ORIGINAL ARTICLE

Wanagama honey modulate macrophage phagocytic activity in mice

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ABSTRACT

Introduction: Honey is one of the natural ingredients that have long been used in the community to cure certain diseases and to maintain health. Several studies have shown that honey contains many active ingredients that have pharmacological effects, including immunomodulatory effects. The active compound of honey varies depending on the bee that produces honey and also the food source of the bees. Wanagama honey is honey produced in the forest in Gunung Kidul, Yogyakarta, Indonesia. Despite the use of Wanagama honey to improve immune system, there is no study regarding its immunomodulatory effect. This study aimed to study the immunomodulatory effects of Wanagama forest honey.

Materials and Methods: Twenty Balb c mice divided into 4 different groups received different treatment which were water (CONTROL), honey 10 mg/kg BW (DOSE 1), honey 25 mg/kg BW (DOSE 2), and honey 50 mg/kg BW (DOSE 3). The immunomodulatory effect evaluation was using the carbon clearance assay. Complete blood count and delta body weight were also measured.

Results: Mice in DOSE 3 group showed lowest delta body weight. the complete blood count parameters were not different significantly between groups of treatment. however, the phagocytic index was higher significantly in the groups receiving honey compare to those without honey treatment.

Conclusion: This study showed the potential of Wanagama honey as immunomodulator.

KEYWORDS:
Wanagama honey; immunomodulator; carbon clearance assay; phagocytic index; weight gain

INTRODUCTION

The immune system is the system in the body which responsible to maintain integrity of the body in the face of dangers that can be caused by the environment. Macrophages are the effectors in the immune system that act as pathogens or germs that will damage systems in the body[1], either directly through intracellular phagocytosis or indirectly by releasing Nitric Oxide (NO), Intermediate Reactive Oxygen (ROI) and cytokines.[2]

Immune system can be enhanced with immunomodulators. Immunomodulators are pharmacological agents that can partially modulate the immune response induced by the immune response and on the other hand it inhibits several other immune systems.[3] One of the immunomodulators that have long been used is honey. Honey is a natural product, rich in nutrients, which also has economic and ecological benefits, and has been used in traditional medicine since prehistoric times.[4] A small clinical trial showed that honey administration for 2 weeks resulted in increase of antioxidant agents, increase serum iron, decrease plasma ferritin, increase percentage of blood parameter, and reduce immunoglobulin E.[5] The pharmacological effects and biochemical constituents of various types of honey have been extensively investigated.

The pharmacological activity and biochemical composition of honey is highly dependent on its content, which is largely influenced by bee species, nectar source, geographic origin, and post-harvest processing.[6] Indonesia is a tropical country that has abundant types of honey and honey bees. Apis cerata is one of the native bee species that is widely used for traditional honey production, including Wanagama forest honey. Wanagama forest honey, harvested from Wanagama Forest in Gunungkidul, Yogyakarta. This honey is widely marketed and traditionally consumed by local communities as health supplement to enhance immunity.

Wanagama forest, unlike natural forest, is a rehabilitated, man-made ecosystem established by Universitas Gadjah Mada as an ex-situ conservation and land restoration project. The vegetation is composed of seven distinct main stands-pine (Pinus merkusii), mahogany (Switenia mahagoni), kesambi (Schleichera oleosa), teak (Tectona grandis), cajuput (Melaleuca leucadendron), glicidia (Gliricida sepium), and mixed stands which differ significantly in floral composition, canopy structure, and microclimate. These variations leading to a highly heterogenous floral ecosystem that differs markedly from natural tropical forest.[7]

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The mosaic of introduced and local flowering plants in Wanagama forest from which the Apis cerana forage makes the nectar and pollen composition of Wanagama forest honey is expected to be unique, potentially yielding distinct phytochemical profiles and immunomodulatory effects compared with other honey. However, despite its popularity, no scientific investigation has yet been conducted to evaluate the immunomodulatory activity of Wanagama forest honey. Therefore, this study aims to explore the potential immunomodulatory effect of Wanagama forest honey.

