COVID-19 is caused by a coronavirus and was first identified in Wuhan, China. Clinical symptoms of COVID-19 patients show a significant decrease in lymphocytes. Decreased lymphocyte count is a factor that increases severity in COVID-19 disease. COVID-19 patients who died reported a very low number of lymphocytes compared to COVID-19 patients who recovered. Increasing lymphocytes to normal levels again, especially in the initial phases of infection, is one of the keys to healing COVID-19 patients. The immune system can be used as a strategy for COVID-19 treatment. Still, immunomodulators must be given at the right time at the beginning of infection, with the right dose, not to cause deterioration in the patient. Immunomodulator administration in the final stage can cause severity in COVID-19 disease due to increased cytokine storm. Therefore, Immunomodulator screening, which increases lymphocyte counts, is essential for COVID-19 treatment strategies in the initial stage. Lymphocytes are easily isolated from human blood and consist of NK (Natural Killer) cells, T cells (for cell-mediated, adaptive cytotoxic immunity), and B cells (for humoral, antibody-driven adaptive immunity). Lymphocytes can be isolated from the blood and cultured in vitro. Animal intervention studies showed that immunomodulatory substances could be used for immunomodulator screening candidates for COVID-19 in initial stage therapy. Some candidates for active ingredients that can be used as immunomodulators are honey. Honey has been shown to increase mouse T cell proliferation. This systematic review shows that honey can increase lymphocyte proliferation and have an anti-inflammatory function by lowering IL-6 levels in several in vivo studies.

Keywords: COVID-19; COVID-19 Initial Phases Treatment; Honey; Immunomodulatory.
COVID-19 patients who died, the amount of interleukin continued to increase, whereas, in COVID-19 patients who recovered, the amount of IL-6 returned to normal when recovered. An increase in excessive IL-6 in the lungs can be associated with damage to the parenchyma and endanger patients' lives. In addition, with the severity of the disease and a higher comorbidity index, we also associated with higher serum levels of IL-6, and IL-10. This shows that the immune system can be used as a strategy for COVID-19 treatment. Still, the use of immunomodulators must be given at the right time at the beginning of the infection, at the proper dosage so as not to cause worsening to the patient, if given at the final stage when a storm occurs cytokines.

Many studies in vitro and in vivo show that bee products, such as honey, propolis, and royal jelly, can increase lymphocyte proliferation. This systematic review will describe and discuss the advantages and disadvantages of the bee products in overcoming lymphopenia and the factors that play a role.

Hopefully, this study will provide an overview to assess the potential of honey as an immunomodulatory on early COVID-19 patient treatment.

MATERIAL AND METHODS

Search Strategy

Report writing in the form of a systematic review. We identify animal studies on honey intervention. The searches were done from February to June 2021, using keywords with the Boolean method on MEDLINE Pubmed. The searching keywords were done following PICO (Population Intervention Comparison Object). Here are the searching keywords with Boolean searching methods: ((honey) AND (lymphocyte OR T cell OR B cell OR NK cell OR cell-mediated immunity OR adaptive cytotoxic immunity )) AND (in vivo OR animal OR cat OR mouse OR rat OR guinea pig OR primate OR dog OR snail). Another searching strategy was also used, using keywords "in vivo", "honey" in combination with "lymphocyte."

We were also searching from the Garuda portal (Garba Rujukan Digital) with the link http://garuda.ristekbrin.go.id/. Garuda Portal was an Indonesian website from the Indonesian government, who manages all nationally standardized publications throughout Indonesia. The searching keyword were written in Bahasa Indonesia: “madu”, “limfosit”, “in vivo”, and “eksperimen binatang”.

The title and abstracts for eligible studies and excluded duplicates Potential studies underwent full-text screening before inclusion to the data system. Following PRISMA (Preferred Reporting Items for Systematics Reviews and Meta-analysis), the search algorithm was selected and analyzed.7

Eligibility Criteria

The studies included in vivo studies with mice, rats, rabbits, cats, dogs, pigs, cats, snails, and non-human primates. The investigation includes the effect of a honey intervention on lymphocyte proliferation and the immune system (direct and indirect interaction). The intervention could be honey alone or combined with other active substances, with oral administration.

In vitro experiments, clinical studies, review articles, and studies without honey intervention were excluded. In addition, non-original research, conference paper, and research articles written in languages other than English were excluded.

