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Exploring the Role of Propolis on Lipid Profile of Wistar Rats with Induced Dyslipidemia

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Obesity has now become a global epidemic with a high annual death rate. In many cases, it is accompanied by dyslipidemia, which may further result in complications. To alleviate dyslipidemia, various alternative complementary therapies have come to light, with propolis being one of them. However, the research on propolis effects on dyslipidemia is limited. Therefore, in this study, we aimed to analyze the impact of propolis supplementation in modulating lipid metabolism in high-fat diet (HFD)-model rats to explore its potential as an alternative complementary treatment for HFD-induced obesity in the near future. To measure lipid profile, blood was collected from the tail of male , separated into four groups, and underwent 12 weeks of obesity induction, continued by propolis supplementation 300 mg/kg for 4 weeks, then analyzed for lipid profile after the serum is separated. Using calorimetric assay, samples are mixed with Diasys reagent and run through Tecan Infinite M200 at a wavelength between 546 and 500 nm. The results showed that propolis supplementation at a dose of 300 mg/kg for 4 weeks effectively modulates the lipid profile of HFD-induced dyslipidemia. This was portrayed by a significant decrease in Total Cholesterol (TC) level ($p = 0.001$) and Triglyceride level ($p = 0.01$), accompanied by a rise in HDL in the HFDP group ($p = 0.001$). To summarize, propolis may serve as a highly potential alternative complementary treatment for obesity and metabolic syndrome through pathways involved in dyslipidemia.

Keywords: Dyslipidemia; High-fat diet; Lipid profile; Metabolism; Propolis.

Obesity is the accumulation of fat in the body exceeding the suggested normal range, usually in body fat percentage, due to an imbalance of energy intake and expenditure.[1–3] World Health Organization (WHO) stated that the incidences of obesity between 1975 – 2016 have nearly tripled, with more than 1.9 billion adults 18 years and above listed as overweight and over 650 million classified as obese.[1] In Indonesia, research conducted by Riset Kesehatan Dasar (Riskesdas) in 2018 showed a rise in overweight percentage from 8.6% to 13.6% and obese from 10.5% to 21.8% between 2007 and2018.[4] This proves that obesity needs better handling on its root cause to end this epidemic.[1]

Obesity leads to a hyperlipidemic state, which is a condition of fat excess reflected by the increased level of total cholesterol (TC), fasting triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) with decreased high-density lipoprotein (HDL).[5,6] The underlying microenvironment disruption in hyperlipidemia will lead to more changes, including microvascular dysfunction that might as well lead to macrovascular disease.[7] As a result, many complications may arise, such as atherosclerosis, emboli formation, cardiovascular disease, or even stroke.[6,8]

As the condition of cholesterol and other associated lipid markers have been known for more than two centuries, countless attempts have been made to create drugs to alleviate dyslipidemia.[9–11] Statins, fibrates, bile acid sequestrants, and even novel drugs are being developed to modulate lipid profiles by targeting different pathways in lipid metabolism. However, as effective as they are right now, chemical drugs come with each of their own risk for adverse effects, including hepatic dysfunction and myopathy.[9,10] Therefore, there are herbal medicines that are potentially used as an alternative therapy and it is coming to light, including propolis.[12–21]

Propolis is a sticky substance collected from the beehive, created from gathered plant resin and exudates by the bees. It, therefore, has wax, resin, essential oils, balsam, and plant metabolites (phenolics, amino acids, vitamins, terpenoids, tannins, and alkaloids) as its constituents.[21] Propolis has been gaining attention as more research has been done about its effect on various physiologic pathways. For instance, studies have reported its role as an antioxidant, anti-inflammatory, and transcription factor modulator.[20,22-25] Since obesity is accompanied by multiple adaptations of how the body performs, each role plays a significant part in ameliorating obesity and the metabolic disturbances that result from it.[19,20,222–29]

Despite having a lot of therapeutic benefits, there are limited studies on the effectiveness of propolis extracts on modulating lipid profile in rats induced by high-fat diet (HFD). Hence, this study explores the role of propolis in regulating lipid metabolism as a potential treatment for HFD-induced obesity in the near future.

