

ACTIVITY OF RED GINGER EXTRACT (*ZINGIBER OFFICINALE* VAR. RUBRUM) AGAINST INTERLEUKIN-6

DIRA HEFNI*, YULMA HERDALINA, NETTY SUHARTI

Faculty of Pharmacy, Andalas University, Kampus Limau Manis, 25163, Padang, West Sumatra, Indonesia

*Email: dirahefni@phar.unand.ac.id

Received: 15 Nov 2022, Revised and Accepted: 05 Jan 2023

ABSTRACT

Objective: Knowing that it contains gingerol chemicals, red ginger (*Zingiber officinale* var. Rubrum) has the potential as a natural immunomodulator. Immunomodulating substances can affect how the human immune system responds by helping to keep the body in a homeostatic state and rebalancing immune system imbalances. The goal of this study was to find out of the extract of red ginger inoculated by Arbuscular Mycorrhizal Fungi (FMA) in different concentrations affected the reduction of IL-6 levels in test animals' serum *in vivo*.

Methods: Twenty-four white male mice were used in this study. Additionally, they were split into six groups, with the negative control group received 0.5% Na CMC. The experimental group received red ginger extract at doses of 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW, and "Stimuno" 50 mg/kgBW, whereas the positive control received the Pfizer vaccine. The Pfizer vaccination was administered intramuscularly on day 0 to experimental animals in the positive control and experimental groups. Following that, they underwent a 14-day oral red ginger extract test. Using the Enzyme-linked immunosorbent assay (ELISA) technique, the test was carried out to measure the concentration of IL-6 in the serum.

Results: The findings illustrated that the dose variations of 100, 200, and 400 mg/kgBW had a significant impact on the drop in IL-6 levels ($p < 0.05$).

Conclusion: The findings indicate that red ginger ethanol extract, in a variety of dosages, may lower IL-6 levels. The largest reduction in IL-6 levels was seen in the extract test group at a dose of 100 mg/kgBW.

Keywords: Red ginger (*Zingiber officinale* var. Rubrum), Immunomodulator, IL-6, ELISA

© 2023 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>) DOI: <https://dx.doi.org/10.22159/ijap.2023.v15s1.04> Journal homepage: <https://innovareacademics.in/journals/index.php/ijap>

INTRODUCTION

The immune system is the body's defense system to protect the body from the dangers that can be caused of pathogen attacks. Pathogens such as bacteria, viruses, fungi, and parasites can cause infection. Infectious diseases are one of the leading causes of death in the world. The entry of those pathogenic elements into the body triggers the formation of proinflammatory mediators, one of which is the cytokine interleukin-6 (IL-6) [1]. IL-6 is the main precursor of macrophages produced by a number of cells such as monocytes, macrophages, T cells and B cells, polymorphonuclear leukocytes and mast cells [1]. The mechanism of IL-6 formation in infected lesions will be recognized by pathogen recognition receptors (PRRs) so that immune cells stimulate various pathways including NF- κ B and increase cytokine mRNA transcription, especially IL-6 [1].

The body's defense system can be activated by giving a compound called an immunomodulator. Immunomodulators are able to modulate the human immune system by playing a role in maintaining the body's homeostatic condition and helping the body repair its immune system imbalance. The utilization of plants as traditional medicine is considered to have its own advantages, namely low toxicity, easy to obtain, cheap in price and relatively non-toxic and less side effects [2]. This advantage has also been empirically proven in traditional human direct use [2].

One of the plants that has been known to contain a number of bioactive components as immunomodulators is ginger. In Indonesia, there are three types of ginger plants that are cultivated, namely red ginger, large white ginger/elephant ginger, and small white ginger/emprit ginger. Red ginger is more widely used as a treatment because it has the highest essential oil and oleoresin content among the three types of ginger. Red ginger contains several chemical compounds, including gingerol, shogaol, and zingerone which have anti-inflammatory, analgesic, antioxidant and anticarcinogenic activities [3].

In this study, the red ginger was inoculated with Arbuscular Mycorrhizal Fungi (AMF). Inoculation with mycorrhizae is one of the efforts to obtain quality red ginger rhizomes and have resistance to

pathogen attack. Arbuscular Mycorrhizal Fungi (AMF) is a mutualistic symbiotic association between soil fungi and the roots of higher plants. There are at least five benefits of mycorrhizae for the development of host plants, namely increasing nutrient absorption from the soil, as a biological barrier against root pathogen infection, increasing growth-promoting hormones, increasing host resistance to drought, and ensuring the implementation of biogeochemical cycles [4]. Red ginger rhizomes inoculated with mycorrhizae are expected to have higher secondary metabolite content than red ginger rhizomes that was not inoculated with AMF. Secondary metabolites, apart from functioning to maintain their existence in the environment in which they are located, can also be used as lead compounds in the discovery and development of new drugs. In addition, red ginger inoculated with Arbuscular Mycorrhizal Fungi (AMF) was able to suppress the development of bacterial wilt disease caused by *R. solanacearum* ras 4 in ginger plants with varying effectiveness in reducing disease [5].

