Honey improves lipid profile of diet-induced hypercholesterolemic rats

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ABSTRACT

BACKGROUND

Coronary heart disease (CHD) is a major cause of morbidity and mortality throughout the world, including Indonesia. One of the risk factors for CHD is hypercholesterolemia. One of the natural products that has been developed for the treatment of hypercholesterolemia is honey. Honey contains fructooligosaccharides, various vitamins, minerals, and enzymes which are supposedly able to lower blood cholesterol levels. This research aimed to study the influence of honey on the levels of blood total cholesterol, triglyceride, and low density lipoprotein (LDL) levels in Wistar rats.

METHODS

This study was of experimental post test control group design. Twenty-four male Wistar rats (*Rattus norvegicus*) were randomly divided into 4 groups. K1 was the negative control group (with normal diet), K2 was the positive control group (with high-fat diet), P1 was fed a high-fat diet for 7 days, followed by high-fat diet plus honey for the next 7 days. P2 was fed a high-fat diet for 7 days, followed by regular diet plus honey for the next 7 days. After completion of this treatment, total cholesterol, triglycerides, and LDL levels were measured by the cholesterol oxidase phenol+aminophenazone (CHOD-PAP) method using enzymatic spectrophotometry principles.

RESULTS

There were significant differences in total cholesterol, triglyceride, and LDL levels between all groups after day 15 (p<0.05).

CONCLUSION

Honey supplementation was able to reduce the blood levels of total cholesterol, triglycerides, and LDL. Honey supplementation accompanied by non-cholesterol feeds could more effectively lower total cholesterol, triglycerides, and LDL serum levels in Wistar rats.

Keywords: Cholesterol, triglycerides, LDL, honey, hypercholesterolemia, rats

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Madu memperbaiki profil lipid pada tikus yang diinduksi diet hiperkolesterolemia

ABSTRAK

LATAR BELAKANG

Penyakit jantung koroner (PJK) merupakan penyebab utama kesakitan dan kematian di seluruh dunia, termasuk Indonesia. Salah satu faktor risiko PJK adalah hiperkolesterolemia. Salah satu bahan alami yang banyak dikembangkan dalam pengobatan adalah madu. Madu mengandung fruktooligoakarida, berbagai vitamin, mineral, dan enzim yang diduga dapat menurunkan kadar kolesterol dalam darah. Penelitian ini bertujuan untuk mempelajari pengaruh madu terhadap kadar kolesterol total, trigliserida, dan LDL darah.

METODE

Penelitian ini merupakan penelitian eksperimental menggunakan post test control group design. Dua puluh empat ekor tikus Wistar (Rattus norvegicus) jantan dibagi secara acak dalam 4 kelompok. Kelompok K1 adalah kontrol negatif (diberi pakan biasa), K2 adalah kontrol positif (diberi pakan tinggi lemak), P1 adalah kelompok yang diberi pakan tinggi lemak selama 7 hari dilanjutkan pakan tinggi lemak ditambah madu selama 7 hari berikutnya, dan P2 adalah kelompok yang diberi pakan tinggi lemak selama 7 hari dilanjutkan pakan biasa ditambah madu selama 7 hari berikutnya. Setelah selesai perlakuan, darah diambil untuk diperiksa kadar kolesterol total, trigliserid, dan LDL menggunakan metode CHOD-PAP dengan prinsip spektrofotometri enzimatis.

HASIL

Terdapat perbedaan bermakna kadar kolesterol, trigliserida, dan LDL setelah hari ke-15 antara keempat kelompok ($p < 0,05$).

KESIMPULAN

Pemberian suplementasi madu dapat menurunkan kadar kolesterol total, trigliserida, dan LDL dalam darah pada tikus. Pemberian suplementasi madu disertai dengan pemberian pakan non-kolesterol dapat menurunkan kadar kolesterol total, trigliserida, dan LDL dalam darah secara lebih efektif.