MATERIALS AND METHODS

All the procedures regarding animal study have received approval by ethical committee of Faculty of Medicine, Public health and Nursing Universitas Gadjah Mada with ethical approval number KE/FK/0251/EC/2021

WanagamaHoney

Wanagama honey is honey obtained from Wanagama Forest located in Banaran Village, Playen District, Gunung Kidul Regency It is produced by Apis cerana bees. The honey was harvested by taking honeycomb and then extracted by squeezing.

Chromatography test

Chromatography test were conducted using Gas Chromatography and Mass Spectroscopy (GCMS). For gas chromatography (GC) analysis, the honey sample was diluted with distilled water, followed by liquid–liquid extraction using ethyl acetate to separate volatile compounds. The organic layer was then filtered through anhydrous sodium sulphate to remove residual water, and the filtrate was concentrated under reduced pressure before injection into the GC system.

Carbon suspension preparation

A total of 1.6 g of dried Chinese ink, suspended with 25 mL of tween 80 (1%) w/v in 0.9% physiological NaCl solution, until a solution concentration of 64 mg/mL was obtained.

Carbon clearance assays

The study involved 20 Swiss albino mice age 3 – 4 weeks old. They received different treatment based on their group which are group which receive water or negative control group (CONTROL), group which received honey 10 mg/kg BW (DOSE 1), group which received honey 25 mg/kg BW (DOSE 2), and group which received honey 50 mg/kg BW (DOSE 3). They received the treatment for 14 days. The honey sample was diluted in distilled water and administered perorally to mice at a volume of 0.1 mL per 10 g body weight using an oral gavage needle to ensure uniform delivery. In the end of the treatment, the mice received intravenous injection of 0.1 ml carbon suspension via tail vein. Blood collection through orbital vein was conducted before and 15 minutes after carbon suspension injection. The carbon concentration was measured using spectrophotometer UV-Vis 650 nm. Optical density (OD) measured was used to calculate the phagocytic index using the following formula:

$$(K) = \frac{\text{Log A (n)} - \text{Log A (n-1)}}{\text{t (n-1)} - \text{t (n)}}$$

K = Phagocytic constant
A = optical density
t = time
n = time after carbon injection (15 minutes)
n -1 = time before carbon injection (0 minutes)

$$PI = \frac{\text{K mice X}}{\text{Mean K}}$$

PI = Phagocytic Index
K = Phagocytic constant

Hematological examination

The blood examination was conducted using Automatic Veterinary Hematology Analyzer.

Statistical analysis

The data presented are mean ± Standard Deviation. The mean of carbon clearance assay and blood parameter value were analyzed using ONE-WAY ANOVA and continued with Tukey's multiple comparisons test.

RESULTS

Chromatography test

Table I and Figure 1 presents the results of the GC-MS test on Wanagama honey. GC-MS chromatogram (Figure 1) shows the presences of twenty phytochemical constituents. The content of fatty acids and hydrocarbons is 76.45%, the value of furan compounds and other components is 20.42% and 3.15%, respectively.

Body weight examination

The body weight measurement shows the increase of the mice body weight in all groups except in DOSE 3 group. However, the difference of the body weight before and after treatment between groups was not significantly difference. The change of body weight before and after treatment is presented in Figure 2.

Blood examination

Complete blood count examination also shows that the blood parameters were similar among the group. Table II presents the complete blood count result.

Carbon clearance assay

Phagocytic index calculation shows that all group treated with honey have higher phagocytic index compared to those on negative control group. Figure 3 shows the phagocytic index measured using carbon clearance assay.

