THE EFFECTIVENESS OF TETRAGONULA HONEY COMBINATIONS Aff.biroi AND ROYAL JELLY AS IMMUNOMODULATORS: IMUNOMODULATORS MODELLING IN FACING THE PLAGUE OF COVID-19

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ABSTRACT

Corona-19 epidemic caused serious concern around the world. Innate and adaptive immunity prevents occurring acute infection. Immunomodulatory activity tests and increased leucocyte cells in rat have been carried out using honey (H) and royal jelly (RJ). This study aims to prove the combination of honey and royal jelly in increasing the leucocyte profile in male Sprague Dawley rats. This study used 28 rats divided into 7 groups, namely the negative control and positive, single dose group H and single dose RJ and the combination dose 1, dose 2, and dose 3, then on day the 8th and 11th rats were injected intraperitoneally with 2% sheep red blood cell antigen as much as 0.5 ml. Leucocyte profile were carried out on the 8th and 15th days. The results showed significant difference on leukocyte count (p = 0.000), but there was a decrease in the normal range on day 15th. The highest leucocyte count was showed on the honey and the combination 3 group. Leukocyte differential showed no significant difference (p> 0.05). The honey group and the dose combination 3 showed an increase in the number of segment neutrophils. The difference in lymphocyte reduction was seen between the honey and royal jelly group, with the dose combination 3, respectively. There are significant differences in granulocyte cells enhancement between the honey group and the dose combination 3. We concluded that single dose honey can be an immunomodulatory. Dose combination 3 can be an alternative for further research for immunomodulatory supplement product.

Keywords: honey Tetragonula sp; immunomodulator; royal jelly

INTRODUCTION

Since WHO announced the COVID-19 case as a pandemic, cases of people with suspected, close contact and those who have confirmed COVID-19 have continued to increase. Data at the end of March 2020, the mortality rate for Indonesia was the highest in Southeast Asia at 8.9 percent. Human-to-human spread of SAR-CoV-2 is the main source of transmission. Viral and host factors play a role in SARS-CoV infection. Inadequate causes viral replication and tissue damage.

Innate and adaptive immunity prevent infection or prevent acute infection from occurring.¹ This is what causes many people to live a healthy lifestyle in order to maintain stamina and increase the body’s immune system ¹ the way to restore and repair impaired immune function can be by using substances that are able to reverse the imbalance of the immune system called immunomodulators.²

Public trust in natural ingredients to boost the immune system is still quite high. One of these natural ingredients is a product produced by honey bees, including honey and royal jelly. Honey is a sweet liquid derived from plant nectar, which is processed by worker bees into honey and stored of carbohydrates. Honey is a natural source of nutrition and has an effect on human health because of its flavonoid content which is believed to be an immunomodulator by stimulating T-lymphocytes and activating
neutrophils, honey has high antioxidant activity, antimicrobials, wound healing effect, antiinflammatory, antimutagenic, antidiabetic, anticancer, antifungal and anti-tumor. The bioactive components of honey, which mainly consist of carbohydrates (82.4%), fructose (38.5%), glucose (31%), water, and protein. There are minor components in honey, such as organic acids, glucose oxidase enzymes and catalase which can destroy bacteria.

Royal Jelly is a yellowish white liquid, has a sour taste, and a jelly-like texture secreted from the hypopharyngeal salivary glands and mandibles in worker honey bees between 5 and 14 days old and as a food source for queen bees. Royal jelly contains protein, namely Major Royal Jelly Protein/ MRJP-3 which has the ability to increase cell proliferation, immunomodulatory and anti-allergic effects. The main component of royal jelly is water ranging from 60% to 70%, followed by 11-23% carbohydrates, 9-18% protein, lipids (especially 10-hydroxy-2-decenic acid) 4-8%, vitamins and mineral salts ranging 0.8 to 3%, amino acids, proteins, sugars, vitamins (thiamine, niacin, riboflavin) and minerals (iron and calcium).

The combination of honey and royal jelly was able to increase stamina in mice compared to a single dose of honey. Provision of forest honey was able to significantly influence lymphocyte proliferation in male Wistar rats. Giving royal jelly to rat and mice showed immunomodulatory properties by stimulating antibody production.

This research is expected to strengthen the effect of the combination of honey and royal jelly in boosting the immune system so that the potential of the two natural ingredients can be considered as immunomodulators during the COVID-19 pandemic.