Kata kunci*: Kolesterol, trigliserida, LDL, madu, hiperkolesterolemia,tikus*

INTRODUCTION

Coronary heart disease (CHD) is a major cause of morbidity and mortality throughout the world, including Indonesia. During the last fifty years, the number of CHD patients has continued to increase, with sudden death as final outcome.[1-4] The increased incidence of CHD is associated with increased CHD risk factors such as dyslipidemia, obesity, hypertension, smoking habit, diabetes mellitus, and other risk factors related to lifestyle. In order to decrease CHD cases, the risk factors should be controlled. As the increased levels of blood cholesterol is also a risk factor for CHD, a decrease in total cholesterol levels, especially reduced levels of low-density lipoprotein (LDL), is one of the therapeutic targets to prevent and decrease the incidence of CHD.[1,3,5]

The American College of Cardiology and the American Heart Association (ACC/AHA) have issued guidelines for cholesterol therapy (also known as Cholesterol Treatment Guidelines, CTG). The main treatment for hypercholesterolemia is by the use of statins. Statins act to lower blood cholesterol levels by

inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase.[6] Several other substances used to lower cholesterol, are among others bile-acid sequestrants (inhibiting the absorption of bile acids), vitamin E, and gemfibrozil. So far, the use of statins is the most effective strategy to lower blood LDL levels.[1] Statins can lower blood cholesterol in more than 30% of patients with hypercholesterolemia. However, the use of statins and other anti-cholesterol drugs, especially in the long term, might cause permanent side effects, such as those associated with gastrointestinal symptoms, skin rash, anxiety disorders, and hepatic dysfunction.[7,8] Therefore, controlling risk factors by improving lifestyle and performing early monitoring is the best measure to prevent CHD caused by hypercholesterolemia.

Various studies on the benefits of honey have been performed. Honey is widely used empirically for its anti-inflammatory, antibacterial, antifungal, antiviral, antihypertensive, antioxidant, cardioprotective, hypoglycemic and other actions.[3,9,10] However, scientific evidence supporting honey as a medicine still needs to be developed to optimize its utilization. Various literature reports mention that honey contains a complete array of nutrients and has the potential to be developed into a medication. Various vitamins, minerals, fructo-oligosaccharides, polyphenols, amino acids, and enzymes contained in honey are expected to be useful for lowering blood cholesterol levels.

This research aimed to study the influence of honey on the levels of total cholesterol, triglycerides, and LDL in the blood of diet-induced hypercholesterolemic Wistar rats (*Rattus norvegicus*).

METHODS

Research design

This was an experimental laboratory research using post test control group design. The study took place in the Physiology Laboratory, Faculty of Medicine, Islamic University of Indonesia. The study was conducted from January to May 2010.

Animals

A total of twenty four male Wistar rats (*Rattus norvegicus*) aged 15 weeks, weighing 200-250 grams, were obtained from the animal house at the Integrated Research and Testing Laboraory *(Laboratorium Penelitian dan Pengujian Terpadu,* LPPT*)*, Gadjah Mada University, Yogyakarta. The number of rats needed was determined based on the 3Rs principle (replacement, refinement, and reduction) and was calculated using the following equation:

$$E = N\text{-}T$$

where: E = a constant between 10-20, N = planned number of animals per group multiplied by number of treatment groups, T = number of treatment groups.

This study used 4 groups. If N or the number of animals per group = 6, then E = (6x4) -4, therefore E=20, which is still included in the specified range of values between 10-20. Thus, the total number of rats was 24 and each group contained 6 rats.[11] Before the treatment was performed, the rats were isolated for an adaptation stage of 7 days. They were placed in cages with regulated indoor light intensity, consisting of 12 hours light and 12 hours dark periods. During this stage, feed and water were given ad libitum.

Treatment

Rats were randomly assigned into four groups, namely the negative control group (K1), the positive control group (K2), treatment group 1 (P1), and treatment group 2 (P2). Each group of six rats was assigned randomly. Group K1 was given regular feed from the beginning to the end of the study. Group K2 was given a high-fat diet for 14 days. Group P1 was given high-fat feeds for 7 days, followed by high-fat feeds plus honey for the next 7 days. Group P2 was given high-fat feeds for 7 days, followed by regular feed plus honey for the next 7 days. All rats were weighed daily to determine changes in body weight from day to day.