Based on the research from (Ho *et al.*, 2013) Ethanol extract from fresh ginger 0.5 mg/ml can reduce the secretion of TNF- α , IL-1 β , and IL-6 in microglia culture models as much as 46.3%, 79.4%, and 77.8%, respectively. It is known that shogaol and gingerol compounds can inhibits IL-1, IL-6, and TNF- α [6]. In a study (Luhurningtyas *et al.*, 2021) explained that the content of phenolic compounds gingerol, zingerone, and shogaol in red ginger rhizome is known to be able to reduce levels of TNF- α and IFN- γ in the treatment group [7]. In this study, the extract of red ginger inoculated by Arbuscular Mycorrhizal Fungi (FMA) in different concentrations was tested to determine its effect on decreasing IL-6 levels in test animals' serum *in vivo*.

MATERIALS AND METHODS

IL-6 level determination

The animal model were grouped into six groups consisting of 4 mice in each group. The test preparation was carried out for 14 d. There were six groups in this study, they are was a negative control group that was only given 0.5% Na CMC suspension, the second group was

a positive control group that was only induced with 0.1 ml intramuscular Pfizer vaccine (obtained from the Padang City Government Health Department) on day 0. The third group, namely the comparison group, which was previously induced with the Pfizer vaccine and then given "Stimuno" (obtained from pharmacies in the city of Padang) at a dose of 50 mg/kgBW. Three other test groups were administered with Pfizer vaccine inducer on day 0 and red ginger (harvested and collected from the village of Bari, Padang Pariaman, West Sumatra) extract suspension at doses of 100 mg/kg body weight, 200 mg/kg body weight, and 400 mg/kg body weight on day-1 to day-14 orally. On the 15th day the mice were sacrificed and blood was taken by cutting the neck veins. The blood was collected into a tube, then centrifuged at 3000 rpm for 20 min. Examination of the concentration of IL-6 in serum was carried out using the Enzyme-linked immunosorbent assay (ELISA) method.

Data analysis

Data analysis was performed using SPSS 26 statistical software using one-way ANOVA method. If there is a significant difference, then proceed to use the Duncan Post Hoc Test to identify differences between group.

Ethical consideration

This study has passed the ethical approval of the Research Ethics Committee of Medical Faculty Andalas University in order to protect human right and welfare of medical health research subject (Number 745/UN.16.2/KEP-FK/20220).

RESULTS AND DISCUSSION

In this study, the Pfizer vaccine was used, which is one of the SARS-CoV-2 vaccines. Pfizer vaccine is known to increase levels of proinflammatory cytokines, especially IL-6 in the body [8]. Pfizer vaccine is an mRNA-based Covid-19 vaccine, it is known that the released mRNA is translated by the ribosome to produce a target protein after the target protein is secreted by the cell, the protein will be recognized by the immune system and stimulate an immune response. Protein S can trigger a proinflammatory response via TLR4, specifically induces the production of IL1- β and IL-6 [9, 10]. While the test preparation given in this test is expected to reduce the concentration of IL-6 in the treatment. High concentrations of IL-6 in humans can be associated with various systemic diseases such as

malignancy, diabetes, central nervous system disease, inflammation and autoimmunity. The administration of Pfizer vaccine by means of the Pfizer vaccine dose in humans is known to be 0.3 ml, then the quantity is converted to a mouse dose.

In the comparison group, stimulation was given. Stimuno was chosen because it contains the active substance of *Phyllanthus niruri* L. plant extract and is a class of phytopharmaceutical drugs and several studies have shown that *P. niruri* L. herbs have properties to increase the activity of the immune system or immunomodulators. The content of flavonoids contained in *P. niruri* L. in stimuno will work when the activity of the immune system decreases, the flavonoid content of *P. niruri* L. will send intracellular signals to cell receptors to increase its activity. On the other hand, if the immune system works excessively, the *P. niruri* L. will be efficacious in reducing the work of the immune system, in other words, the *P. niruri* L. will function as a balancer for the immune system. In addition, it is known that *P. niruri* L. as an immunostimulant can increase the phagocytic activity of macrophages, due to an increase in the levels of proinflammatory cytokines (IFN- γ , TNF- α and IL-6), and the number of CD4+ and CD8+ cells, which can increase the proliferation and activation of immune cells. If phagocytosis in the body increases, the immune system will increase, this is because the pathogens that enter the body have been destroyed [11, 12]. To determine the concentration of IL-6 cytokines after administration of the extract, a standard curve of IL-6 was determined with a concentration series of 0; 7.8; 15.6; 31.3; 62.5; and 125 pg/ml. The maximum absorption length was measured at a wavelength of 450 nm. The results of the determination of the standard curve were carried out using the four parameter logistic curve (4PL) method according to the instructions in the kit and is a regression model that is often used to analyze bioassays, especially ELISA. Analysis using 4PL has the advantage that it produces a wide range of curves so that it can be used to predict the analyte content of the sample accurately even though it is outside the standard concentration range [13]. 4PL calculations require special software to be able to find out the results. For this, the author uses a website, namely <https://www.aatbio.com/> which provides 4PL logistic regression calculations. The results of the determination of the IL-6 standard curve can be seen in fig. 1.