**MATERIAL AND METHODS**

The design of this study used a pre-post test experimental method with a control group design held in the Laboratory Pharmacology and Therapy Faculty of Medicine, Padjajaran University on July, conducted in the laboratory using honey, royal jelly and white male rats (Rattus norvegicus) of Sprague Dawley strain previously induced with sheep red blood cells, then blood sampling for the total number of leucocyte and differential count of leucocyte as immune response. The honey (H) was obtained from Tetragonula aff. biroi species dari North Luwu, North Sulawesi packaged by CV Nanobiotek Indonesia, Depok while the royal jelly (RJ) was obtained from corn flower pollen purchased from honey farmers in Sragen, Central Java.

The bioactive chemical testing simplicia has been tested in Laboratory of the Centre for Agricultural Research and Development, Ministry of Agriculture, Bogor that include examination of total flavonoids, total phenols and water content.

Prior to the experiment, Sprague Dawley strain rat were adapted for one week and each group of rats was kept in a cage, fed standard feeding ad libitum. Then each group was sensitized with 10% of red blood cell sheep (SRBC) 10% suspension intraperitoneally on days 8 and 11 after treatment. The samples were taken from animal experiments with a minimum sample size of 28 tails calculated on the Federer formula. Then divided into 7 groups namely 2 control group given Na-CMC 0.5% as negative control and Levamisol 10 mg/kgBW/day with 2 mL CMC 0.5% as positive control and 5 treatment groups consisting of honey group dose 1 mL/200gBW rat, RJ group dose 0.5 mL/200gBW rat and honey dan RJ combination with dose of 0.3 mL honey+0.05 mL RJ, 0.6 honey+RJ 0.1 mL and 1.2 mL honey+RJ 0.2 mL/kgBW/day was suspended with 0.5% Na CMC 2 mL, then administered to the orally daily rats for 14 days.

According to Yulianti, R cit Nurmaya Effendi (2018), fresh sheep red blood cells (SRBC) were obtained from the Pharmacology and Therapy Laboratory of the Faculty of Medicine, Padjajaran University, then they were accommodated in a clean, dry and tube has been sterilized which contains powder EDTA as an anticoagulant. For 1 mL of sheep’s blood, 1 mg is needed EDTA. SRBC separated from plasma by centrifuge at a speed of 3000 rpm for 10 minutes. Next the blood cells on wash it by adding PBS in large
quantity and the tube contains the suspension is reversed several times and mess around back. Do the most washing at least three times. After forming two layers of plasma and red cell deposits, then the plasma fluid is removed by using a micropipette. After obtaining SRBC 100%, then prepare antigen to be used by diluting 1 mL SRBC 100% suspension with 9 mL of PBS to obtain 10 mL antigen suspension (SRBC 10%).

Rat blood that comes from the heart immediately taken and accommodated on a 1.8 ml Eppendorf tube dripped two drops with EDTA 4%. The pipette is then shaken for three minutes until homogeneous. Blood sampling was performed on days 8 and 15. Take the blood from the tube by inhalation using a pipette until the mark 0.5 and the tip of the pipette is cleaned with a tissue. Then suction the Turk solution with the same pipette until it reaches the limit of the number 11. Before the solution is put into the count chamber, remove the first two or three drops. After the solution is put into the counting chamber, wait for one minute, the next leucocyte is calculated using magnification 10 times or 40 times on the objective lens. The leukocyte count formula is number of cells x 50. Total lymphocyte counts were performed by using the multiplication of leukocyte count and leucocyte differential count. The calculation of the absolute number of each leukocyte type is calculated by the formula namely the number of cell of the leukocytes type divided by 100 x total number of leukocytes.

Statistics The significance of differences between means of the groups were tested by the paired Student's t test using a statistical package, the Statgraph (Statgraphs Inc., Version 4.2). Results were considered statistically significant if p< 0.05.

RESULT AND DISCUSSION

Identification of the test material through a non-specific parameter test in the form of an overview of the water content in the honey and royal jelly ingredients, the water content of honey (21.21%) was less than that of royal jelly (66.44%), while the organoleptic specific parameter testing showed that honey was a liquid with a thick, coloured texture brown and has a sweet and sour taste, while Royal Jelly is a white-yellowish liquid, has a thinner texture and a sour taste as shown in Figure 1.