MATERIAL AND METHODS

This was done in the Animal Science Laboratory, Sekolah Pasca Sarjana, and Central Laboratory, Universitas Padjadjaran, West Java, Indonesia.

Animal Selection

In vivo, an animal study with healthy male Wistar rats 8 weeks of age weighing 220 – 250 grams as subjects were used and were divided into four groups with four rats each, which are control population with standard chow diet(control, CD group), population with standard chow diet and propolis supplementation (CDP group), obesity population (HFD group), and obesity population with propolis supplementation (HFDP group). The HFD feed and propolis used in this study is identical to the one utilized in a previously published study.[30]

The rats were placed in rooms under a 12-h light-dark cycle with a temperature of approximately 22-24°C and allowed access to water ad libitum. The CD groups were given a chow diet containing 65.5% carbohydrates, 25% protein, 7% fat, roughly 5% micronutrients, and minerals. In comparison, the HFD groups were given a standardized high-fat diet containing a total fat content of 99.5%, consisting of 70-80% palmitic acid (Optima 100) for its fat content.[30] Propolis was administered via gastric lavage at 300 mg/kg daily for the CDP and HFDP groups.[31]

Before being given determined treatment for each group, rats underwent an adaptation period of 3 months to induce obesity in HFD groups. Rats with no weight changes during the adaptation period, present hair fall before treatment, or other illnesses that may alter experiment results are excluded. Primary data were collected using a random sampling method to give every rat in the population group the same chance of being selected and examined, hence resulting in data that could represent the whole population.

Blood Analysis

After 4 weeks of propolis supplementation, at the age of rats are 24 weeks, venous blood was taken from the rat's tail and measured using a Diasys reagent kit for TC (ref. 1 1350 99 10 021), TG (ref. 1 5760 99 10 021), HDL (ref. 1 3540 99 90 885) level in calorimetric assay which is done

according to manufacturer instructions, continued by spectrophotometry by Tecan Infinite M2000 that were recorded at a wavelength between 546 and 500 nm to measure the color changes.

Data Analysis

Data collected from this study is analyzed according to descriptive statistics and processed using IBM® SPSS® Statistics 25. Data is later presented in the following results graphs.

RESULTS

Cholesterol

The effect of propolis on cholesterol levels in Wistar rats given CD and HFD is presented in Figure 1. In general, the administration of HFD results in a higher level of cholesterol in comparison to CD. After 4 weeks of propolis supplementation, the HFDP group showed a significant decrease in cholesterol level compared to the HFD group,

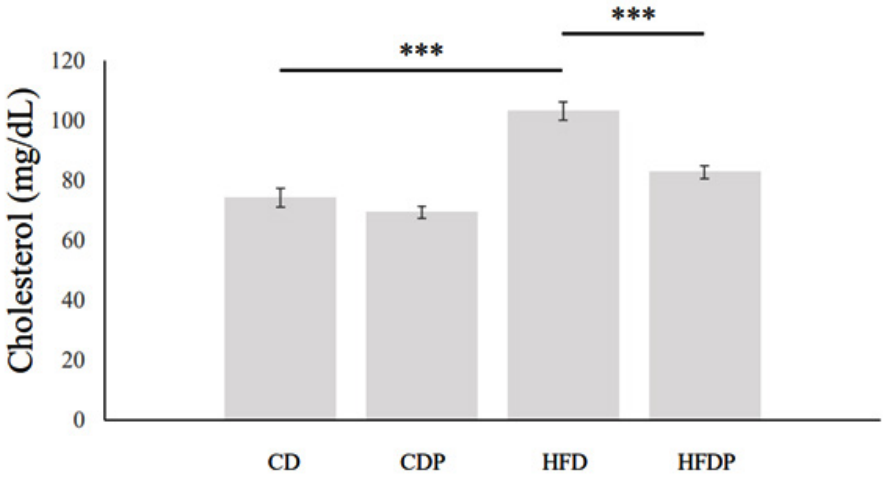

Fig. 1. Effect of 4 weeks propolis supplementation on cholesterol level. Wistar rats were given propolis at 300 mg/kg once daily by gastric lavage. TC level is calculated according to the standard in calorimetric assay. Data presented as Mean ± SD. *: Significant difference between groups (*** $p \leq 0.001$)