Analyzed Features

For the eligible study, we recorded publication (i.e., journal, authors, date, affiliation details), animal variables (i.e., strain, sex, age, sample size, weight), protocol details (the frequency of delivery, administration time, honey bee species), and outcome measures (lymphocyte proliferation, IL-6 elevation). Focus on the study was identified as elevation of lymphocyte level and immune system.
**Data Collection and Analysis**

Data were extracted and analyzed using Microsoft excel. Descriptive analyses by species, honey bee species are summarized in tables and figures; no hypothesis tests were performed. Studies explicitly determined that species or standard models were based on complete records. The distribution of lymphocyte proliferation and immune system elevation shows density estimates in the table.

**RESULT**

A total of 1267 papers were searched by title and abstract. Of these, 51 studies were included in the data system. After the full-text screening, seven articles were undergone final analysis (Fig.1).

![Flowchart of study selection](image)

**Figure 1.** Flowchart of study selection

Three articles combine honey with active substances from other bee products from these seven articles taken. So, it is rather difficult to measure the effect of honey alone from the three papers. However, it can be seen that the resulting trend has been produced due to honey intervention and its combinations. And can be input for further research as well.

The selected paper (7 articles) years were obtained from 2004 - 2020. These papers are in vivo experiments with various experimental combinations using the honey intervention.
RESULT AND DISCUSSION

Table 1. Animal Variable

| Animal          | Strain     | Sex    | Age            | Weight (gr)  | Sample size/treatment | Ref. |
|-----------------|------------|--------|----------------|--------------|-----------------------|------|
| Quail chicks    | NM         | NM     | NM             | NM           | 8                     | 8    |
| Swiss mice      | NM         | both   | 8-10 weeks     | 28.33±3.44   | 7                     | 9    |
| rats            | Sprague Dawley | NM | 7 weeks        | 250-290      | 6                     | 10   |
| rats            | Wistar     | male   | NM             | 198±111.4    | NM                    | 11   |
| rabbit          | NM         | NM     | NM             | 1000-1300    | 6                     | 12   |
| mice            | BALB/C     | female | 6 weeks        | NM           | 5                     | 13   |
| rat             | Wistar     | male   | 8-12 weeks     | 100-300      | 5                     | 14   |

Note : NM = Not Mention

In vivo, honey intervention experiments were carried out on animals: Quail chicks, mice, rats, and rabbits. Most of the experiments were carried out on rats due to convenience in terms of maintenance and experimenting. Experiments with mice used rats aged seven weeks, equivalent to humans aged 20 years, while the mice used six weeks old. At the age of 6 weeks, the rats have reached the age of young adults and can breed so that the organ system can respond well to the intervention that will be given and for this honey study.

Table 2. Comparison of Methodology

| Administration time of intervention | Combination Intervention | Honey bee species | Dosage | Ref. |
|-------------------------------------|--------------------------|-------------------|--------|------|
| Every day for 42 days, at 21 days were challenging against H9N2, New Castle Disease, and Shee Red Blood Cells. | Propolis, pollen powder, royal jelly, virginiamycin | NM | NM | 8 |
| Honey was given daily during the study | No combination, only honey | NM | Fed daily with honey administration 0.8 g honey/ kg body weight | 9 |
| Regular fed with different dosages of honey administration (4.6 and 9.3 g/ kg of body weight/day) for 30 days | No combination, only honey | Stingless bee (Trigona sp.) from Johor Baru, Malaysia | Honey with different dosage (4.6 g/kg body weight and 9.3 g/kg body weight) | 10 |
| Bee bread was given daily for 28 days | Bee bread (consist of honey and pollen) | NM | Bee bread combined with toxic aluminum chloride with a maximum dosage of 0.75 g/kg body weight | 11 |
| Honey was given one hour before vitamin C, B, and CdCl2 treatment combination. The length of this research is only one day | No combination, only honey | NM | Honey was given 0.09 g/kg body weight | 12 |
| Honey bee larvae were administrated orally for 22 days (the treatment combination with inoculated tumor cells) | Honey bee larvae powder (consist of honey and bee larvae) | NM | Honey bee larvae were given 6 g/kg body weight/day | 13 |
| Honey was given daily with different dosages for 18 days | No combination, only honey | Jungle bee from Kalimantan | Different dosage honey were 0.27 ml/0.2kg, 0.54 ml/0.2 kg, and 1.08 ml/0.2 kg body weight | 14 |

Note : NM = Not Mention
In all studies in Table 2, the honey was administered orally every day without other treatment. However, only one study (to observe honey’s effect on cadmium toxicity) was given 1 hour before cadmium administration. Three articles combine honey with other bee products to examine their inflammatory effects, immune response, and protection against toxins. This shows that other bee products also have a lot of potential as immunomodulators. The combination of other bee products is expected to increase the immunomodulatory effect of honey.