In chemical testing, the total flavonoids and total phenol of honey by the spectrometer method had a higher value than royal jelly as shown in Table 1.

| Assay (mg/100g) | Honey | Royal jelly |
|----------------|-------|-------------|
| Total Flavonoids | 57.93 | 10.63       |
| Total Phenol | 2.55 | 0.88        |

Antigen is a foreign object that comes from outside or from inside the body. The injection of a foreign substance into the animal's body will trigger the body's response to produce a non-specific or non-specific immune system. These immunogens will signal the sending of signals to cells that play a role in making antibodies. The more foreign the antigen used, the more effective it is in causing an immune response. Sheep red blood cell is a polyclonal antigen which is a protein with a greater determinant of potential, is insoluble and given intraperitoneally or intraplantarally so that it will pass through the surface barrier and the inflammatory response then diffuses from the peritoneal cavity into the vascular space through the lymph vessels, then it will be phagocytosed by macrophages as antigen presenting cell (APC) into an immunogenic peptide then presented via MHS class II to Th0 cells to further differentiate into Th1 and Th2 cells. Sheep red blood cells are used in this study because they are easy to obtain in the form of a uniform and measurable suspension, quite stable and their lysis can be seen easily and their antigenic properties are high. with the entry of antigens into the body, there will be body defence against
foreign objects that enter both non-specific and specific defences.

In the calculation of leukocyte cells with the blood smear method using Giemsa's solution as a dye, which then uses emersion oil to clarify the shape of the observed cells, visible stem neutrophil cells, segment neutrophils, eosinophil cells, monocyte cells, lymphocytes, while basophil cells are not alkaline, can be observed because these cells are soluble in Giemsa dye.

**Leukocyte count test results.** Leukocyte cells are active components of blood that play a role in the body's defense system and the leukocyte cells produced in the bone marrow will go to the blood vessels and are able to leave the circulation to the tissues that need them.²

The results of measuring the mean number of rat blood leukocytes after seven days and fourteen days of treatment respectively can be seen in Table 2 and Figure 2.

### Table 2. Average Value of Total Leucocyte Count (x10^3/mm³)

| GROUP                    | Leucocyte cell (x10^3/mm³) |
|--------------------------|-----------------------------|
|                          | Day-8 | Day-15 |
| Negative control         | 9.5   | 4.9    |
| Positive control         | 16.0  | 6.3    |
| Honey                    | 14.1  | 6.7    |
| Royal Jelly              | 8.8   | 5.9    |
| Combination dose 1       | 10.9  | 4.7    |
| Combination dose 2       | 8.1   | 6.3    |
| Combination dose 3       | 9.8   | 6.3    |

Note: combination dose 1: M 0.3 mL and RJ 0.05 mL/200g rat in Na-CMC 0.5% solution; combination dose 2: M 0.6 mL and RJ 0.1 mL/200g rat in Na-CMC 0.5% solution; combination dose 3: M 1.2 mL and RJ 0.2 mL/200g rat in Na-CMC 0.5% solution.

Based on Table 2 on day 8 to day 15, it shows a tendency to decrease the number of leukocytes. After the 7th day of treatment, it was seen that the group giving a single dose of honey showed an immunomodulation effect on the non-specific immune system through an increase in leukocyte cells above normal, namely 14.1 x 10^3 / mm³ (5-13 x 10³ / mm³)¹⁷ compared to the Royal jelly group and positive control, where the drug levamisole has the ability to increase the effect of mitogens, lymphokines and chemotactic factors to stimulate lymphocytes, granulocytes and macrophages,² whereas the combination group showed that the dose combination group 2 and 3 seemed to maintain the immunomodulation effect of immune cells in the normal range compared to the dose combination 1 and the negative control group. The decreasing trend of leukocyte cells from all treatment groups on the 15th day showed that after the mice received repeated sheep red blood cell antigen sensitization, it was seen that the leukocyte cells worked to get rid of these antigens so that there was no severe acute inflammatory process due to antigen sensitization. It appears that after repeated exposure to antigen, all treatment groups did not show systemic symptoms due to acute inflammation. This proves that the inflammatory process can be overcome.

