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73 Immunomodulator of Extract (Cymbopogon nardus L.) Increase Cells a Precaution Against SARS-CoV-2 Putri Ayu Ika Setiyowati 1* , Rofiatun Solekha 1 , Sri Bintang Sahara 2 , Reny Rosalina 3 1Departement of Biology, Faculty of Sains and Technology Universitas Muhammadiyah Lamongan, Indonesia 2Departement of Pharmacy, Faculty of Health Science Universitas Muhammadiyah Lamongan, Indonesia 3Biomedical Sciences Program, Khon Kaen University, Thailand A R T I C L E I N F O A B S T R A C T Article history: Received 30 April 2021 Received in revised form 15 June 2021 Accepted 21 July 2021 Available online 31 October 2021 Keywords: Cymbopogon nardus L., Immune response, Immunomodulator, Coronaviruses. *) Corresponding author: putriayuikasetiyowati@gmail.com Introduction: In humans, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) can damage some tissue when the immune systems was decrease. Natural product from the plant often used to improve immune response against microorganism including virus.

This study aimed to determine the potential antioxidant of lemongrass extract (C . nardus) with various dosage that can provide immunomodulatory effects and find an optimal dosage to be used. Methods: The method used observasional analytic, using animal model of 30 male mice strain BALB/C, weight 25-30 gram, divided into 5 groups; the positive control group was given 0.05 mL of 0.05% CMC within 14 days, negative control group was given IMBOOST® tablet 200 mg/kg body weight (bw) within 14 days, treatment groups were given C. nardus extract with various doses 50 mg/ kg bw, 150 mg/kg bw, and 300 mg/kg bw.

In day 21 all group were injected with 0,2 ml of pathogen bacterial (S. aureus). Blood samples were taken three times: 7 th day, 14 th day, and 21 th day. Results: The results showed that lemongrass extract (C. nardus) able influence leukocyte and count

significant ($p<0.05$). optimal is mg/kg weight. Conclusion: The antioxidant compounds that contain in the C. nardus extract have an ability to increasing the immune system in the dose 150 mg/kg bw , but in the dose 300 mg/kg bw became toxic that can make a skin injury or death in animal test.

Introduction In humans, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) SARS-CoV-2 primarily infects cells in the airways that line the alveoli. SARS-CoV-2 will bind to receptors to enter the cell. The glycoprotein which is owned by this virus will bind to the ACE-2 protein in the cell.1 In SARS-CoV, Protein S was reported as a significant determinant of virus entry into host cells. Viral and host factors have a role in SARS-CoV infection. 2 Inadequate immune response leads to viral replication and tissue damage, on the other hand, an excessive immune response can cause tissue damage. 3 Although until now there has not been found the right drug or vaccine for handling SARS- CoV-2.

efforts maintain immune must be done before this virus can successfully enter the cells cause proinflammatory 4 For this reason, many studies have examined the potential of a plant as the body's defense against viruses. The genus of Cymbopogon including Cymbopogon nardus (C. nardus) as a nature plant has three basic ingredients flavonoids, and 5 The of phenol, and in can as anti-microbial, and anti-oxidant, as well as an immunomodulator to modulate immune response. 6 Some study before explain about effect lemongrass (C. citratus) on the of in chickens increasing number blood The of C. nardus and C. citratus are in the colour of stem morphology and the percentage activity of antioxidant. C.

nardus has brown colour of stem and C. citratus is white. In the percentage activity of antioxidant, C. nardus has activity antioxidant 60,8% and C. citratus is 50%.7,8 Although, there had been studies of percentage activity of antioxidant in C. nardus. but studies have not been reported on the activity of C. nardus ethanolic extract as an imunommodulator against microorganism with enhance a leukocyte, monocyte, and lymphocyte counts. In this study, an observation of one of the immunomodulatory parameters will be carried out in the form of an increase in the number of leukocytes, the Biomolecular and Health Science Journal Available at https://e-journal.unair.ac.id/BHSJ ; DOI: 10.20473/bhsj.v4i2.26619 This work is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License. 74 percentage of monocytes and lymphocytes from the extract of C.

nardus with dosage variations of 50, 150, and 300 mg/ kg bw, in this study we using commercial immunostimulants as a comparison also pathogen bacteria Staphylococcus aureus (S. aureus) that given in day 21 for knowing the effectiveness C. nardus extract

when exposure by some antigen. target of study to the potential as well as the optimal dose of the use of C. nardus extract in its activity as an immunomodulator. Methods Ethical clearance All procedures in this in-vivo study has been approved by Ethical Review Committee, Research and Community service Departement, Universitas Muhammadiyah Lamongan, East Java, Indonesia.