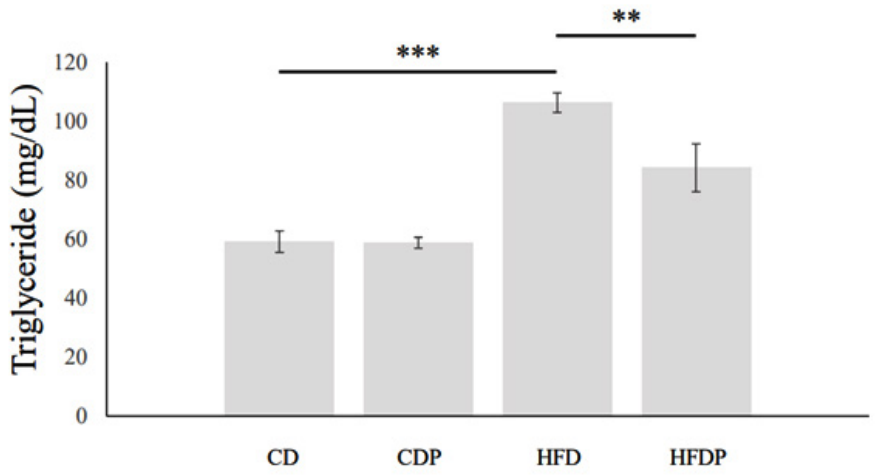

Fig. 2. Effect of 4 weeks propolis supplementation on triglyceride level. Wistar rats were given propolis at 300 mg/kg once daily by gastric lavage. TG level is calculated according to the standard in calorimetric assay. Data presented as Mean ± SD. *: Significant difference between groups (** $p \leq 0.01$, *** $p \leq 0.001$).

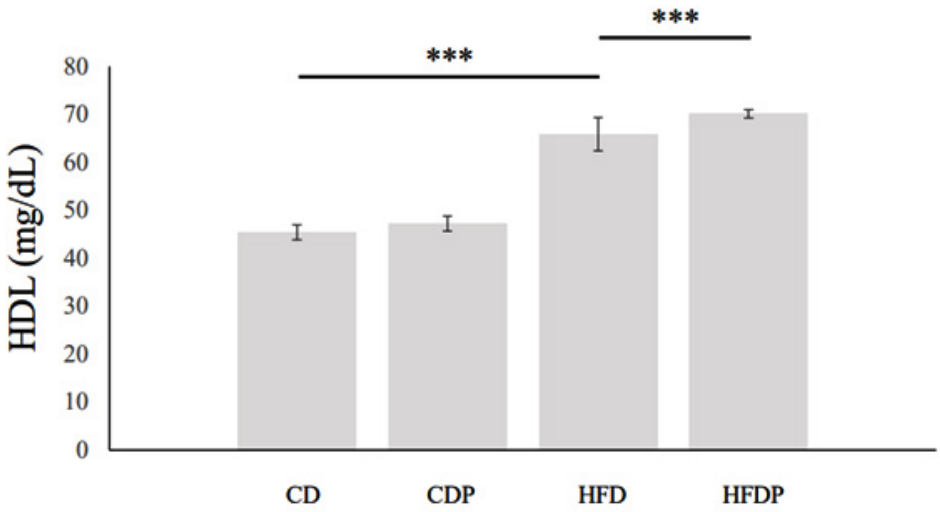

Fig. 3. Effect of 4 weeks propolis supplementation on HDL level. Wistar rats were given propolis at 300 mg/kg once daily by gastric lavage. HDL level is calculated according to the standard in calorimetric assay. Data presented as Mean ± SD. *: Significant difference between groups (*** $p \leq 0.001$)

which is not treated with propolis. This reduction is more significant than that of the groups given CD.

Triglyceride

Similar to the results in TC level, TG level after 4 weeks of propolis supplementation in Wistar rats shows a significant decrease in the HFDP group compared to the HFD group. However, this steep difference is not seen in groups given CD. The effect of propolis supplementation on TG level is seen in Figure 2.

HDL

To measure HDL level, the blood of Wistar rats is collected after 4 weeks of propolis supplementation. HFD significantly elevated HDL levels in comparison to groups given CD. Supplementation of propolis for 4 weeks shows a slight increase of HDL in CDP compared to the CD group, while a significant rise is noted from HFDP compared to the HFD group.