Changes in the total number of leukocytes indicate an immunomodulation effect of the immune system with an increase in leukocyte cells. Then after antigen sensitization was seen on day 15 there was a decrease in the number of leukocyte cells, which indicates that the
leukocyte cells work in a destructive manner through the phagocytosis process. This is a response in protecting against the presence of antigens that enter the body, where there is migration of leukocytes from peripheral blood to the fingers that need it. In normal / healthy conditions during the research process in experimental animals, there was a decrease in leukocyte cells as seen in the negative control group, because the condition of the body and age of the experimental animals could affect the production of leukocytes even though the number of leukocytes was in the normal range.

This is related to the pathogenesis of COVID-19, where there is a severe acute inflammatory reaction due to cytokine storms. Therefore, honey supplementation, either single or combined with royal jelly, can be used as an immunomodulator so that it can protect the body from cytokine storms.

On day 15, it was seen that the single dose of honey given group was able to maintain the number of leukocyte cells compared to the negative control group, the combination group and the positive group. The results of the ANOVA statistical test with a significance value of p = 0.000 (p <0.005) showed that on the 15th day the mean of all treatment groups was significantly different and the Post Hoc Tukey test showed that the highest average number of leukocyte cells was in the honey group and in the 3 dose combination group. Thus allowing the content of honey in single form or in combination with royal jelly can mutually strengthen antioxidant properties that play a role in counteracting free radicals by maintaining the function of the immune system of Sprague Dawley mice against antigen attack so that they can maintain the number of leukocyte cells in the normal range.

The mechanism of antioxidant compounds to maintain the number of leukocyte cells in the normal range indicates that these compounds provide a protective effect against attack by Sprague Dawley rat antigens. According to research besides flavonoids, phenols, as well as 90% of the total protein in RJ are major royal jelly proteins (MRJPs) that have biological activity as immunomodulators and antibacterials, while the specific fatty component of 50% of the total fatty acids in royal jelly is 10-Hydroxy-trans-2-decenoic acid (10H2DA) which is often referred to as royal jelly acid which has anti-inflammatory and immunomodulatory benefits where it is associated with production inhibition activity. IL-6 and Nitric Oxide (NO) are induced by LPS (lipopolysaccharide) inhibition which causes no binding with CD14 on the phagocyte surface, thereby inhibiting signal transmission to the nucleus to activate Nucleus Factor kappa B (NFkB), a transcription factor that triggers production cytokines and chemokines.

In addition, 10H2DA works to inhibit interferon-γ which is stimulated by the macrophage response. Interferon-γ produced by the main MAC cytokine has activity in immunity as well as inflammatory processes and is able to activate macrophages to kill foreign bodies that are phagocytic and trigger inflammatory reactions. Although interferon has the ability to kill foreign bodies through macrophage activation and inhibits viral replication directly, if the activated macrophages become excessive due to the increased IFN-γ levels, it will trigger an inflammatory reaction. Interferon-γ according to the classification based on its cell source is a lymphokine family because IFN-is produced by T lymphocytes and LGL cells during an immune response. As a result of inhibition of 10H2DA and MRJPs, the activity of macrophages to secrete proinflammatory cytokines (IL-1, IL-6, TNF-α) is decreased so that the inflammatory process can be suppressed or inhibited. Thus, if royal jelly supplements in a single dose or combined with honey will have a good effect in suppressing the cytokine storm caused by the Corona-19 virus.

Honey has high antioxidant activity. The content of flavonoids and phenols in pure honey has anti-inflammatory properties by inhibiting the release of pro-inflammatory cytokines, by modulating the immune system and acting as an antioxidant that functions to neutralize free radicals that occur in the inflammatory process. The mechanism of antioxidants in neutralizing free radicals by
donating one electron by converting highly reactive Reactive Oxygen Species (ROS) into molecules that are not harmful to the body. 19

To see ability of *Tetragonula aff biroi* and *Royal jelly* in the hematopoietic system related to the immune system, namely the total number of leucocytes and tissue cells derived from leucocytes, including lymphocytes, monocytes and granulocytes. The results of measuring the mean number of differential count leucocytes in each treatment group are shown in Table 3a,3b and Figure 3a,3b.