DISCUSSION

The GC-MS results show the volatile compounds of honey. The compounds are grouped into furans, hydrocarbons and fatty acids, sugars, and other components. Fatty acids and hydrocarbons dominated in Wanagama honey, followed by furan group compounds. Honey mainly consists of carbohydrates which contribute to more than 90% of its dry weight. Other main compounds of honey include proteins, vitamins, amino acids, minerals, and organic acids. Honey also contains flavonoids, polyphenols, alkaloids, glycosides, and volatile compounds. [8–10]

Table 1. The results of GC-MS test of Wanagama Honey

Ret.Time	Area %	Compound	SI
10.63	3.15	Pyranone	92
13.25	20.42	HMF (hydroxymethylfurfural)	93
24.64	3.07	Myristic acid	95
26.38	0.46	Pentadecanoic acid isomer	95
27.89	14.24	cis-9-Hexadecenoic acid	96
28.24	27.07	cis-11-Eicosenoic acid	93
30.99	0.76	9-Hexadecenoic acid	88
31.06	0.89	cis-Vaccenic acid	88
31.32	0.52	Aqua Cera	88
36.01	1.83	Eicosane	95
36.93	0.35	Triphenylphosphanoxid	84
37.32	0.26	Tetracosane	87
38.59	13.22	Heptacosane	96
39.79	0.45	Octacosane	89
40.97	6.86	Tetratetracontane	96
41.34	0.41	13-Methylheptacosane	79
46.44	0.64	Palmitaldehyde	81
47.09	4.24	9-Eicosyne	88
47.36	0.65	2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	76
47.48	0.53	Tetratriacontane	76

Table 2. Complete Blood Count Result after 7 days treatment. There was no statistical different among CBC parameter measured between groups.

Parameter	Control	Dose 1	Dose 2	Dose 3
RBC	9.52	9.09	9.18	9.27
Hb	14.85	14.48	14.52	14.62
Ht	49.23	47.68	49.05	48.66
MCV	51.77	52.53	53.53	54.98
MCH	15.60	15.92	15.95	16.40
MCHC	30.17	30.37	29.85	29.95
RDW	16.77	16.42	16.70	16.63
PLT	1210.67	1143.00	1162.00	1171.89
MPV	4.55	4.43	4.72	4.55
PDW	15.70	15.75	15.90	15.87
PCT	0.51	0.51	0.55	0.57
WBC	2.12	2.10	1.87	2.03
Granulocyte	26.30	40.34	16.30	20.08
Limphocyte (%)	63.60	47.46	73.78	69.45
Monocyte (%)	10.10	12.20	9.92	10.47
Granulocyte (0.29-1.42)	0.72	1.20	0.30	0.32
Limphocyte (0.49-3.92)	1.18	0.94	1.37	1.13
Monocyte (0.0-0.08)	0.22	0.30	0.17	0.15

RBC (Red Blood Cell), Hb (Haemoglobin), Ht (Haematocrite), MCV (Mean Corpuscular Volume), MCH (mean Corpuscular Hemoglobin), MCHC (mean Corpuscular Hemoglobin Concentration), RDW (Red Cell Distribution Width), PLT (Platelet), MPV (mean Platelet Volume), PCT (Platelecrit), WBC (white Blood cell)

Although, volatile compounds are generally low in concentration, approximately 600 volatile compounds have been reported to exhibit pharmacological effect.[11] The volatile compound found in the highest percentage in Wanagama honey, cis-11-Eicosenoic acid, has been known to act as an immune system stimulator.[12] Another major volatile compound, cis-9-Hexadecenoic acid, possessess antiinflammtory properties.[13] Based on these findings, the chemical profile of Wanagama honey supports its potential immunomodulatory activity.

This study examined the potential of Wanagama honey as immunomodulator using carbon clearance assay in mice, where the phagocytic index was determined. The phagocytic index evaluates the capacity of reticular endothelial systems (RES), a systemic network of phagocytic cells, to clear exogen materials from systemic circulation. The injected carbon suspensionis cleared cleared by macrophage, and a higher phagocytic index reflects better RES function.[14] In this study, Wanagama honey modulated macrophage phagocytic activity in vivo at all tested doses (10 mg/kg BW, 25 mg/kg BW, and 50 mg/kg BW).