The results of measuring the concentration of IL-6 in the test treatment with the ELISA is listed in table 1.

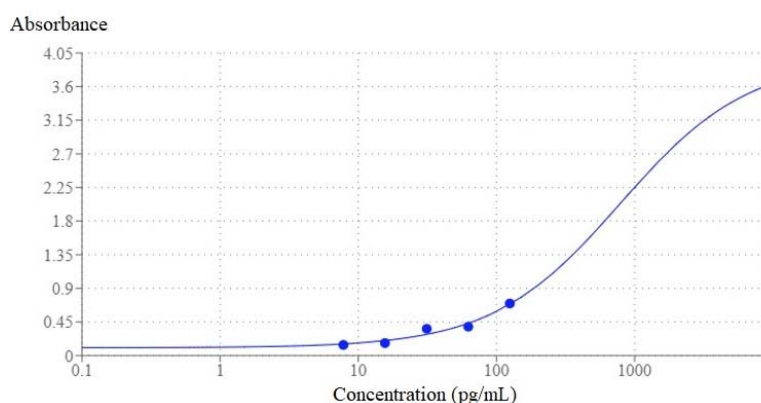


Fig. 1: IL-6 standard curve

Table 1: Concentration of IL-6

Replicate	Concentration of IL-6 (pg/ml)					
	Control (-)	Control (+)	Stimuno 50 mg/kgBW	100 mg/kgBW	200 mg/kgBW	400 mg/kgBW
1	9.012	45.923	5.331	7.824	17.315	26.669
2	10.214	37.299	6.318	7.151	17.862	28.986
3	9.869	43.634	5.988	8.331	15.146	31.132
4	5.988	47.600	7.655	7.319	10.559	33.697
Average	8.771	43.614	6.323*	7.656*	15.220	30.121
±SD	1.923	4.512	0.978	0.533	3.321	3.001

*P<0.05 compared with negative control group

Based on the table of the average concentration of IL-6 in each group, it can be seen that the positive control treatment group had the highest average value of IL-6 cytokines, which was 43.614 pg/ml. The increase in value is due to positive control group was only given the Pfizer vaccine inducer as an antigen that has the potential to induce an immune response and was not treated with drugs or extracts, so there was no decrease in the concentration of IL-6. It is known that the concentration of IL-6 in serum under normal conditions ranges from 0-7 pg/ml [14]. Gingerol compounds found in red ginger extract can inhibit cytokines in increasing inflammatory cells through direct suppression of the expression of the proinflammatory mediator COX2 and can inhibit the increase in NF- κ B which is one of the components of inflammation and is a transcription factor that controls the release of a number of important genes in the process of immunity and inflammation including IL-2, GM-CSF, IL-6 and TNF- α [15]. In the table the dose of 100 mg/kgBW has an average IL-6 concentration of 7.656 pg/ml, this value indicates a significant decrease in IL-6 concentration compared to the negative control. At higher doses of 200 mg/kgBW and 400 mg/kgBW, the average concentration values were 15.220 pg/ml and 30.121 pg/ml, respectively. The inconsistency between increasing the dose and the inhibition of IL-6 concentration can be explained according to the literature that at increasing doses after a certain period the modulation is not dose dependent but the modulation forms a characteristic level of effectiveness [16]. In the study of natural immunomodulators conducted by (Wang et al., 2020) there was also an inconsistency where the administration of alkaloid compounds isolated from the orchid plant (*Dendrobium aphyllum*) where at the lowest dose concentration resulted in the highest decrease in IL-6 cytokines, while the highest dose administration resulted in a decrease in cytokines. IL-6 was lower, this was due to the activation of NF- κ B at high doses which stimulated the production of proinflammatory cytokines so that the effectiveness of alkaloid compounds at high doses was reduced [17].

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the administration of various doses of ethanol extract of red ginger (*Zingiber officinale* var. *Rubrum*) significantly affected the decrease in the concentration of IL-6 in male white mice ($P < 0.05$) based on the results of the one-way ANOVA test. The highest decrease in IL-6 concentration was found at the extract dose of 100 mg/kgBW.