Tabel 3. Research Outcome

| Humoral immune response | Lymphocyte proliferation | IL-6 elevation | Ref. |
|-------------------------|--------------------------|----------------|------|
| -Antibody titer was the highest against H9N2 (Avian Influenza virus). | - Ratio of Heterophil/Lymphocyte was the lowest. | ND | 8 |
| Antibody against New Castle Disease and Sheep Red Blood Cells was an increase compared to control but was not the highest compared to another ingredient | -Honey does not affect lymphocyte proliferation | ND | 8 |
| -Antibody titer was higher than control treatment on honey intervention treatment against Sheep Blood Red Cells and E. coli. | ND | ND | 9 |
| | - Ratio of Neutrophil/Lymphocyte decreases concomitantly with the increase of honey doses. -Honey increase lymphocyte proliferation compared to another study in this study | - The higher of honey doses, the lower of IL-6 | 10 |
| | ND | in terms of neutralizing the toxicity of aluminum, bee bread combined with aluminum did not induce lymphocytes proliferation. | ND | 11 |
| | ND | honey can significantly protect lymphocyte damage from CdCl2 toxicity, such as vitamin C and B | ND | 12 |
| Administration of HLP (Honey Larvae Powder) significantly elevates CD4+ T cells in the peripheral blood to favor tumor suppression | ND | HLP significantly reduced IL-6 production with a maximum inhibition of 36.29% for 125 µg/ml, thus showing anti-inflammatory activities | 13 |
| | ND | Giving honey at a dose of 0.54 ml /0.2 kg body weight significantly affects increasing lymphocyte proliferation on 48-hour measurement. | ND | 14 |

Note: ND = Not Determined

The intervention of honey could increase the formation of antibody titer against New Castle Disease virus antigen in Japanese Quails. Still, the combination with propolis and pollen powder can further increase the immune system compared to honey alone. So were the antibody titers against E. coli in another study. This shows that honey can increase the humoral immune response against viruses and bacteria. Although honey seems to increase the humoral immune response and antibody titers, the addition of other bee products such as propolis and pollen powder should be...
considered considering the results shown in the above article. Furthermore, the addition of bee larvae to honey in the in vivo intervention study can also increase CD4+ cells in response as an antitumor. This shows that many bee products can be combined to get the maximum immunomodulatory effect for maximum treatment.

In the study of Babaei et al., 2016, there was no increase in lymphocytes when only honey was added. But in this study, honey increased antibody titers. This is possible because, in chickens, there are stressors that can reduce lymphocytes and increase heterophils and corticosterone. Another study showed that the stress hormone in chicken is produced due to the infection. This makes choosing a chicken test animal to measure the increase in lymphocytes should be considered carefully.

In the research of Ranneh et al., 2019 and Senas et al., 2012, it is clear that honey intervention increases lymphocyte proliferation in vivo. Both of these studies were conducted in rats and showed the same and significant results. This study shows rats performing intervention studies better for lymphocyte enhancement experiments.

Furthermore, giving honey can protect against lymphocyte damage caused by toxic substances such as CdCl2. This shows that apart from being an immunomodulator, honey also functions to maintain the body's immune system so that it is always balanced. This is possible because honey can act as an antioxidant. Honey contains phenolic acids and alkaloids, which can function as antioxidants and inhibit cell damage due to CdCl2 poisoning.

Research by Ranneh et al., 2019 and Li, 2020 shows that giving honey suppresses the increase in IL-6, a marker of inflammation. This shows that honey can have anti-inflammatory properties. The results of this study indicate that honey has potential as an immunomodulator by increasing lymphocytes and decreasing IL-6 to overcome Lymphopenia in COVID-19 patients without increasing inflammatory markers such as IL-6.