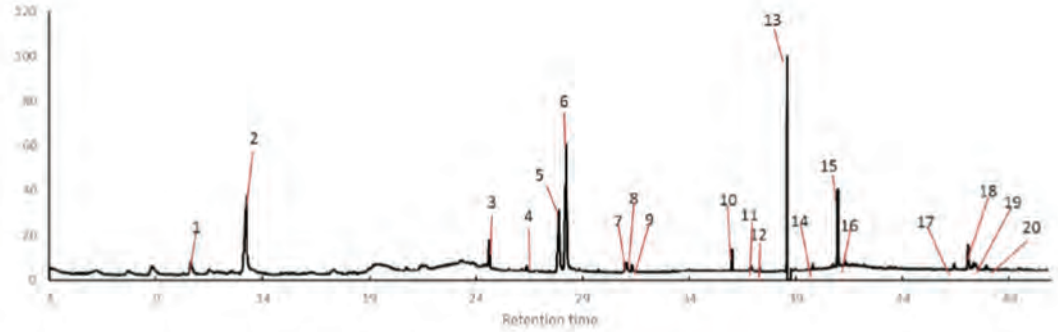

Figure 1. GC-MS result of Wanagama honey

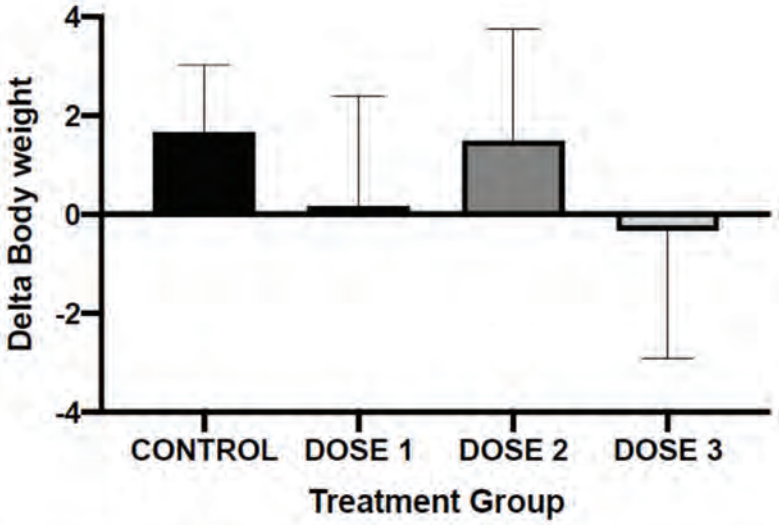

Figure 2. Difference in body weight before and after treatment with honey in different concentration. There was no statistical different of weight change between groups.