Preparation of honey

Honey from one local honey store was chosen to be used in the study. Based on the printed label of the packaging, the honey used for this study contained glucose, fructose, sucrose, amino acids, zinc, anti-oxidant, potassium, phosporus, iron, vitamins (B1, B2), and some enzymes. The honey was given daily by gavage at a dose of 0.8 mL/day. The dose of honey for the rats was obtained by multiplying the usual dose of honey for daily human consumption by a conversion factor. The usual dose for human consumption as printed on the packaging and empirically considered adequate to reduce cholesterol was 2 tablespoons (30 mL) each day. After multiplying the dose for humans by the conversion factor of man to rat (0.018), a dose of 0.8 mL/day was obtained. Each dose of honey was dissolved in water to a volume of 2 ml. The honey used in this experiment belonged to the *kapok* tree type of honey, which had been commercialized with a specific brand.

Preparation of high-fat diet

High-fat feed was prepared by mixing the regular feed with white butter at a ratio of 5:1, i.e. 5 parts of feed (20 g/day/rat) was mixed with 1 part white butter (4 grams/day). The dose of white butter was obtained from a preliminary study. White butter was chosen in this study on the basis of economic and accessability aspects. The feed and butter were evenly mixed and then placed in a feed container in each cage, to be consumed by the rats. The amount of feed consumed was determined daily by measuring the feed left in the container.

Biochemical analyses

Blood sampling of the rats was performed three times, i.e. before treatment, on day 7 of treatment, and on day 15, after completion of the treatment. Blood was taken at the retro-orbital plexus using 2 ml hematocrit pipettes. The blood samples were taken to the Laboratory of Nutrition, Central Inter-University Laboratory, Gadjah Mada University, to check the levels of total cholesterol, triglycerides, and low density cholesterol (LDL) using the cholesterol oxidase phenol + aminophenazone (CHOD-PAP) method, based on the principles of enzymatic spectrophotometry. The results were expressed in mg/dL.

Statistical analysis

The data on total cholesterol, triglycerides, LDL, and HDL obtained from laboratory tests were analyzed using statistical software and presented as mean ± SD. The distribution of the obtained numerical data were also examined using Shapiro Wilk test followed by Levene test in order to determine the homogeneity of the data. If the data were distibuted normally, then analysis of variance (ANOVA) test was used, and Kruskall Wallis test was used if the data were not normally distributed. Differences were considered significant when the probability value was less than <0.05 ($p < 0.05$).

Ethical clearance

This study was approved by the Ethical Commision of the Islamic University of Indonesia.

RESULTS

Before treatment, blood sampling was performed for all groups in order to measure the initial levels of total cholesterol, triglycerides, and LDL. The mean levels of total cholesterol, triglycerides, and LDL are presented in Table 1. The data analysis began with the Shapiro Wilk normality test and homogenity test using Levene test. The Shapiro Wilk test for cholesterol, triglycerides, and LDL indicated that the data had a normal distribution. Therefore, one way Anova test for cholesterol, triglyceride, and LDL was applied. There were no siginificant differences between groups before treatment ($p > 0.05$). The statistical analysis indicated that the subjects were in similar condition before treatment.

During the first seven days, K1 rats were given standard feed and water ad libitum, while

Table 1. Mean total cholesterol, triglyceride, and LDL levels by treatment groups at base line

Group	K1	K2	P1	P2	p
Cholesterol (mg/dL)	104.78 ± 1.06	109.43 ± 1.68	111.29 ± 1.80	112.48 ± 1.61	0.062[a]
Triglycerides (mg/dL)	64.09 ± 2.91	75.49 ± 2.70	70.34 ± 3.57	67.52 ± 3.88	0.314[a]
LDL (mg/dL)	13.27 ± 0.52	15.15 ± 1.27	17.71 ± 1.21	17.86 ± 1.32	0.074[a]

K1: standard feed control group, K2: high fat feed control group, P1: high fat feeds for 7 days followed by high-fat feed plus honey for the next 7 days, P2: high-fat feeds for 7 days followed by regular feed plus honey for the next 7 days. [a] Anova test

K2, P1, and P2 rats were treated with high-fat feed and water ad libitum. On day 7, blood sampling was again performed. Mean total cholesterol, triglyceride and LDL levels on day 7 are shown in Table 2.