Animal Model Thirty male mice (Mus musculus) strain BALB/C, body weight 20-30 and age weeks were provided by Laboratory animal, Universitas Muhammadiyah Lamongan. All mice were fed and watered ad libitum, acclimated in 30-35 oC with 12-h light cycle at Pharmacology Laboratory, Universitas Muhammadiyah Lamongan. Plant Material and Extraction The C. nardus were acquired from Materia Medica, Batu, East Java. The stalks of C. nardus were cut into small parts and dried under the sun for 3 days.

Dried stalks were blended into a powder (100 g) then soaked in 1000 mL of ethanol 96% in a closed container within 3x24 hours room The was and concentrated by heating in a water bath at temperature of 60oC in order to get a thick extract of C. nardus. a. Preparation of 0.5% Na-CMC Suspension Five hundred milligrams of Na-CMC were weighed, then dissolved in some warm distilled water, stirred, and added with distilled water while continuing to stir using a stirring rod. After dissolving all the remaining distilled water is added to obtain a volume of 100 ml Na-CMC solution using a 100 ml measuring flask. b. of nardus and Suspension 1. C. nardus extract suspension The Extract were homogenously crushed with 0.5% Na- CMC.

The extract concentrations made were 1%, 3%, and 6 %. This concentration can be determined by the formula: 9 Concentration: (Dose (mg/kg bw))/(%VAO (ml/g)) The percentage volume of sub-cutan drugs administration (VAO) is 2%. 2. Imboost® suspension One tablet of Imboost® contains 250 mg of the active ingredient, 2 tablets Imboost® (500 mg) were put in a mortar, crushed. Then added with 0.5% CMC-Na suspension to taste and homogenized. After that poured into 100 volumetric and up 1% CMC-Na suspension to the mark line. The suspension was homogenized again by shaking lightly. The concentration of Imboost® suspension was obtained 0.25%.

Experimental Design After 2 weeks of acclimatization, a thirty male mice were randomly into groups, positive group was given 0.5% Na-CMC injection of 0.5 ml / kg bw for 14 days, the negative control group was given commercial immunostimulants, namely Imboost® tablet at a dose of 200 mg / bw for 14 days, then the group consisted of treatment which differentiated on the dose of C. nardus extract. The various doses are 50 mg / kg bw, 150 mg / kg bw, and 300 mg / kg bw. The extract was given for 14 consecutive days subcutaneously. Every 7th day, 14th day, and 21th day, blood is drawn through the tail vein.

On the 21st day, the mice were injected with S.aureus (0.5 Mc Farland) 0.2 mL intraperitoneally, one hour later the mice were drained of blood through the tail vein after that the mice being sacrified. Leukocyte Counts The total leukocyte counts was calculated using a hemocytometer with a 1:20 dilution. Because the depth of the Neubauer compute chamber is 0.1 mm and the area is 4 mm2 (consisting of 4 rooms each with an area of 1 mm2 so a total of 4 mm2). Then the volume of the squares is 0,4 mm3. Therefore, the formula is: Leukocytes count per mm 3 = Nx dilution factor / volume of squares = Nx20 / 0.4

mm3 = 50 N N = count of leukocytes from 4 counting rooms. Lymphocyte and Monocyte Count The fresh blood sample was dropped on a glass slide and a was dry air, with stained with Giemsa dye at a 1: 9 dilution, washed using distilled water and allowed to dry then examined under a microscope at magnification. percentage monocyte lymphocyte from the total 100 leukocyte were determined with the following formula: % or = lymphocyte monocyte / 100 x 100% Furthermore, the percentage both of lymphocyte and monocyte were converted into count of lymphocyte and monocyte with the following formula: N = % lymphocyte or monocyte x leukocyte count N = count of lymphocyte or monocyte Statistic analysis Analysis of leukocyte, monocytes, and lymphocytes count using (Analysis Variance).

significance level was 95% a value 5% = the of showed < than was considered statistically significant. Results Leukocyte Counts There was significant change ($p<0.05$) of leukocyte counts of the mice in treatment groups when compared with those of the positive control group. Within the groups, there was significant ($p<0.05$) leukocyte from 7 to day 21 in all the groups except positive control group (Table 1.) 75 Figure 1. Comparison of leukocyte counts between mice given C. nardus extract and each control group towards day of experiment. (Graph with one or more symbol * show significant level ($p<0.05$) with control positive group). a.

