nonspecific immune system. Carbon clearance method is usedto measure the activity of phagocytic cells killing pathogenic organisms that enter the body and count the number of leukocyte cells (Marbun et al., 2018).

2 METHOD

Place of research

The extract making is carried out in Chemical laboratory, at Pharmacy Faculty, Institut Kesehatan Medistra Lubuk Pakam

Research time

This research is carried out on the month (January 2019 to August 2019).

Material

Pirdot leaf, carboxy methyl cellulose (CMC), chloroform, 2 N hydrochloric acid, Mayer reagent (mercury (II). chloride and potassium iodide), Dragendorff reagent (bismuth, nitric acid and potassium iodide), Maych reagent (mercury (II) chloride and potassium iodide), Dragendorff reagent (bismuth, nitric acid and potassium iodide), Bourchardat reagents, mercury (II) chloride and potassium iodide), Dragendorff reagents (bismuth, nitric acid and potassium iodide), Bourchardic reagents, iodine and potassium iodide, Zn powder, concentrated hydrochloric acid, amyl alcohol, concentrated sulfuric acid, Molish reagent, iron (III) chloride, n-hexane, Liebermann Burchat reagent (anhydrous acetic acid and concentrated sulfuric acid), chloroform, chlorohydrate, n-hexane, distilled water, carbon ink (Pelikan B17), Imboost®, sodium citrate, 1% acetic acid, and 0.9% NaCl and distilled water.

Sample Processing

Saurauia vulcani Korth. are cleaned of dirt by washing under running water until clean then drained to dry after it is sliced into small pieces dried in a drying cabinet / in the room (aerated). *Saurauia vulcani* Korth. that have dried are blended until they become powder weighed by weight and are called simplicia. As much as 1 kg of pirdot leaf simplicia powder (Saurauia vulcani Korth.) Was put into a closed vessel, added 7.5 liters of 96% ethanol then the vessel was closed and left for 5 days protected from light while frequently stirring. Then filtered and the pulp is rinsed again with 96% ethanol until 100 parts are obtained. Maserat is accommodated in a dark bottle, left in a cool place and protected from light for 2 days then filtered. Then the extract was concentrated using a rotary evaporator. Each extract was dried with a freeze dryer.

Group test

In the test, 4 dosage variations will be used, namely doses of 50, 100, 200, and 400 mg / kgBW. Weighed 1 gram of EEDP extract. Put into mortar, then pour gradually CMC Na 0.5% suspension while crushed until homogeneous, after homogeneous poured into a 100 ml flask and filled with 0.5% CMC Na suspension up to the mark line. Obtainedpirdot leaf extract concentration of 1%. For positive control use Imboost®.

3 RESULT AND DISCUSSION

3.1 Test Results Evaluation of Carbon Elimination Rate

Based on EEDP phytochemical screening, it was found that all parameters (flavonoids, tannins, saponins, glycosides, steroids / triterpenoids) except alkaloids have positive simplicia andextracts. According to Salihin (2017) that Pirdot Leaf Ethanol Extract contains saponin compounds which have the potential as antidiabetic. The chemical content of the pirdot leaf ethanol extract, namely flavonoids, is reported to have an effect as oat cancer and as a chemotherapy companion agent. The active compound of the flavonoid group is reported as an inhibitor of the enzyme DNA topoisomerase. This inhibitor results in damage to the DNA of cancer cells, further influencing the processes in the cell.

Carbon elimination rate is a method used to measure phagocytic activity in mice. The amount of carbon in the blood decreases with time, due to phagocytic events by leukocyte cells, especially neutrophils, monocytes, macrophages, and eosinophil (Bao et al., 2013; Baratawidjaja, 2010). Data on carbon elimination rates can be seen in the Table 1 below.

