

Generating the Responses Immune with Honey, Saussurea costus, and Nigella Sativa in Cellular and Humoral May Resolve COVID-19?

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Generating the Responses Immune with Honey, *Saussurea costus*, and *Nigella Sativa* in Cellular and Humoral May Resolve COVID-19?

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ABSTRACT

Backgrounds: Covid-19 has become pandemic in the world, including Indonesia. Some Indonesia Covid-19 people tried to consume *Honey*, *Saussurea costus*, and *Nigella sativa* for curing the Covid-19 disease. From many testimonies, citizen Indonesia people with no need to take hospitalization have reported a cure if they consumed it.

Aims: To make evidence-based medicine that *Honey*, *Saussurea costus* dan *Nigella* may cure the Covid-19

Methods: We used to post only control design and mice as an animal model. The research divided mice into two groups, and the first group as control received PBS as a placebo. Then the second group, we gave *Honey*, *Saussurea costus* dan *Nigella sativa*. All of the regiment enters the mouth with special sonde to reach the gastrointestinal organ. After administration regiments a long three weeks, we sacrificed the mice. We evaluated cellular immune responses that are Th2, Th17, and NK cells. We check for humoral immune response, TGF- β , IL-17A, sIgA, IL-4, IL-4, B-def, and IgG.

Results: We got deference Th2 and Th17 between control with treatment group ($p < 0.05$) statistically from the cellular immune response results. Then there was no statistical difference of NK cells between the control with the treatment group ($p > 0.05$). For markers, humoral immunity all has deference between control with treatment group ($p > 0.05$) statistically, but one (IL-17A) have no statistical difference.

Conclusions: We want to continue studying immune responses in humans with COVID-19 if giving *Honey*, *Saussurea costus*, and *Nigella sativa*.

Keywords: Respons immune, Honey, *Saussurea costus*, *Nigella sativa*, COVID-19

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INTRODUCTION

Sarcov2 causes Coronavirus Disease-19 (COVID-19), which indicates an acute respiratory infection and is highly contagious. Fever with a temperature above 38⁰ C is a sign of COVID-19 sufferers. Besides these signs, we can also find other symptoms such as dry cough, fatigue, dyspnea, and difficulty breathing (1,2,3). COVID-19 was first discovered in December 2019 in the South China Seafood Market in Hubei Province, China (4).

Until now, scientists in the health sector have not been able to overcome the disease, which is still a pandemic. Based on World Health Organization data dated January 17, 2021, 93,194,922 confirmed cases of SARS-CoV-2, with 2,014,729 deaths in 220 countries globally. In Indonesia alone, there were 896,642 confirmed cases with 25,767 deaths (3.1%). Treatment of COVID-19 at our hospital, namely the General Hospital dr Iskak Tulungagung Indonesia, uses several types of standard drugs. The drug includes avigan/favipiravir/oseltamivir as an antiviral, levofloxacin/azithromycin as an antibiotic, and high doses of vitamins C, D, and there is more as symptomatic medicine. We have also used passive antibodies for the challenging disease (5). Our hospital also performs this convalescent plasma therapy method.

Indonesia has around 250 population people and is the most significant Muslim population in the world. They refer to the Holly Quran 16:69 that Honey can improve body health (6). Meanwhile, the Hadiths of Buchori 5715 dan 5696 noted that *Saussurea costus* and *Nigella sativa* could cure lung disease (7). In believing this statement, many Indonesian Muslim people try to cure COVID-19

used *Honey*, *Saussurea costus*, and *Nigella sativa* (HSN). Many cases of COVID-19 use these preparations that do not require hospital treatment. Some reports are not well documented; many say a complete recovery. Of the many testimony's researcher want to reveal herbs' role above, is it possible to cure COVID-19. The results of this evidence base study, if they can prove the benefits of these herbs for COVID-19 therapy, will be a breakthrough.

This study used the immunity paradigm infection in cellular and humoral immune responses and a phase one clinical trial using mice.