One way Anova test results for cholesterol on day 7 showed significant differences between groups (p=0.000). LSD post hoc test showed significant differences (p <0.05) between groups K1 in comparison with groups K2, P1 and P2. The results of one way Anova test for triglyceride levels on day 7 showed significant differences between groups (p=0.000). LSD post hoc test showed significant differences (p<0.05) between group K1 in comparison with groups K2, P1 and P2. Levels of LDL on day 7 also showed significant differences between groups (p=0.008) using Kruskal Wallis test (Table 2). Thus, the administration of high-fat feeds in groups K2, P1 and P2 led to significantly increased levels of cholesterol, triglycerides, and LDL compared with the negative control group (group K1).

The results for day 14 indicated that the levels of cholesterol, and triglycerides, and LDL showed significant differences between groups (p=0.000; p=0.000; and p=0.001) (Table 3).

In this study, total cholesterol, triglycerides, and LDL levels were highest in group K2 (the group receiving high-fat feeds without honey). This showed that the induction with high-fat feeding in rats led to an increase in the levels of total cholesterol, triglycerides, and LDL.

DISCUSSION

The induction with high-fat feeds causes an increase in cholesterol synthesis and eventual accumulation of blood cholesterol (hypercholesterolemia). Increase in cholesterol synthesis is facilitated by the enzyme HMGCoA-reductase. Tomkin and Owens [12] state that the decrease in cholesterol synthesis resulting from inhibition of HMGCoA-reductase enzyme activity reduces LDL levels and vice versa. HMGCoA-reductase inhibitory activity is shown by statins used in the treatment of hypercholesterolemia. If the cholesterol level in the blood increases, LDL as a carrier of cholesterol to cells throughout the body will also increase. Furthermore, the accumulation of cholesterol followed by free radical activity causes oxidative damage to various tissues. In addition, LDL is a substance that is readily oxidized. Oxidized LDL binds to macrophages and subsequently forms foam cells to cause atherosclerotic lesions. The hypercholesterolemic

Table 2. Mean total cholesterol, triglyceride, and LDL levels
by treatment groups after 7 days of treatment

Group	K1	K2	P1	P2	p
Cholesterol (mg/dL)	106.24 ± 1.14	217.93 ± 2.70	215.54 ± 2.31	213.95 ± 1.77	0.000[a]
Triglycerides (mg/dL)	65.56 ± 2.81	124.99 ± 2.41	123.42 ± 2.06	121.41 ± 1.78	0.000[a]
LDL (mg/dL)	3.00	11.50	15.30	12.20	0.008[b]

K1: standard feed control group, K2: high fat feed control group, P1: high fat feeds for 7 days followed by high-fat feed plus honey for the next 7 days, P2: high-fat feeds for 7 days followed by regular feed plus honey for the next 7 days; [a] Anova test; [b] Kruskall Wallis test

Table 3. Mean total cholesterol, triglyceride, and LDL levels by treatment groups
after 14 days of treatment

Group	K1	K2	P1	P2	p
Cholesterol (mg/dL)	107.54 ± 1.17	257.80 ± 2.53	152.91 ± 0.70	96.03 ± 0.72	0.000[a]
Triglycerides (mg/dL)	66.91 ± 2.69	140.47 ± 1.20	101.72 ± 0.88	57.75 ± 0.82	0.000[a]
LDL (mg/dL)	4.80	18.00	13.00	6.20	0.001[b]

K1: standard feed control group, K2: high fat feed control group, P1: high fat feeds for 7 days followed by high-fat feed plus honey for the next 7 days, P2: high-fat feeds for 7 days followed by regular feed plus honey for the next 7 days; [a]Anova test; [b]Kruskall Wallis test

condition can lead to atherosclerosis, resulting in complications such as myocardial infarction and stroke.[12,13]