Monocyte and Lymphocyte Counts The results indicated that the monocyte and lymphocyte counts were on normal range, it shown by monocyte and lymphocyte percentage before converted to monocyte and lymphocyte counts. The percentage of monocyte were in 2% from leukocyte counts while on lymphocyte were in 82% from leukocyte counts. The normal range of monocyte percentage in mice about 0-2% and lymphocyte about 65 - 87% of leukocytes count. 11,12 There significant ($p<0.05$) of and lymphocyte count of the mice in treatment groups when compared with those of the positive control group, except on 14 monocyte was significant).

the there a increase of monocyte and lymphocyte counts between day 7 to day 21 in all

the groups except on positive control group (Table 2.). The comparison of each group within monocyte and lymphocyte count towards day of experiment were presented in (Figure 2 and 3). Figure 2. Comparison of monocyte counts between mice given C. nardus extract and each control group towards day of experiment. (Graph with one or more symbol * show significant level ($p<0.05$) with control positive group). Figure 3. Comparison lymphocyte counts between mice given C. nardus extract and each control group towards day of experiment.

(Graph with one or more symbol * show significant level ($p<0.05$) with control positive group). Table 1. Effect of C. nardus extract on leukocyte count of mice Leukocyte count (µl/mm3) Day 7 Day 14 Day 21 Positive control (Na-CMC 0.5%) 4600 ± 894 5981 ± 1297 4519 ± 116 Negative control (IMBOOST tablet 200 mg/ kg bw) 8237 ± 101? 9000 ± 410? 10575 ± 409†? C. nardus extract (50 mg/ kg bw) 7843 ± 697? 8381 ± 121? 10012 ± 441†? C. nardus extract (150 mg/kg bw) 9175 ± 837? 10456 ± 523†? C. nardus extract (300 mg/kg bw) 8932 ± 783? Description: Data as ± Mean towards of with † significally ($p<0.05$) in nardus 150 mg/kg bw, with symbol †† is significally different) .

Mean values toward positive control with one or more symbol ? are significally different ($p<0.05$). Table 2. Effect of C. nardus extract on monocytes and leukocytes count of mice. a. Effect of C. nardus extract on lymphocytes count of mice 3) Day 7 Day 14 Day 21 Positive control (Na-CMC 0.5%) 3658 ± 717 4829 ± 1049 3570 ± 92 Negative control (IMBOOST tablet 200 mg/ kg bw) 6817 ± 844? 7560 ± 350? 9017 ± 401†? C. nardus extract (50 mg/ kg bw) 6349 ± 501? 6914 ± 104? 8436 ± 397†? C.

nardus extract (150 mg/kg bw) 8889 ± 470†?? 10159 ± 424††?? 76 Discussion The results of this research is similar with with previous study that revealed the bioactive of natural constituents from lemongrass (C. citratus) tea increasing the number of white blood cells in human with the optimal dosage 4 g within 30 days. 13 The leukocyte counts in our research are at the normal high limit, increases leukocytes counts show that the immune system sufficient in blood to infection. 14 The immune system of the human body is generally divided into two parts.

The first is the innate immune response, this is includes defense mechanisms that are encoded in the host's genes. The second is the organs involved in these systems are mostly secondary lymphoid organs. Leukocytes, which are the innate immune system, play an important role in protecting the body from attack by microorganisms, particularly useful in the field of conservation physiology because they are altered by stress and can be directly related to stress hormone levels. 15 We observed that C. nardus extract on 150 mg/kg bw can increasing the number of leukocytes but still on the normal range.

The total number of leukocytes on day 7, day 14 and day 21 significant (p for treatment group, except in positive control group. The system effector that the ability to destroy of microbial cells and to clear both of toxic and allergenic substances. Monocyte act as cells that are able to recognize, attack microbes, and cancer cells and also produce cytokines, exerting defense in response to infection. 16 The high monocyte counts in the blood plays an important role in protecting the body from attack by microorganisms. 17 In this research use S. aureus to stimulate the immune response. In treatment of C.

nardus 300 mg/kg bw extract) have decreasing on monocyte counts in day 21. We assumed that it's because the affect high of C. nardus. As we know that common lemongrass have an antioxidant compound that can decrease an oxidative stress, but consumed high dose of extract, cause effect us or decreasing the immune system. 18,5 This agree with 19 that reports on the occurrence of dermatitis caused by contact with dry leaves of C. citratus . Infection from viruses can stimulates immune reactions in the host, some of immune cells that can produce an antibody is lymphocyte.