Table 1. Elimination Carbon Clearence

Group (mg/kgbb)	No	Rate of Carbon elimination			
		Minute 5	Minute -10	Minute -15	Minute -20
CMC Na 0,5%	1	0,14	0,14	0,14	0,13
	2	0,14	0,14	0,13	0,13
	3	0,14	0,13	0,13	0,13
	4	0,14	0,12	0,13	0,13
	5	0,14	0,14	0,13	0,13
Mean		0,14± 0,01	0,14± 0,01	0,12±0,01	0,13±0,01
Imboost	1	0,10	0,08	0,07	0,06
	2	0,10	0,09	0,08	0,05
	3	0,10	0,08	0,07	0,05
	4	0,10	0,08	0,08	0,06
	5	0,11	0,08	0,07	0,05
Mean		0,10±0,01	0,08±0,01	0,07±0,01	0,05±0,01
50	1	0,14	0,13	0,12	0,12
	2	0,14	0,13	0,13	0,12
	3	0,14	0,13	0,12	0,12
	4	0,14	0,13	0,12	0,12

	5	0,14	0,13	0,12	0,12
Mean		0,14±0,01	0,13±0,01	0,12±0,01	0,12±0,01
100	1	0,14	0,13	0,12	0,11
	2	0,14	0,12	0,10	0,09
	3	0,14	0,12	0,11	0,10
	4	0,14	0,12	0,12	0,09
	5	0,14	0,12	0,12	0,10
Mean		0,14±0,01	0,12±0,01	0,11±0,01	0,10±0,01
200	1	0,13	0,10	0,09	0,07
	2	0,13	0,10	0,08	0,07
	3	0,13	0,10	0,08	0,07
	4	0,12	0,09	0,08	0,07
	5	0,12	0,10	0,08	0,07
Mean		0,12±0,01	0,10±0,01	0,08±0,01	0,07±0,01
400	1	0,11	0,09	0,08	0,05
	2	0,10	0,09	0,08	0,05
	3	0,11	0,10	0,09	0,05
	4	0,10	0,09	0,07	0,05
	5	0,10	0,09	0,07	0,06
Mean		0,11±0,01	0,09±0,01	0,08±0,01	0,056±0,01

Based on EEDP phytochemical screening, it was found that all parameters (flavonoids, tannins, saponins, glycosides, steroids / triterpenoids) except alkaloids have positive simplicia andextracts. According to Salihin (2017) that Pirdot Leaf Ethanol Extract contains saponin compounds which have the potential as antidiabetic. The chemical content of the pirdot leaf ethanol extract, namely flavonoids, is reported to have an effect as oat cancer and as a chemotherapy companion agent. The active compound of the flavonoid group is reported as an inhibitor of the enzyme DNA topoisomerase. This inhibitor results in damage to the DNA of cancer cells, further influencing the processes in the cell.

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Table 2. Results of value carbon elimination rate, fagocytic index, and stimulation index

	Group treatment (mg/ kgBB)	Carbon elimination (MEAN±SEM)	Fagocyti index	Stimulation index
1	CMC Na 0,5%	0,0021 ±0,0002 (+)	6,34	1,14
2	Imboost 32,5	0,0151±0,0039 (*)	9,25	1,41
3	EEDP 50	0,0061±0,0014 (+)	7,68	1,12
4	EEDP 100	0,0083±0,0013 (*,+)	8,15	1,19
5	EEDP 200	0,0110±0,0010 (*,+)	8,45	1,37
6	EEDP 400	0,0176±0,0013 (*,+)	8,92	1,45

Based on the above table, the results of carbon elimination rate data are used to calculate the value of carbon elimination constant. The carbon elimination constant is one of the parameters used to determine the rate of phagocytosis. The greater the value of the carbon elimination constant, the higher the rate of carbon clearance, which means the faster the phagocytic cells carry out the process of phagocytosis (Hamdy et al., 2015). Non-specific immune system is the body's leading defense against antigens. One of the body's efforts to defend against antigen entry is to destroy the antigen by the process of phagocytosis. Phagocytic cells attack through several processes, namely recognition of foreign objects to be digested, movements toward objects (chemotaxis), attachment, ingestion (ingestion), and digestion (digestion). Phagocytosis is carried out mainly by mononuclear, neutrophil and eosinophil phagocytes. Mononuclear phagocytes are produced by stem cells (steam) cells in the bone marrow. In the bone marrow it proliferates and is released into the blood after one period through the monoblast phase - the promonocyte phase - the monocyte phase. The monocyte is only briefly in the blood then the cell moves to the tissue and differentiates into macrophages (Bao et al., 2013).