Uncontrolled hypercholesterolemia can develop into cardiovascular disease. Hypercholesterolemia is strongly associated with high total cholesterol and LDL levels in the blood. High levels of total cholesterol and LDL in the blood attract attention because they are correlated with the incidence of coronary heart disease.[1,3] Cardiovascular disease, such as coronary heart disease, is one of the leading causes of worldwide death. Mortality and morbidity due to cardiovascular disease are aggravated by a variety of risk factors, such as: minimal physical activity and exercise, smoking, poor dietary habits (diets high in fat and low in fiber), alcoholism, and others.[14,15] Several studies were developed to address the conditions of hypercholesterolemia and maintain cholesterol homeostasis. Cholesterol homeostasis is maintained via a complex arrangement in terms of absorption, anabolism, catabolism, and excretion.[3,7,14]

According to previous studies, cholesterol levels in the blood may be reduced through several mechanisms. First, through competition with intestinal Niemann Pick C1-like 1 (NPC1L1). Around 1200-1700 mg of cholesterol enters the lumen of the small intestine every day. Approximately 300-500 mg of the cholesterol comes from the diet and the rest comes from bile acids. Cholesterol absorption in the small intestine is mediated by NPC1L1. Once absorbed, cholesterol is transported by NPC1L1 from the lumen of the small intestine into the enterocytes.

Cholesterol is then converted into cholesteryl ester (CE) by intestinal acyl-CoA cholesterol acyltransferase 2 (ACAT2). In addition, the CE will be packaged into chylomicrons by microsomal triacylglycerol transport protein (MTP). Eventually, chylomicrons head into the circulatory system in the form of very-low density lipoprotein (VLDL). Next, VLDL is degraded forming LDL to deliver cholesterol to cells throughout the body. The addition of substances that are structurally similar to the NPC1L1 may reduce the levels of cholesterol and LDL.[7,12,16]

The second mechanism is inhibition of intestinal acyl-CoA cholesterol acyltransferase 2 (ACAT2). In mammals, ACAT2 is important for lipid esterification in enterocytes and the liver to produce cholesteryl ester which is useful for the formation of chylomicrons and VLDL. Inhibition of ACAT2 may lower total cholesterol and LDL. The third mechanism is inhibition of 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase, which is required in the biosynthesis of cholesterol. Thus, inhibition of HMG-CoA reductase may inhibit the cholesterol synthesis pathway so that the levels of cholesterol and LDL in the blood are reduced.[7,12,16]

The fourth mechanism is activation of LDL receptors. Increasing the number and activity (up-regulation) of LDL receptors may improve the cholesterol-LDL clearance from the circulation. Therefore, activation of the receptors may reduce the levels of blood cholesterol and LDL. The fifth mechanism is inhibition of bile acid reabsorption. This is done through the binding of bile acids in the intestine by certain substances to form insoluble complex compounds that can not be

reabsorbed into the liver and are eventually excreted. Inhibition of bile acid reabsorption lowers cholesterol levels and increases the hepatic synthesis of bile acids from cholesterol. The increase in hepatic bile acid synthesis triggers blood cholesterol influx into the liver. This is also caused by LDL receptor activation due to decreased hepatic cholesterol levels. Thus, cholesterol levels and LDL in the blood will decrease.[7,12,16]

The sixth mechanism is activation of cytochrome P450 7A1 (CYP7A1) or cholesterol-7á-hydroxylase. Activation (up-regulation) of CYP7A1 is theoretically able to lower the cholesterol in the liver, causing blood cholesterol influx, and ultimately lowering cholesterol levels in the blood.[7,12,16]

The last mechanism is inhibition of plasma cholesteryl ester transporting protein (CETP). CETP inhibition could be expected to lower blood cholesterol and LDL levels because the transportation of cholesteryl ester as a raw material for making chylomicrons is inhibited.[7,12,16]

Research on the mechanisms of cholesterol-lowering therapy is important to determine the substances and the working points which are expected to lower total cholesterol and LDL levels in the blood. One substance that is widely studied as cholesterol-lowering agent is honey. Studies on honey as an anti-hypercholesterolemia agent has been widely developed, in both humans and experimental animals with varying results. In the present study, administration of honey in group P1 and P2 resulted in significantly lower cholesterol, triglycerides, and LDL levels in comparison with those in group K2. Group P2 (the group receiving high-fat feeds followed by normal feed plus honey) showed lower results compared with group P1 (the group receiving high-fat feeds followed by high-fat feeds plus honey). In fact, total cholesterol, triglycerides, and LDL levels of rats in group P2 were almost identical with those in group K1 (the negative control group receiving regular feed up to the end of treatment).