DISCUSSION

Obesity induction by HFD for 3 months showed a successful result, as proven by the significant increase of TC, TG, and HDL in the HFD group compared to the CD group. This may occur due to several underlying pathways, one of which is high saturated fat content that influences the decrease of LDL clearance by tampering with LDL receptor and stimulating lipogenic gene expression via the expression of proliferator-activated receptor gamma coactivator 1 beta (PGC-1â), sterol responsive element binding protein 1a (SREBP1a), and sterol responsive element binding protein 1c (SREBP1c), which together increase the level of circulating triglycerides and cholesterol in VLDL particles.[32] At the same time, there may also be disturbances of molecular pathways that are involved in lipid metabolism, such as upregulation of adipose triglyceride lipase (ATGL) along with suppression of hormone-sensitive lipase (HSL) and AMP-activated protein kinase (AMPK).[33] Therefore, an increase in blood cholesterol and triglyceride concentration is possible due to a diet high in saturated fats.[32,34] Meanwhile, HDL levels also increase as a response to a high amount of saturated fats by increasing HDL-cholesterol (HDL-C) and apolipoprotein A-1 (Apo A-I) levels, involving the role of ATP binding cassette transporter A1 (ABCA1), which is also increased in another study using HFD.[35,36]

After a successful induction, propolis supplementation was administered for 4 weeks. At the end of week 4, we found that the cholesterol and triglyceride levels decreased significantly

in the HFDP group. This result is similar to findings in research conducted by Oršoliæ and colleagues, also by Zheng and colleagues, which may involve propolis properties concerning several underlying mechanisms.[20,37] As mentioned in previous studies, in obesity, adipokines, and hormone synthesis are increased, consistent with the amount of adipose tissue present.[38] This leads to a low-grade systemic inflammatory state due to a decrease of adiponectin alongside excessive secretion of tumor necrosis factor-alpha (TNF-á), interleukin-1-beta (IL-1â), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and activation of other pro-inflammatory mediators.[38–41] In addition, increased free fatty acid (FFA) from hydrolysis of TG within adipocytes may also increase oxidative stress by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and further dysregulate the production of proinflammatory cytokines. Propolis may also ameliorate insulin sensitivity and dyslipidemia as an anti-inflammatory agent by suppressing the proinflammatory cytokines.[19,24, 28,37] However, in groups given CD, this series of inflammatory mediators dysregulation has not yet occurred, explaining the insignificant reduction in TC and TG levels as the propolis anti-inflammatory effect does not occur. Propolis may also act as an antioxidant by preventing further aggravation of inflammation through reduction of ROS level so that the activation of protein kinase c (PKC), c-JUN n-terminal kinase (JNK), or nuclear factor kappa B (NF-êB) does not take place.[20,22,39,442] Aside from the two, propolis is also a potent transcription factor regulator proven by its ability to interrupt cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and CREB-regulated transcriptional coactivator 2 (CRTC2) interaction in a study conducted by Chen and colleagues. CREB works by binding to a cAMP-responsive DNA enhancer element (CRE) either in its non-phosphorylated state, which is weaker, or in a phosphorylated state, which results in a more potent transcriptional activity.[25] High levels of hepatic CRTC2 in humans are revealed to be associated with cholesterol synthesis, while liver-specific knockout of CRTC2 liver-specific knockout (CRTC2-KO) mice showed an improvement in energy metabolism.[443] Hence, CREB/CRTC2 complex may act as a regulator of glucose and lipid metabolism.[44–46] One of the bioactive components in Brazilian propolis, artepillin C (APC), which acts as one of the potential agents to inhibit CREB-CRTC2 interaction, also reduces lipid synthesis through modulation of the SREBP family.[25] This was proved by the remarkable decline in mRNA levels of hepatic SREBP1a, 1c, and 2, along with other target genes involved in cholesterol and FFA synthesis.[25,44,45] In another study conducted by Nishikawa and colleagues, a consistent finding regarding the effect of APC on lipid homeostasis was also seen, this time from induction of brown-like adipocyte formation.[26] Brown adipose tissue (BAT) is a specialized adipose tissue present alongside white adipose tissue (WAT) in mammals. While WAT stores excess energy as triglycerides, BAT releases excess energy through thermogenesis and, by such means,suppresses body weight gain and related metabolic disorders.[34,47] Brown-like or beige adipocytes are one of the two types of brown adipocytes, another one being classical brown adipocytes.[47] Brown-like adipocytes develop from myogenic factor 5-negative precursor. They are also known for being inducible brown adipocytes since their development can respond to chronic cold, b3-adrenergic receptor, or peroxisome proliferator-activated receptor-gamma (PPARã).[48,49] Despite all that, PPARã agonist drugs, which were previously tested to treat obesity, showed that they indeed induced the formation of brown-like adipocytes but also act strongly in white adipocytes and cause body fat accumulation.[26] However, Nishikawa and colleagues displayed a remarkable result of propolis APC-induced brown-like adipocyte formation by acting as PPARã direct ligand and stabilizing PRD1-BF-1-RIZ1 homologous domain-containing protein-16 (PRDM16), which is essential in the process.[26,48] In addition, propolis also upregulates the expression of thermogenin or uncoupling protein 1 (UCP1) in mice inguinal WAT, which allows mitochondrial protons to flow back into the matrix and dissipate energy through heat production instead of ATP formation.[26,34,49] Another study by Berbée and colleagues supported this finding through another experiment, which shows a positive relation between the browning of adipose tissue and a decrease in plasma TC and TG levels. Possible pathways may include selective uptake of fatty acid from TG-rich lipoproteins into BAT and accelerating cholesterol-enriched