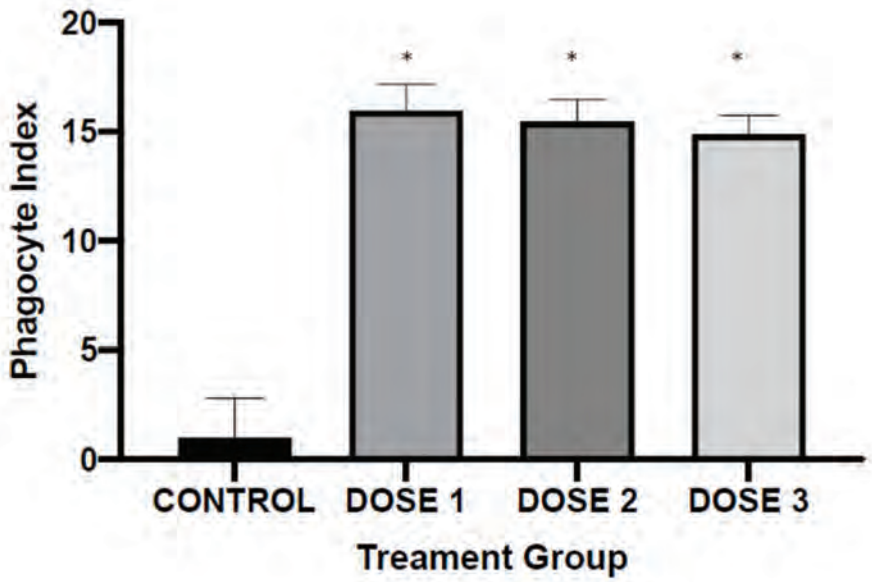

Figure 3. Phagocyte Index measured by carbon clearance assay. * P < 0.05 vs control

A previous study in rat showed that 14 days administration of honey increased several cytokines including cytokines IL-4and IL-10.[15] Ripened honey has high concentration of glucose and fructose and low water content, which prevents the spoilage.[16] Stimulation of cytokines release by honey is partly atributed to H_2O_2 production from glucose oxidation catalyzed by honeybee glucose oxidase (Gox). Low concentration of H_2O_2 is known to stimulate immune responses, while honey also serves as energy substrate for macrophages, enhancing their phagocytic performance.[16-17] furthermore, the sugar content of honey can be metabolized into short chain fatty acids (SCFA), which also possess immunomodulatory properties.[18]

Honey also contains non sugar components with antioxidant effects. The immune system uses reactive oxygen species (ROS) to eliminate pathogens, however, excessive ROS can lead to oxidative stress, impairing immune function and causing tissue damage. Antioxidant compounds in honey may therefore support immune balance by mitigating oxidative stress.[19-20]

In this study, the effect of wanagama forest honey administration on carbon clearance was not showing clear dose response relationship. According to lawa of mass action all pharmacological effect is inherently concentration dependent; however, in practice, some responses may appear non–dose-related when the dose–response curve reaches a plateau at relatively low doses.[21] In our study, the lower and medium doses of Wanagama honey might have been sufficient to achieve near-maximal macrophage activation, such that higher doses produced no additional effect. This saturation or plateau effect is common in immunomodulatory studies where receptor occupancy or cytokine signaling reaches maximal efficiency at submaximal concentrations.

Honey administration in several different doses also potentiated the several hematological parameters such as Hb, RBC, PCV, lymphocytes, and eosinophils in rat model of breast cancer. In our study, all of the blood parameters measured were not significantly different with those on CONTROL group. We suggest that it is due to the different animal model, dose and length of honey treatment. In study by Ahmed et al., 2018[17], the honey administration is higher, 0.2-2g/kg BW, and longer, >120 days. In our study, the honey dose was 10-50 mg/kg BW and the duration of treatment was 14 days.

The absence of significant differences in leukocyte, lymphocyte, or monocyte counts after 7 days of honey administration also suggests that the immunomodulatory effect occurs at the functional level rather than through hematopoietic stimulation. We suggest that Wanagama forest honey modulate immune cell function—such as phagocytosis and cytokine release—without necessarily changing circulating cell numbers.

Avoiding consumption of high-sugar foods often discourages honey use despite its health benefits. However, in this study, mean body weight did not differ significantly between groups. Interestingly, mice in the highest dose group (50 mg/kg BW) showed the lowest or even negative weight gain. This finding aligns with Nemoseck et al. (2011), who reported that after 33 days of honey treatment, rats exhibited reduced weight gain and adiposity, possibly due to decreased food intake compared with sucrose-fed controls.[22]

CONCLUSIONS

This study demonstrated that Wanagama forest honey, produced by Apis cerana in a unique rehabilitated forest ecosystem, contains bioactive volatile compounds with potential immunomodulatory properties. In vivo findings showed enhanced macrophage phagocytic activity at all tested doses, supporting its role as a natural immunomodulator. Further studies with longer duration and positive controls are recommended to confirm its mechanism and therapeutic potential.

CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

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