The mechanism of action of lymphocytes for the immune system functions to provide immune substances by antigens specific on cell membrane. 20 In this research, lymphocyte counts in all treatment group except C. nardus 300 mg/kg bw were increase on day 21 after injected by S. aureus, but they are still in a normal range of lymphocytes, this reason have a correlation with the intensity of infection by pathogens will help the increasing need for white blood cells (lymphocytes).

Some cases found that infection of some microorganism or viruses may can decrease lymphocyte counts below a normal range (lymphophenia). The by about effect fraction of lemongrass (C. nardus) against viral activity with the three treatment methods were designed to indicate whether the antiviral actions of fractions occurs before, during or after viral entry into the cells. The result is at 0.1 LC50, the C. nardus were effective cells with the fractions before being inoculated with the virus. Lymphocytes are cells that are involved in the activity of specific responses. are main to immune that able fight agents.

23 There are two types of immunity, namely humoral and cellular immunity. Humoral immunity involves the role of circulating antibodies as gamma globulin, which is carried out by B lymphocytes. While cellular immunity is the defense system carried out by T lymphocytes, is responsible for delayed allergy reactions and rejection of foreign tissue transplantation, forming the main defense against viral, fungal and some bacterial infections. 24 C. nardus is one of the potential plants that can increase the immune system. The compounds of secondary metabolites in methanol and ethyl acetate fraction from C. nardus stalks are flavonoids, phenolic and terpenoids.

The n-hexane fraction only contains steroid compounds. Polyphenol, tannins and flavonoid mayor that antiviral in the Cymbopogon sp.25,26,27 Some studies mention that tannins have a potential against microbial with increasing the phagocytic cells. 28,29 The mechanism of common lemongrass extract against the immune system has not been clearly estimated by increasing lymphocyte activation and proliferation or by increasing macrophage and T-helper lymphocytes. 30 The increase in lymphocytes indicates that C. nardus extract has immunomodulatory activity. C. nardus has an ability against microbials, in this research, we know that the number of leucocytes increase the of by S. aureus that given on day 21.

The C. nardus extract can promote the C. nardus extract (300 mg/kg bw) 8932 ± 783? b. Effect of C. nardus extract on monocytes count of mice Monocyte count (cell/mm3) Day 7 Day 14 Day 21 Positive control (Na-CMC 0.5%) 78 ± 23 132 ± 24 90 ± 52 Negative control (IMBOOST tablet 200 mg/ kg bw) 165 ± 20†? 190 ± 8 205 ± 54? C. nardus extract (50 mg/ kg bw) 154 ± 9? 168 ± 2 200 ± 9†? C. nardus extract (150 mg/kg bw) 183 ± 17? 209 ± 10 235 ± 8†? C. nardus extract (300 mg/kg bw) 16±5? 20±17? 130 ± 58 Description: Data as ± Mean towards of with † significally ($p<0.05$) in nardus mg/kg with †† significally Mean toward positive with or symbol ? are significally different ($p<0.05$).

77 leukocytes to the before microbial effect. So that, the treatment by inducing of lemongrass extract (C. nardus (L.)) for 3 weeks can prevent the damage of immune system. 31 In this research, we compare the effectiveness of C. nardus extract with commercial supplement from Echinacea purpurea extract. According to 32 revealed that E. purpurea contain of antioxidant compound. Alkamides is the compound that can increasing the immune system. Conclusion Cymbopogon nardus extract at a dose of 50 mg / kg bw, 150 mg kg and mg kg was to the leukocyte, monocyte and lymphocyte count with an optimal dose of 150 mg / kg bw after injected by S. aureus. So, it can be said that C.

nardus extract has immunomodulatory activity. Acknowledgement We thank to Rofiatun Solekha and Sri Bintang Sahara who performed field analyzed data. express our gratitude to Majelis Diktilitbang Muhammadiyah for providing the grant of this research. Conflict of Interest The author stated that there is no conflict of interest. References 1. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin - converting enzyme 2 (ACE2) as a SARS - CoV - 2 receptor : molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;2. doi:10.1007/s00134-020-05985-9 2. Li G, Fan Y, Lai Y, et al.

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