Based on the data analysis perfomed in this study, honey was proven to be able to reduce the levels of cholesterol, triglycerides, and LDL. This is in line with previous studies conducted on various experimental animals. Nemoseck et al.[17] stated that honey has many advantages that would be promising in the world of health. One of the benefits of honey that is described in their study was to lose weight and lower blood triglyceride levels in SD strain rats. In contrast, the other lipid profile parameters were increased or were not significantly different from those of the positive control group or the group given additional sucrose diet.

In addition, Alagwu et al.[13] suggest that the administration of pure Nigerian honey can reduce levels of blood LDL and total cholesterol in rats. However, Alagwu's research showed elevated levels of triglycerides and VLDL in the blood.

Adnan et al.[1] also suggest that honey from the acacia tree (*desi kikar*) may lower the levels of triglycerides, total cholesterol, and LDL in diet-induced hypercholesterolemic albino rats, as well as in the group treated with simvastatin. The group treated with a combination of acacia honey and simvastatin showed greater reductions in lipid profile, approaching the values in the negative control group.

Research on the effects of different variants of honey on the lipid profile has also been conducted in humans. A research study by Mushtaq et al.[18] mentions that the consumption of 40 grams of honey was shown to improve lipid profiles in obese adult subjects. Yaghoobi et al.[19] also found that 70 grams of pure honey daily for 30 days was proven to reduce cardiovascular disease risk factors and did not cause weight gain in overweight or obese subjects. In addition, studies on healthy young subjects with similar dose and duration (70 grams daily for 4 weeks) also showed a decrease in total cholesterol, triglycerides, and LDL compared with the control group.[20]

Honey is a nutrient-rich food that is produced mainly by the honeybee (*Apis mellifera* L.). Honey has been used by humans since

ancient times. Various literature reports mention that honey has antibacterial, antifungal, antiviral, anti-inflammatory, antihypertensive, antioxidant, antitumor, cardioprotective, hepatoprotective, and hypoglycemic effects. Honey contains much carbohydrate (glucose and fructose—85% content of honey, sucrose, maltose, isomaltose, maltulose, and others), vitamins (thiamine, riboflavin, pyridoxine, vitamin A, niacin, pantothenic acid, vitamin E, vitamin C), trace elements and minerals (aluminum, arsenic, lithium, sulfur, iodine, cobalt, sodium, calcium, potassium, magnesium, phosphorus, zinc, copper, iron, selenium, manganese, and others), polyphenols, organic acids, amino acids (proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine, isoleucine, and others), enzymes (glucose oxidase, invertase, amylase, catalase, and acid phosphatase), polyphenols (phenolic acids, flavonoids, phenolic acid derivatives [quercetin, chrysin, galangin, luteolin, kaempferol, apigenin, and others]), nitric oxide (NO), and other substances. The composition of the substances contained in honey is influenced by the type of crop, climate, and environmental conditions.[10,15,21]

The hypotriglyceridemic effects of honey may be caused by fermented nondigestible-oligosaccharides (NDOs), such as fructooligosaccharides (FOS) or other similar carbohydrate isomaltulose which are contained in honey. Nondigestible-oligosaccharides (NDOs) are not hydrolyzed in the small intestine, but are degraded by microflora in the colon. In the colon, the degradation process will produce short chain fatty acids that will affect the normal flora in the colon. The normal flora of the colon is important and has a beneficial effect on lipid metabolism.[17] Polysaccharides that can not be digested and short chain fatty acids resulting from the fermentation process by the normal flora will be able to reduce the levels of LDL cholesterol in the blood by inhibiting HMG-CoA reductase [7] and bile acid reabsorption into the liver.[3,7,9,22] In addition, the normal flora species which are beneficial and present in sufficient numbers in the colon, such as *Lactobacillus,*

Bifidobacterium, Enterococcus, and *Streptococcus,* will be able to help lower blood cholesterol levels by inhibiting the reabsorption of bile acids into the liver.[7,22] Bogdanov et al.[9] mention that honey can increase *Lactobacillus* in the small intestine and colon.