THE METHODS

We use the post control only design method and the animal model in mice. We take six mice in every group, and our research requires two groups. Ideally, each group at least contained 20 mice. The content mice in each group were six because the study was part of our umbrella study. In our umbrella study, there are seven treatment groups. So, with the consequence of our report, every group contains six mice. We expect this writing to be more focused by separating its title from the other's treatment. The umbrella of this research is to find alternative therapies and prevention of covid-19.

The first group was mice treated with HAS, while the second group gave phosphate buffer saline (PBS). Our study gets the Honey from the beekeeping in Pati, Central Java, Indonesia for the refined powder *Saussurea costus* we buy from Saudi Arabia. Meanwhile, *Nigella Sativa* we get from a local company in the form of soft capsules. Based on existing testimony for COVID-19 therapy, the dosage for

adults, (HSN) 5 ml, 1 gram, and two soft capsules, respectively. This study makes 1 gram of Nigella sativa with 50 ml boiling water. Citizen Indonesia took this HSN three times a day.

To calculate the dose for the animal model, we do as follow, and we divided the HSN dosage by the number 5000 ml (human blood volume)/5 ml (mouse blood volume) to find the dose according to the mice. So, we found a Honey dose of 5/1000 ml = 0.005 ml, *Saussurea coctus*. 50/1000 ml = 0.05 ml, and *Nigella sativa* 2/1000 ml = 0.002 ml for each administration. Researchers gave HAS following the calculation of the oral dose every morning at 07.00 am. We gave HAS to the first group, while in the second group, we gave normal saline. The duration of administration is three weeks. How to check cellular immunity, we used to refer method in our previous study (8). We examined three types of cells, namely: NK cells, TH2 cells, and Th17 cells. We assessed the absolute counts of Th17, NK cell, and Th2 used flow cytometry, respectively. , using the antihuman CD4 + IL-17APE antibodies (BioLegend, San Diego, CA, USA) and anti-human CD4 + CD25 + Foxp3-PE antibodies (BioLegend, San Diego, CA, USA) with standard method. Peripheral blood mononuclear cells s was adjusted to the concentrations of 1×10^6 cells / L and incubated with various antibodies. We analyzed all the samples used BD Cell- Quest™ Pro software (BD Biosciences).

The method of checking for humoral immunity is also the same as referring to our previous method (8). In general,

we convey the following: Enzyme-linked immunosorbent assay (ELISA) for measuring s-IgA, β -defensin, and IL-17. We cut the ileum, and the mucous was collected and suspended with the same volume of PBS. Then we centrifuged at 6000 rpm at a temperature of 4 ° C for 30 min. Then we measured s-IgA and β -defensin, using the s-IgA ELISA kit from Elabscience (Donghu HiTech Development Area, China) (E-EL-M1040) and β -defensin ELISA kit from MyBioSource (MyBioSource, Inc. San Diego, USA) (MBS2886605) with the standard method. Blood was collected from the mice's heart and stored for 10 min at 70 ° C. IL-17 was measured from blood plasma using the IL-17A ELISA kit from BioLegend (San Diego, CA, USA) standard method.

Data analysis

We performed all statistical analyses using IBM® SPSS® version 23.0 (International Business Machines Corporation, Armonk, New York, USA) software. If the research $p < 0.05$ achieved, we considered it statistically significant.

RESULTS AND DISCUSSION

Our first study aimed to determine the cellular immunity profile when we supplemented the HAS as the instructions we wrote above. We investigated a cellular profile of immunity that included only Th2, Th17, and NK cells. We can see the results of the examination of cellular immunity in Fig 1.