Based on the results obtained indicate that the greater the dose the phagocytic index value increases. Increasing the phagocyte index indicates an increase in phagocytic activity of macrophages and an increase in non-specific immunity. Macrophages are responsible for the process of elimination, especially in the liver and the rest are in the spleen. Lymph as a secondary lymphoid organ contains B lymphocyte cells and T lymphocytes which play a role in the process of specific immune responses. In addition, there are also dendritic cells and macrophages in the lymph that act as APC (Antigen Presenting Cell) which serves to present antigens to lymphoid cells. Increased immune cells are correlated with lymphatic weight. This increase in relative lymphatic weight indicates the effect of binara herbaceous ethanol extract on immunostimulatory activity. Based on the results of statistical tests, the graph shows that the negative control group CMC Na 0.5% suspension with phagocytosis index 6.34 has a significant difference ($p < 0.05$) with other treatment groups. EEDP 50 mg / kg body weight with phagocytosis index of 7.68 had a significant difference ($p < 0.05$) with other treatment groups. EEDP 100 suspension with phagocytosis index 8.15 and 200 mg / kgBB with phagocytosis index 8.45 did not have a significant difference ($p > 0.05$) but significantly different from EEDP 400 mg / kgBB with phagocytosis index 8.92 and Imboost ® with phagocytosis index 9.25. EEDP

suspension 400 mg / kgBB and Imboost® did not have a significant difference (p> 0.05). The main mission of phagocytic cells is to maintain (Murray et al., 2011). In addition, they are also involved in innate immune cell responses (immune to bacterial, fungal, parasitic and viral infections).

3.2 Test Results Evaluation of Stimulan Index

The stimulation index is the result of a comparison between the test group and the control group. Previous research stated an immunostimulant substance if the stimulation index is greater than 1 and immunosuppressant if the stimulation index is smaller than 1. EEDP suspensions of 50, 100, 200, 400 mg / kgBW indicate that there is a relationship between the increase in dose and the stimulation index value, ie the greater the dose increases, the value of the stimulant index obtained increases. This shows that EEDP is an immunostimulant substance. Compounds that may act as immunostimulants are flavonoids. This is in accordance with the research of Prastiwi et al. (2015) which states that the saponin compound, tannins have immunostimulant activity so that it is suspected that the immunostimulant activity of pirdot leaves might be caused by flavonoid content. Compounds that act as immunostimulants are flavonoids, alkaloids. This is in accordance with the research of Prastiwi et al. (2015) which states that alkaloid compounds, saponins, tannins have activity as immunostimulants. so it is suspected that the immunostimulatory activity of pirdot leaves might be caused by flavonoid content.

Research Marbun, 2017 also reported that flavonoid compounds have immunostimulatory activity by increasing IFN-sel cell stimulation. IFN-γ produced by NK cells can function as a mediator of innate immune responses, the main function of IFN-γ in regulating the immune system is as a potential activator for mononuclear phagocytes. IFN-mengind directly induces the synthesis of enzymes that play a role in respiratory bursts, so macrophages can kill the microbes that are ingested by the mechanism of phagocytosis. IFN-γ also activates neutrophils and increases respiratory bursts. According to Hamdy (2015), EEDP contains flavonoids, alkaloids, and saponins which have anti-inflammatory, antioxidant and can also reduce serum TNF-α levels. EEDP stimulation index values obtained are still under positive control (Imboost).

Each imboost tablet contains 250 mg of Echinacea purpurea. E. Purpurea is reported to have the ability to increase phagocytosis because of the content of polysaccharides that can activate macrophage cells and NK cells and have been tested preclinically and clinically as immunostimulants besides Flavonoids are compounds that function as immunostimulants including triterpenoids and polysaccharides. Thus, both EEDP and Echinacea purpurea have activity as immunostimulants but with different strengths. Leukocytes form the innate and adaptive immune system and respond, for example against infection, inflammation and tumor growth growth (Gajewski et al., 2013).

4 CONCLUSIONS

Conclusion of this study is Ethanol extract of pirdot leaves (Saurauia vulcani Korth.) can increase phagocytic activity not significantly different from Imboost available in the market. Macrophage cells in male rats injected with carbon suspension. EEDP doses of 50, 100, 200, and 400 mg / kg body weight can influence phagocytic activity compared with negative control CMC-Na 0.5% (p <0.05). EEDP 400 mg / kgBB gives phagocytosis effect which is almost the same as the positive control of Imboost® which shows the rate of carbon elimination at a dose of 400 mg / kgBB 0.0065 ± 0,0005 and Imboost 32.5 mg / kgBB 0.0041 ± 0,0002. This shows an insignificant difference between a dose of 400 mg / kgBW and Imboost. Based on the results of the study, ethanol extract of pirdot leaves increased the immunostimulatory activity of immune cells of male rats

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