The content of antioxidants in honey is also predicted to have a beneficial effect on lipid metabolism. Nevertheless, the content of antioxidants in honey varies greatly depending on the type of plant and its environment. Honey with darker colors have higher antioxidant content than the honey with a lighter color.[1,17] According to Chen et al.,[7] antioxidant compounds, such as quercetin, kaempferol, galangin, and various other flavonoid compounds, are able to help lowering LDL and cholesterol levels of blood. The antioxidant compounds work by inhibiting ACAT2, activating (up-regulating) LDL receptors, and activating CYP7A1.

Chepulis and Starkey [23] found that although there was an increase in HDL-cholesterol in honey-fed rats compared with rats fed sucrose or a sugar free diet, but there were no other differences in lipid profiles. Nemoseck et al.,[13] Alagwu et al.[17] and Majid et al.[20] also suggest that the supplementation of honey will increase the excretion of cholesterol through bile acids thereby lowering blood cholesterol levels. In addition to antioxidants, honey also contains niacin. The content of niacin in honey could be expected to lower blood cholesterol by inhibiting the mobilization of deposits of triglycerides from adipose cells and through inhibition of diacylglycerol acyltransferase enzymes in liver cells that are involved in cholesterol synthesis in the HMG-CoA reductase pathway.[13,18,20] Yaghoobi et al.[19] suggest that antioxidants in honey, in addition to their role in lowering blood cholesterol and LDL levels, are also advantageous by inhibiting the formation of atherogenic plaques. Inhibition of atherogenic plaque formation is also determined by a variety of trace elements, minerals, vitamins, and NO contained in honey. Some trace elements that prevent the formation of atherogenic plaques are

copper and zinc. Meanwhile, some of the vitamins contained in honey playing a role in preventing oxidative stress and atherosclerotic plaque formation are vitamin E (niacin), vitamin C, vitamin B, and vitamin A.[20,24]

Honey is a food supplement with a complete array of substances, of which some are potentially of use as antihypercholesterolemic agents. Honey contains several amino acid components.[15] According to Chen et al.,[7] protein has the ability to lower of blood cholesterol and LDL cholesterol levels by inhibiting the reabsorption of bile acids, inhibiting HMG-CoA reductase and activating LDL receptors. In addition, honey also contains calcium. Supplementation of calcium in the diet is beneficial to lower cholesterol levels by inhibiting intestinal NPC1L1, activating CYP7A1, and increasing bile acid excretion via feces.[7,25] Meanwhile, there are still many substances in honey which have not been scientifically proven to be beneficial in lowering blood cholesterol levels.

In our study, the group treated with honey followed by a normal diet had the lowest levels of cholesterol, triglycerides, and LDL among all treatment groups. Moreover, the level was almost identical with that in the negative control group. This indicates that in addition to adequate therapy with antihypercholesterolemia agents, dietary modification also has a positive effect on the prevention of cardiovascular disease.[7,14,22]

This study had a limitation in the dose of honey that should be given to rats. Further studies should use a number of honey doses. The results of this study may be useful to researchers and clinicians as a reference in the use of honey supplementation for those who are dyslipidemic.

CONCLUSIONS

Honey supplementation can reduce levels of total cholesterol, triglycerides, and LDL in the blood. Supplementation of honey accompanied by a non-cholesterol diet can more effectively lower total cholesterol, triglycerides, and LDL in the blood.

CONFLICT OF INTEREST

There was no conflict of interest with other institutions.

ACKNOWLEDGEMENT

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