Table 3a Average number of neutrophil, granulocytes, lymphocytes, and monocytes 0n day-8

| Group            | Measurement of Cells on Day-8 | Neutrophil | Granulocyte | Lymphocyte | Monocyte |
|------------------|--------------------------------|------------|-------------|------------|----------|
| Negative control |                                | 18.75      | 19          | 74.5       | 1.75     |
| Positive control |                                | 24.5       | 25.25       | 74.5       | 0.5      |
| Honey            |                                | 34         | 34.5        | 64.25      | 1.25     |
| Royal Jelly      |                                | 22.75      | 25          | 74         | 1        |
| Combination dose 1 |                             | 23.25      | 23.5        | 75         | 1.5      |
| Combination dose 2 |                             | 21.25      | 21.75       | 77.5       | 0.75     |
| Combination dose 3 |                             | 15.5       | 16          | 84         | 0        |

Table 3b Average number of neutrophil, granulocytes, lymphocytes, and monocytes 0n day-15

| Group            | Measurement of Cells on Day-15 | Neutrophil | Granulocyte | Lymphocyte | Monocyte |
|------------------|--------------------------------|------------|-------------|------------|----------|
| Negative control |                                | 18.25      | 18.75       | 78.25      | 1.75     |
| Positive control |                                | 34.75      | 35          | 61.5       | 3.5      |
| Honey            |                                | 45.5       | 45.75       | 52         | 2.25     |
| Royal Jelly      |                                | 18.5       | 20          | 77         | 3        |
| Combination dose 1 |                             | 20         | 20.25       | 78.5       | 1.75     |
| Combination dose 2 |                             | 28.25      | 29.25       | 69.5       | 1.25     |
| Combination dose 3 |                             | 23.25      | 24.25       | 74         | 1.75     |

Noted: combination dose 1: M 0.3 mL and RJ 0.05 mL/200g rat; combination dose 2: M 0.6 mL and RJ 0.1 mL/200g rat; combination dose 3: M 1.2 mL and RJ 0.2 mL/200g 200g

From the results of statistical tests, it can be seen that the mean differential measurement values of leukocyte cells are not significantly different (p> 0.05) in the four types of cells, namely neutrophils, granulocytes, lymphocytes and monocytes as shown in Table 4.

Table 4. Statistic test

|                  | Neutrophil | Granulost | Lymphocyte | Monocyte |
|------------------|------------|-----------|------------|----------|
| Statistic test   | One way    | Kruskal   | Kruskal    | Kruskal  |
| Asymp. Sig.      | 0.289      | 0.655     | 0.982      | 0.226    |
| Post Hoc         | Tukey      |           |            |          |
| Asymp. Sig.      | 0.226      |           |            |          |

**Neutrophil**

Neutrophils constitute 70% of the number of circulating leukocytes that have a function in the body's defence against antigens. Neutrophils are divided into two, namely stem
and segment neutrophils, the difference between the two is that rod neutrophils are a form of young neutrophils from segment neutrophils. Segment neutrophils are also called polymonuclear (PMN). PMN cells circulating in the circulation can migrate to tissues that need a stimulus, for example by macrophages which detect microbes through acute inflammation and are classified as capable of phagocytosis such as macrophages, but the difference is that neutrophils do not produce cytokines like macrophages. The results of the study, the mean total number of neutrophils are shown in Tables 2a and 2b, while the results of the mean values for the total number of neutrophils are shown in Table 5.

Table 5. Mean Neutrophil Cells (%)

| Parameter          | Day | Negative control | Sub Polymorphonuclear | Royal Jelly | Combination dose 1 | Combination dose 2 | Combination dose 3 |
|--------------------|-----|------------------|-----------------------|-------------|--------------------|--------------------|--------------------|
| Segment neutrophils| 8   | 22.75            | 24.25                 | 33          | 22.75              | 21.25              | 30.5               | 15.5               |
|                    | 15  | 17.5             | 22.5                  | 32          | 17.5              | 18.75              | 24.5               | 22.25              |
| difference         | 5.25| 7.75             | 10.00                 | 5.50        | 4.50              | 4.00               | 6.75               |
| Sub Neutrophils    | 8   | 0.5              | 0.25                  | 1           | 0                 | 0.75               | 0                  |
|                    | 15  | 2.5              | 2.75                  | 2.5         | 1.25              | 1.25               | 3.75               | 1                  |
| difference         | 2.00| 2.50             | 1.90                  | 1.25        | 1.25              | 3.00               | 1.00               |