CONCLUSION

The study showed that the intervention of honey could significantly increase lymphocyte proliferation in vivo experimental, especially in the rat. Not only the number of lymphocytes, but honey can also increase antibody titers as a humoral immune response to viruses and bacteria. The intervention of honey in vivo experiments also reduced the amount of IL-6 and had anti-inflammatory properties. In conclusion, honey can treat lymphopenia in the early phase of infection in the patient. Moreover, the studies showed honey could reduce IL-6 and inhibit the severity of the COVID-19. Further experimental studies are needed to evaluate the potential of honey in the management of COVID-19 patients

ACKNOWLEDGEMENT

This work was supported by a Dana Riset Penanggulangan COVID-19 from Universitas Pembangunan Nasional Veteran Jakarta (UPNVJ) 2020.

REFERENCES
1. A. C. et al. Apoptosis-induced T-cell lymphopenia is related to COVID-19 severity. J. Med. Virol. 93, 2867–2874 (2021).
2. Henry, B. M. COVID-19, ECMO, and lymphopenia: a word of caution. The Lancet Respiratory Medicine8, e24 (2020).
3. Yang, X. et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir. Med. (2020). doi:10.1016/S2213-2600(20)30079-5.
4. Saad, N. & Moussa, S. Immune response to COVID-19 infection: a double-edged sword. https://doi.org/10.1080/25785826.2020.1870305 (2021). doi:10.1080/25785826.2020.1870305.
5. Ruan, Q., Yang, K., Wang, W., Jiang, L. & Song, J. Clinical predictors of
mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine (2020). doi:10.1007/s00134-020-05991-x.

6. RL, L. et al. IL-6 and IL-10 are associated with disease severity and higher comorbidity in adults with COVID-19. Cytokine 143, (2021).

7. Moher, D. et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine 6, (2009).

8. Babaei, S., Rahimi, S., Karimi Torshizi, M. A., Tahmasebi, G. & Khaleghi Miran, S. N. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. Vet. Res. forum an Int. Q. J.7, 13–20 (2016).

9. Al-Waili, N. S. & Haq, A. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. J. Med. Food 7, 491–494 (2004).

10. Ranneh, Y. et al. Stingless bee honey protects against lipopolysaccharide induced-chronic subclinical systemic inflammation and oxidative stress by modulating Nrf2, NF-kB, and p38 MAPK. Nutr. Metab. 16, (2019).

11. Bakour, M. et al. Antioxidant activity and protective effect of bee bread (honey and pollen) in aluminum-induced anemia, elevation of inflammatory makers and hepato-renal toxicity. J. Food Sci. Technol.54, 4205–4212 (2017).

12. Abdelaziz, I., Elhabiby, M. I. & Ashour, A. A. Toxicity of cadmium and protective effect of bee honey, vitamins C and B complex. Hum. Exp. Toxicol.32, 362–370 (2013).

13. Li, K. et al. Evaluation of the Immunomodulatory and Anti-Inflammatory Activities of Honey Bee Larva Powder. J. Med. Food23, 772–782 (2020).

14. Senas, K. S. & Linawati, Y. Pengaruh Pemberian Madu Hutan Terhadap Proliferasi Limfosit Pada Hewan Uji Tikus Jantan Galur Wistar. J. Farm. Sains dan Komunitas (Journal Pharm. Sci. Community)9, (2012).

15. Pallav Sengupta. The Laboratory Rat: Relating its Age with Human’s. Int. J. Prev. Med.4, 624–630 (2013).

16. Jackson, S. J. et al. Does age matter? The impact of rodent age on study outcomes. doi:10.1177/0023677216653984.

17. Chan, G. C. F., Cheung, K. W. & Sze, D. M. Y. The immunomodulatory and anticancer properties of propolis. Clinical Reviews in Allergy and Immunology44, 262–273 (2013).

18. Ibrahim, A. A. E.-M. Immunomodulatory effects of royal jelly on aorta CD3, CD68 and eNOS expression in hypercholesterolaemic rats. J. Basic Appl. Zool.67, 140–148 (2014).

19. Scanes, C. G. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poult. Sci.95, 2208–2215 (2016).

20. Khalil, M. I., Alam, N., Moniruzzaman, M., Sulaiman, S. A. & Gan, S. H. Phenolic Acid Composition and Antioxidant Properties of Malaysian Honeys. J. Food Sci.76, (2011).

21. Tanaka, T., Narazaki, M. & Kishimoto, T. IL-6 in inflammation, Immunity, And disease. Cold Spring Harb. Perspect. Biol.6, 16295–16296 (2014).