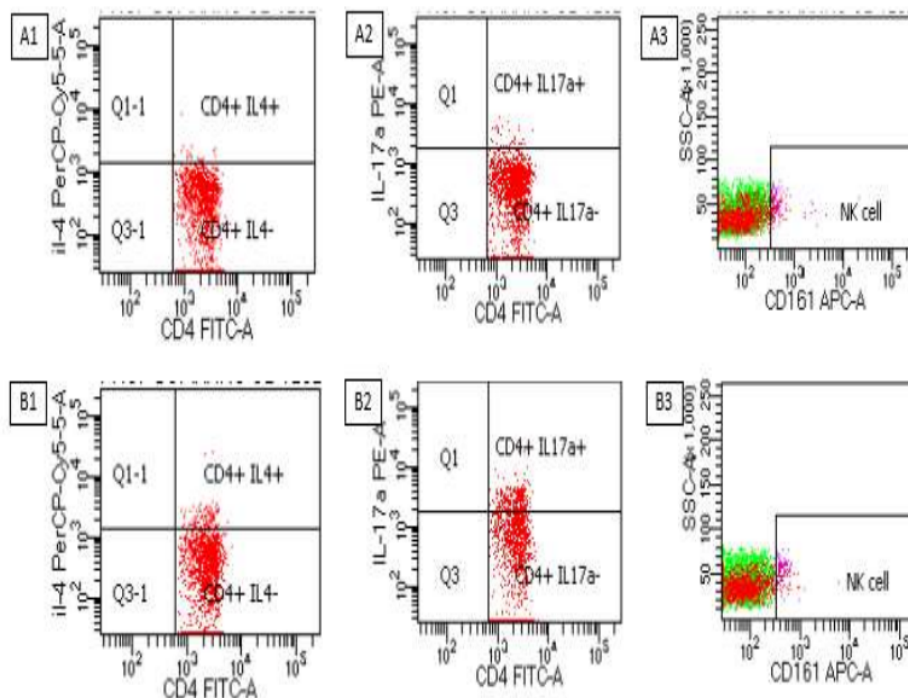


Fig 1: Representative flow cytometry Figs from control group Th2 (A1), group Th2 treatment (B1), from control group Th17 (A2), group Th17 treatment (B2), and from control group NK cell (A3), and group NK cells treatment (B3).

The results of calculating the number of Th2, Th17, and NK cells can see in Table 1.

Table 1. The analysis of differences between the control group and the supplement group by reviewing the cellular responses of Th2, Th17, and NK cells.

PARAMETER	MEAN ±SD	SIG
Control	0.32 ± 0.04	0.000
Th2	1.22 ± 0.13	
Control	0.50 ± 0.12	0.000
Th17	3.54 ± 0.25	
Control	1.16 ± 0.05	0.229
NK cell	1.24 ± 0.15	

Table 1 showed statistically significant differences in Th2 and Th17 between the control and treatment groups with $p = <0.05$. But on NK cell examination results, although there were differences in numbers, it was not significant

($p \Rightarrow 0.05$). To explain the table 1 review, we continue the statistical analysis by displaying a graphic image shown in Fig 2.

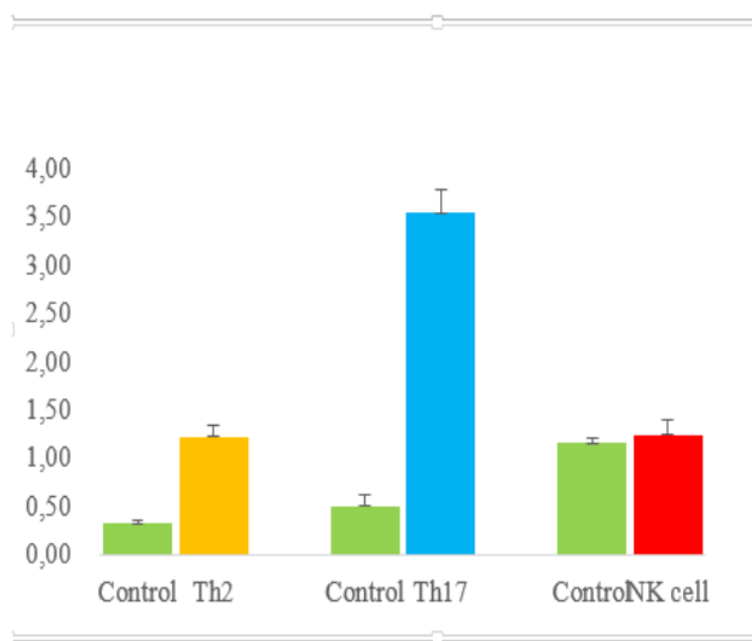


Fig 2. Differences in the results of Th2, Th17, and NK cells between the control and treatment groups.