FUNDING

The research funding by the Ministry of Research and Technology-National Research and Innovation Agency Republic of Indonesia through National Research Priority with the contract number 92/E1/PRN/2020.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Authors declare no conflict of interest in this study.

REFERENCES

1. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295. doi: 10.1101/cshperspect.a016295, PMID 25190079.
2. Lallo S, Mirwan M, Palino A, Nursamsiar N, Hardianti B. Aktifitas ekstrak jahe merah dalam menurunkan asam urat pada kelinci serta isolasi dan identifikasi senyawa bioaktifnya. *IJPF* 2018;5(1):271-8. doi: 10.33096/jffi.v5i1.319.
3. Febriani Y, Riasari H, Winingsih W, Aulifa L, Permatasari A. The potential use of red ginger (*Zingiber officinale* Roscoe) drugs as analgesic. *Indones J Pharm Sci Technol*. 2018;1(1):57-64.
4. Purba PRO, Rahmawati N, Kardhinata EH, Sahar A. Efektivitas beberapa jenis fungi mikoriza arbuskular terhadap pertumbuhan tanaman Karet (*Hevea brasiliensis* Muell. Arg.) di Pembibitan. *J Online Agroekotekol*. 2014;2(2337):919-32.
5. Suharti N, Habazar T, Nasir N, Jamsari D, Arbuskula I Fungi Mikoriza (FMA). *Indigenus pada Bibit Jahe untuk Pengendalian Penyakit Layu Ralstonia solanacearum ras 4*. *J Natur Indonesia*. 2011;14(65):61-7.
6. Ho SC, Chang KS, Lin CC. Anti-neuroinflammatory capacity of fresh ginger is attributed mainly to 10-gingerol. *Food Chem*. 2013;141(3):3183-91. doi: 10.1016/j.foodchem.2013.06.010, PMID 23871076.
7. Luhurningtyas FP, Susilo J, Yuswantina R, Widhihastuti E, Ardiyansah FW. The immunomodulatory activity and phenolic content of red ginger rhizome extract (*Zingiber officinale* Rosc. var. *rubrum*). *Indones J Pharm Nat Product*. 2021;4(1):51-9.
8. Bergamaschi C, Terpos E, Rosati M, Angel M, Bear J, Stellas D. Systemic IL-15, IFN- γ , and IP-10/CXCL10 signature associated with effective immune response to SARS-CoV-2 in BNT162b2 mRNA vaccine recipients. *Cell Rep*. 2021;36(6):109504. doi: 10.1016/j.celrep.2021.109504, PMID 34352226.
9. Nugroho SA, Hidayat IN. Efektivitas dan keamanan vaksin covid-19: studi refrensi. *JKP*. 2021;9(2):61-107. doi: 10.33650/jkp.v9i2.2767.
10. Jugler C, Sun H, Chen Q. SARS-CoV-2 spike protein-induced interleukin 6 signaling is blocked by a plant-produced anti-interleukin 6 receptor monoclonal antibody. *J*. 2021;9(11):1-12. doi: 10.3390/vaccines9111365, PMID 34835296.
11. Perdana PR. Review: aktivitas imunomodulator ekstrak herba meniran (*Phyllanthus niruri* L.). *J Far*. 2022;9(1):50. doi: 10.47653/farm.v9i1.545.
12. Oktarina DR, Susilawati Y, Halimah E. The potential of phyllanthus genus plants as immunomodulatory and potensi tumbuhan genus phyllanthus sebagai imunomodulator dan. *Indones J Biol Pharm*. 2021;1(2):47-77.
13. Arif M, Ferry Fernanda H, Sa'adi A, Sudjarwo. Verifikasi linieritas kurva baku testosteron menggunakan metode Elisa (enzyme-linked immunosorbent assay). *J Res Technol*. 2019;5(1):50-6.
14. Li Q, Xie Y, Cui Z, Tang S, Yuan B, Huang H. Analysis of peripheral blood IL-6 and leukocyte characteristics in 364 covid-19 patients of wuhan. *Front Immunol*. 2020;11(Nov):559716. doi: 10.3389/fimmu.2020.559716, PMID 33329518.
15. Riduan RJ. Pengaruh pemberian ekstrak jahe merah terhadap gambaran histopatologi pankreas yang diinduksi aloksan. *J Majority*. 2015;4(8):11-6.
16. *Imunomodulator Bahan Alami* SE. Yogyakarta: Rapha Publishing; 2017.
17. Wang Q, Liang J, Brennan C, Ma L, Li Y, Lin X. Anti-inflammatory effect of alkaloids extracted from dendrobium aphyllum on macrophage RAW 264.7 cells through NO production and reduced IL-1, IL-6, TNF- α and PGE2 expression. *Int J Food Sci Technol*. 2020;55(3):1255-64. doi: 10.1111/ijfs.14404.