remnants hepatic clearance through activation of BAT.[51] Therefore, if APC is also present as a component of propolis in this study, it may exert its effect on regulating lipid homeostasis.[25,26,49,50]

In HDL measurement, propolis is effective in elevating HDL levels in the HFDP group compared to the HFD-only group. Similar to how HDL increased due to HFD administration, propolis is suggested to induce liver expressions of ABCA1 and ATP binding cassette transporter G1 (ABCG1) via induction of PPARã and liver X receptor (LXR) pathway. This was also confirmed in a study conducted by Yu and colleagues. Hence, cholesterol efflux and HDL formation are made possible.[51] Another study also showed that this increase in ABCA1 expression might occur not only in the liver but also in macrophages.[52] Aside from this pathway, propolis antioxidant properties may also play a role. This could be achieved through inhibition of the NF-êB signaling pathway, enhancement of reactive oxygen species (ROS) scavenging ability, and activation of nuclear factor erythroid 2–related factor 2 (NrF2) regulatory protein, which is associated with propolis antioxidant properties through elevation of antioxidant enzymes that are important in glutathione metabolism.[20,27,553] Active components of propolis that may execute this pathway include caffeic acid phenethyl ester (CAPE), cinnamic acid, and pinocembrin.[24,27,53]

CONCLUSION

In summary, this in vivo study shows that propolis successfully decreases TC and TG levels while increasing HDL levels in dyslipidemia, which is induced by customized standardized HFD containing 70-80% palmitic acid earlier on. Thus, propolis, which has abundant beneficial properties, may serve as a potential alternative treatment for obesity and metabolic syndromes when given at an appropriate dose.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research follows the ethical guidelines for Universitas Padjadjaran Animal Laboratory Guide and Care Protocol, which is aligned with agreement about animal welfare that consists of 3R (replacement, reduction, refinement) and 5F (freedom from hunger and thirst, discomfort, pain, injury, and disease, fear, and distress, also the freedom to express normal behavior). Ethical permission for this study was granted by the Ethics Committee of Universitas Padjadjaran, which has a number of 822/UN6.KEP/EC/2022.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable**.**

Authors' Contribution

Putri Teesa Radhiyanti: Conceptualization, Analysis, Writing – Review & Editing, Funding Acquisition; Kezia Immanuela: Data Collection, Analysis, Writing – Original Draft; Hanna Goenawan: Analysis, Resources, Writing – Review & Editing; Nova Sylviana: Resources, Visualization, Project Administration; Ronny Lesmana: Conceptualization, Methodology, Supervision; Siti Nur Fatimah: Conceptualization, Methodology, Analysis; Felix Zulhendri: Conceptualization, Supervision, Resources

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