In Table 1 and Fig 2, the study results show a significant difference between the control group and the treatment group. There was a difference in the number of Th17 between the control and treatment groups. The proliferation of Th17 will produce IL-17 and IL-22. These two ILs will stimulate the cylindrical epithelium of the intestinal lumen to produce β -defensin (9). While the

expansion of Th2 will produce IL-4, and this IL-4 will stimulate virgin B cells to experience maturation to produce IgM. Upon IL-6 and TGF- β precursors, mature B cells switch to produce IgA dimer (10). Calculating the amount of humoral immune response, namely: TGF- β , IL-17A, sIgA, IL-4, IL-4, B-def, IgG, can be seen in Table 2.

Table 2, Quantitative results of mice humoral immune response due to supplementation with HAS

PARAMETER	MEAN ± SD	SIG.
Control	6.093 ± 0.417	
TGF-β	6.210 ± 0.620	0.000
Control	8.225 ± 0.342	
IL-17A	8.820 ± 0.520	0.067
Control	1.103 ± 0.395	
sIgA	2.584 ± 0.359	0.000
Control	7.104 ± 0.817	
IL-4	9.785 ± 1.027	0.002
Control	1.056 ± 0.056	
β-def	1.364 ± 0.121	0.001
Control	2.728 ± 0.313	
IgG	3.944 ± 0.417	0.001

To facilitate evaluation, study, and analysis table 2, we display the graphic image shown in Fig 3.

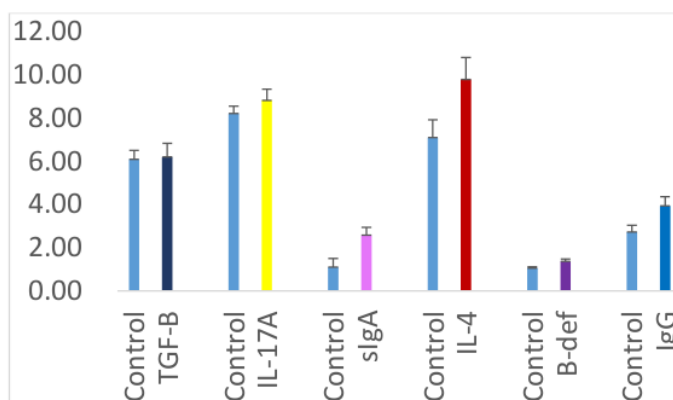


Fig 3. The examination results, TGF-β, IL-17A, sIgA, IL-4, IL-4, β-def, and IgG due to treatment HAS.

The second research objective in this study was to determine the humoral immune response due to supplementation. We measured TGF-β, IL-17A, sIgA, IL-4, IL-4, β-def, and IgG as manifestations of the humoral immune response. By evaluating the research results shown in Table 2 and Fig3 using statistical analysis to get a conclusion. We got results that almost match our hypothesis. The study showed a significant difference between the control group and the group giving HAS on all humoral immune response markers except for one, namely IL-17A ($p < 0.05$, for IL-17A $p = 0.065$ still $> 0, 05$). Our result could be the case that there may be competition with IL-22, which also has a role as a trigger for β-defensin expression produced by mucosal epithelial cells (11,12). We regret not examining the IL-22 marker, which might answer why there were no differences between the control and treatment groups for the IL-17A marker. The β-defensin has a vital role in mucosal immunity by directly lysing microbes, including viruses. The β-defensin molecule's shape has a positive charge, while the microbes have a negative wall charge (13,14). In addition to the direct killing of pathogenic microbes in the mucosa carried out by β-defensin, this killing can also be carried out

indirectly by s-IgA. Specific s-IgA that is present in the mucosa will ozonate and bind to pathogenic microbes. This opsonin will be phagocytosed by dendritic cells, which will then be lysed by the phagolysosome's substance (9). Therefore, β-defensin and s-IgA are critical substances to kill pathogenic microbes that will enter the human body. The research results that we see in Table 2 show that there are significant differences and correlations between the control group and the treatment group, each with $p = < 0.05$. Therefore, the question arises whether Honey, *Saussurea costus*, and *Nigella sativa* can increase the response / immune modulator of the covid-19 vaccine, whether it is commercial or still in the development process, needs explanation.