Based on Table 5, the neutrophil parameters of the segment of day 8 to day 15 showed the greatest increase in the treatment group giving a single dose of honey with a difference of 10 (the mean value of segment neutrophils was 43%) higher than the positive control (total the mean value of segment neutrophils was 32%), whereas in the combination group, the dose group 3 showed a greater difference, namely 6.75 compared to the combination of doses 1 and 2. This shows that in the group giving a single dose of honey 1 ml / 200gBW rats were effective in increasing neutrophils, segment after exposure to sheep red blood cell antigen which can be seen at the largest positive difference and still within the normal range of segment neutrophils is 15.4 ± 4.5%. Segment neutrophils (PMN) have the ability to attack and destroy foreign substances and provide an initial response to bacterial infections.

Honey contains high flavonoids and phenols as seen in Table 1. The benefits of honey flavonoids are thought to be able to increase the immune system, both cellular and humoral immune systems. Honey flavonoids have biological activity as immunomodulators that are associated with their activities as antiviral, antimicrobial, antioxidant, anti-inflammatory, cytotoxic and antiproliferative. The mechanism of action of honey as an immunomodulator is reported that flavonoids can have an effect on T cells, B cells, macrophages, NK cells, basophils, mast cells, neutrophils, eosinophils, and platelets. In addition, it also plays a role in transduction signalling and activation of immune system cells through enzyme systems such as tyrosine kinase, threonine kinase, phospholipase A2, phospholipase C, lipoxygenase, and others. Honey as an antioxidant and antibiotic works by disrupting the function of microorganisms, both bacteria and viruses. The recommended consumption of honey to prevent disease in humans is 1-2 times / day 1 tablespoon.

Giving honey from the beginning of the treatment until the 7th day, then the antigen induction on the 8th day is the dose adjustment stage for the administration of honey, royal jelly or combination so that the body is protected first before giving the antigen. In the second antigen induction, it is expected that the antibodies that have been formed since the first exposure will reach their peak by introducing the antigen by lymphocytes and activating the memory system of the immune system so as to produce antibodies faster than in the first exposure. The introduction phase to the antigen occurs 3-5 days after the antigen is injected. In this study, it is necessary to look at the measurement of immunoglobulin M and immunoglobulin G in order to strengthen honey, royal jelly and the combination of both of them having an effect on the immune system because each antibody is specific for a particular antigen, while the number of neutrophils shows a protective effect against incoming antigens so that the balance of the immune system is maintained. influence of foreign substances by phagocytic foreign substances after being in infected tissue.
Based on Table 5, the stem neutrophil parameters from day 8 to day 15 at each treatment have increased, where the dose combination group 2 experienced an increase by a difference of 3 compared to the negative control, but when viewed in each treatment there were still some in below the normal limit of stem neutrophils which is 2-6%. It is possible that the neutrophil circulation time in the blood circulation is only 10 hours, allowing the neutrophils to have distributed to the tissues.

**Lymphocyte**

Lymphocytes constitute 20% of the total leukocytes in the blood circulation consisting of T cells and B cells, which are the key to controlling the immune system and including the type of leukocyte agranulocytes.

**Table 6 Difference of Lymphocyte Cells**

| Groups            | Lymphocyte (%) | Difference |
|-------------------|----------------|------------|
|                   | Day-8          | Day-15     |            |
| Negative control  | 74.5           | 78.25      | 3.75       |
| Positive control  | 74.5           | 61.5       | -13        |
| Honey             | 64.25          | 52         | -12.25     |
| Royal Jelly       | 74             | 77         | 3          |
| Combination dose 1| 75             | 78.5       | 3.5        |
| Combination dose 2| 77.5           | 69.5       | -8         |
| Combination dose 3| 84             | 74         | -10        |

Based on Table 6 shows an increase in the percentage of lymphocytes in the negative control, royal jelly and the dose combination 1 on the 15th day, whereas, in the positive control treatment, honey and the combination dose 2 and 3 the percentage of lymphocytes decreased on the 15th day. The decrease or increase in lymphocyte percentage showed no significant difference (p > 0.05) based on Table 4.

The decrease in the percentage of lymphocytes is caused by the large number of lymphocytes that move from the blood circulation to the tissues. The increase in the percentage of lymphocytes can be caused by the body’s response to eliminate cell damage or apoptosis and the effect of flavonoids in honey and royal jelly to increase IL-2 activity and lymphocyte proliferation.