NK cells in the tissues or blood can also kill microbes in these areas. But the ability of these NK cells must be supported by IgG, which can bind to NK cells. The IgG will capture if only microbes attached to specific cells in the body bind to NK cells via Fab of IgG. This event is known as ADCC (Antibody-Dependent Cellular Cytotoxicity) (15,9). We saw the study results in Fig 2 and show that the NK cells did not show any significant differences and correlations between the control group and the treatment

group with $p \leq 0.05$. An increase in the humoral immune response, namely Th2, will suppress the cellular immune response (NK cell as a Th1 cellular response), causing the situation in Fig 2. Th2 products manifested for enhancing the humoral immune response, namely IgG and IL-4, which turned out to be a correlation between the control and treatment groups $p \leq 0.05$. Unfortunately, the researcher missed the opportunity to analyze other types of cells that can also kill microbes. The specific type of cell capable of killing these microbes is CTL (Cytotoxic T Lymphocyte). CTL is a way of killing microbes different from NK cells. In CTL, it will kill microbes, especially viruses that have entered specific body cells. The virus that enters the cell causes the cell to express MHC-I molecules. This MHC-I molecule will stimulate a bond with the CD₈ molecule on the surface of the CTL. The bonding of these two molecules will cause the two kinds of cells to lysis; the virus will accompany the cell lysis (16,17). We examined another humoral response marker, namely TGF- β as in Table 2. The results showed a significant correlation between the control group and the group giving HSN the humoral immune response TGF b, $p \leq 0.05$. TGF b and Il-6 cause cell plasma to switch from producing IgM to IgA (18,19).

Recent study Mahmoud (2020) asked may *Saussurea costus* help in the treatment of COVID (20)? They found from one journal that one component, *Saussurea costus*, namely myrcene, acts on ACE receptors (21). COVID-19 has emerged as a pandemic and a public health crisis. The causative agent was named SARS-CoV-2 and was detected from throat swab samples. It enters the cells by endocytosis after attachment to the angiotensin-converting enzyme-2 (ACE2) receptors on cells in the lung, gastrointestinal tract, blood vessels, heart, and kidney (22, 23). Mahmoud's finding is fascinating because, firstly, myrcene can act as a competitive inhibitor with adherence to the epitope in the spike ACE2. Then SARS-CoV-2 cannot enter the cells by endocytosis after attachment. Secondly, if myrcene has function as an epitope, it will produce antibodies attached to ACE. As a result of the extension, then SARS-CoV-2 cannot enter the host cell. Honey and *Nigella sativa* in the immune response can be a modulator, and no report has components like in *Saussurea costus*. The honey and *Nigella sativa* just only, antioxidant activity, antibacterial activity, anti-inflammatory and immunomodulatory activities (24, 25). Our latest research shows that the epitope/peptide protein adhesion of *S. flexneri* can stimulate the production of antibodies. Anti-peptide serum antibodies epitope ATLGATLNRLDFNVNKK (A-K) adhesion molecule *S. flexneri* can inhibit diarrhea caused by *S. flexneri*. We obtained this A-K epitope from a bioinformatics study (26).

Therefore, the result of this completed study will be interesting if it we combine with the most dominant epitope in the Sarcov-2 spike. We can study the immune response, and the most important thing is to confirm whether it has virocydal power. Researchers will determine this virosidal power from the serum (IgG), s-IgA, and beta defensins in the mucosa. We will know the cytopathic effect (CPE) is there have verocydal? (27). Our study's conclusion makes our rationale for evaluating whether Honey, *Saussurea costus*, and *Nigella sativa* (HSN) can be used for therapy or as an immune-adjuvant vaccine for sufferers of COVID-19.

CONFLICT OF INTEREST

There was no conflict of interest in this study.

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