**Monocyte**

Monocytes are cells that originate from the bone marrow and carry out proliferation, including the type of mononuclear leukocytes. After proliferating and maturing, then monocyte cells enter the blood circulation to act as phagocytes. After 24 hours, monocyte cells will move from the bloodstream to their destination in various tissues and will differentiate as macrophages and can last for 4-12 days or months.

The results of measuring the number of monocytes are shown in Table and Figures 3a and 3b, while the difference in monocytes is shown in Table 7.

**Table 7. Difference of Monocyte Cells**

| Groups            | Monocyte (%) | Difference |
|-------------------|--------------|------------|
|                   | Day-8        | Day-15     |            |
| Negative control  | 1.75         | 1.75       | 0.00       |
| Positive control  | 0.5          | 3.5        | 3.00       |
| Honey             | 1.25         | 2.25       | 1.00       |
| Royal Jelly       | 1            | 3          | 2.00       |
| Combination dose 1| 1.5          | 1.75       | 0.25       |
| Combination dose 2| 0.75         | 1.25       | 0.50       |
| Combination dose 3| 0            | 1.75       | 1.75       |

Based on Table 7 shows an increase in the percentage of monocytes in all treatment groups even though it is close to normal (3-8%) seen royal jelly and dose combination 3 showed a higher increase than negative control on day 15. The increase in the percentage of monocytes was not significantly different (p > 0.05) based on Table 4.

The increase in the number of monocytes which are still below the normal range can be caused by the monocyte cells that...
have moved from the bloodstream to the tissues that need them. The increase that occurs can be in the positive group because levamisole has the ability to effect various materials such as antigens, mitogens, lymphokines and chemotactic factors to stimulate macrophages. Royal jelly and dose 3 combination have a protective effect on the immune system because it can increase the number of monocytes / macrophages compared to other treatments.

Granulocyte
Granulocytes are a type of leukocytes that have granulocytes or polymorphs derived from the bone marrow. Granulocytes constitute 60-70% of total leukocytes but can also be found outside the blood vessels because they can penetrate blood vessel walls. Granulocytes according to histological staining are divided into neutrophils, eosinophils and basophils. These cells together with antibodies and complement play a role in the acute inflammatory process with the main function is phagocytosis.

Based on Table 8, it shows the highest increase in the percentage of granulocytes seen in the honey treatment group and followed by the 3 and 2 dose combination groups compared to negative control values, it is seen that the dose combination 3 shows a higher increase than other combinations on the 15th day. The increase in the percentage of granulocytes was not significantly different (p> 0.05) based on Table 4.

Table 8. Difference of Granulocyte Cells

| Groups            | Granulocyte (%) | Day-8 | Day-15 | Difference |
|-------------------|-----------------|-------|--------|------------|
| Negative control  |                 | 19    | 18.75  | -0.25      |
| Positive control  |                 | 25.25 | 35     | 9.75       |
| Honey             |                 | 34.5  | 45.75  | 11.25      |
| Royal Jelly       |                 | 25    | 20     | -5.00      |
| Combination dose 1|                 | 23.5  | 20.25  | -3.25      |
| Combination dose 2|                 | 21.75 | 29.25  | 7.50       |
| Combination dose 3|                 | 16    | 24.25  | 8.25       |

The decreased number of polymorphs is often accompanied by an increased susceptibility to infection. The mechanism of action of eosinophils contains phosphatase and peroxidase enzymes, which play a role in detoxifying bacteria by moving the antigen-antibody complex. Neutrophils are the main component of granulocytes because they have an important role as phagocytosis and are the most abundant in granulocytes. Basophil cells were not found in the blood samples examined, because of the very small percentage of basophils in the blood. MRJP3 has been described as a candidate dominant immunomodulator suppressing cytokine production (especially IL4) from T cells and promoting anti-allergic responses. Flavonoids as antioxidants can increase the number of eosinophils even though they are still within normal limits.

CONCLUSION
A single dose of honey can be an immunomodulator and a combination of 3 doses can be an alternative for further research to become an immunomodulatory supplement product. Further research on the potential of honey and royal jelly directly related to the therapy of patients with COVID-19 is needed to be done.

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