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Research Article

Propolis effectiveness on the reduction of blood glucose level and improvement of body weight in diabetic model's rat

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ABSTRACT

Propolis is an herbal medicinal plant that contains caffeic acid phenethyl ester. This natural polyphenol compound acts as an antioxidant and can reduce blood glucose levels and increase body weight. This study aims to determine the effectiveness of Gunung Lawu propolis in lowering blood glucose levels and increasing body weight in diabetic rats. The Wistar rats were induced with STZ 45 mg/kg BW and nicotinamide 110 mg/kg BW as diabetic model rats. Research subjects were taken randomly and divided into five groups: (1) Normal, (2) DM with no propolis, (3) DM 14 days + propolis 100 mg/kg BW/day (P1), (4) DM 14 days + Propolis 200 mg/kg BW/day (P2), and (5) DM 0 day + Propolis 200 mg/kg BW/day (P3). Propolis extract was given orally once a day for 14 consecutive days (in groups 3 and 4) and 28 consecutive days (in group 5). Blood glucose levels in the treatment group (P1, P2, and P3) decreased significantly (P1: 115.28 ± 4.7 mg/dL; P2: 98.36 ± 4.8 mg/dL; and P3: 87.36 ± 4, 2 mg/dL) compared with that in the DM group (272.07 ± 3.9 mg/dL). The body weight of the treatment group (P1, P2, and P3) increased significantly (P1: 180.60 ± 5.7 g; P2: 180.60 ± 4.2 g; and P3: 208.00 ± 5.1 gr) compared with that in the DM group (160.00 ± 3.2 gr). In conclusion, Propolis is effective in reducing blood glucose levels and improving (increasing) body weight in diabetic model rats.

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INTRODUCTION

Diabetes mellitus (DM) is one of the non-communicable and chronic diseases often found globally, with the number of cases continuing to increase. According to the World Health Organization data, people with DM were around 346 million in 2011 worldwide and are expected to increase to 438 million in 2030 (World Health Organization, 2016). According to the International Diabetes Federation (2021), almost 537 million people worldwide have diabetes. The IDF shows a projection that in 2030 DM prevalence will be 643 million and continue to increase up to 784 million in 2045. Indonesia ranks fourth in the population with a high prevalence of DM after India, China, and America respectively (Jasmani, 2016; IDF, 2021).

DM is one of the top 10 leading causes of death worldwide. A total of 1,5 million people in the world in 2019 died from diabetes (WHO, 2021; Khazael et al, 2023). DM is a metabolic disease when the pancreas does not produce enough insulin, or the body's metabolism is ineffective at using it to its full potential. Hyperglycemia is the effect of uncontrolled diabetes that can damage the body system (Salsabila Z, 2022). Type 2 DM is the most common (90%–95%), characterized by relative insulin resistance and deficiency or insulin secretion defects (Dennedy et al., 2016). In DM conditions with defects in insulin secretion, glucose transport to cells and muscle and fat tissues is disrupted, so energy is obtained via lipolysis and glycogenolysis in the body. Consequently, muscle mass and fat tissue decrease, and body weight (BW) decreases (Rias and Sutikno, 2017).

Propolis is a honeybee product that is widely used to cure several diseases, including DM. It is a natural product derived from plant resins collected by bees. Propolis contains more than 300 chemical compounds. Phenolic compounds were propolis samples' most commonly found compounds (Woźniak et al., 2019). Caffeic acid phenethyl ester (CAPE) has several beneficial biological activities, such as antioxidant, anti-inflammatory, antiviral, antiproliferative, neuroprotective, hepatoprotective, and cardioprotective, and has anti-inflammatory effects (Jia et al., 2019). The CAPE content in Gunung Lawu (Indonesia) propolis extract was $30.24 \pm 3.53 \times 10^{-6}$ g, and quercetin, $4.42 \pm 0.50 \times 10^{-6}$ g (Sarsono et al., 2012). The antioxidant mechanism of Propolis is by way of phenol compounds by giving hydrogen ions to free radicals to protect cells from oxidation reactions. Propolis can protect DNA, lipids, and proteins from damage caused by free radical compounds (Anjum et al., 2019). Research on Propolis from several countries, such as Morroco and Iran, has been conducted with the results that Propolis has hypoglycemic activity (Menyiy et al., 2019; Rivera et al., 2018; Zakerkish et al., 2019).

The composition of Propolis varies and depends on geographical conditions such as plant type, climate, and environmental conditions (Menyiy et al., 2021). This study used Indonesian Propolis from Gunung Lawu, whose CAPE content was $30.24 \pm 3.53 \times 10^{-6}$ g. Still, the other country was 0.63 ± 0.09 mg/mL (with 70% ethanol solvent) and 0.56 ± 0.08 mg/mL (with 96% ethanol solvent) (Sarsono et al., 2012; Woźniak et al. 2019).

This study aims to determine the effect of Gunung Lawu propolis on reducing blood sugar levels and improving BW in diabetic rats.

METHODS

Ethical Approval

This research was conducted based on the Ethics Committee Approval issued by the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada Yogyakarta, with no KE/FK/0560/EC/2020 dated May 11, 2020.

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Experimental design

The research design is an experimental laboratory with a post-test control group design. The research was conducted at the Food and Nutrition Laboratory of PAU UGM. The study was conducted between January and August 2021.

Propolis Extraction

The ethanolic extract of Gunung Lawu propolis was obtained from beekeepers in Kerjo District, Karanganyar, Surakarta, Indonesia. The ethanolic extract of Gunung Lawu propolis was obtained from the maceration and evaporation processes. At the maceration stage, the Propolis was cleaned, blended, and weighed (500 g). Then, a beaker with 70% alcohol solvent was entered (Woźniak et al., 2019). This mixture of ingredients was stored for 7 days by shaking or stirring with a stirring spatula twice a day. This material was then filtered with filter paper and a Buchner funnel to obtain the filtrate. In the evaporation stage, the filtrate was evaporated with a rotary evaporator at 45°C, vacuum pressure (<1 atm) for 4 h, producing a thick propolis extract of ±100 g, evaporated for 24 h in a beaker so that the ethanol evaporated.

Experimental animal and treatment

The study subjects were 25 male white rats, *Rattus novergicus* Wistar strain, aged 2–3 months, had a BW of 200–300 g, and were healthy. The Wistar rats were induced with STZ 45 mg/kg BW and nicotinamide 110 mg/kg BW as a diabetic model (Kottaisamy et al., 2021). Propolis extract given to rats at a dose of 100 mg/kg BW/day and 200 mg/kg BW/day was administered orally with a gastric probe once a day for 14 consecutive days (in groups 3 and 4) and 28 days in a row (in groups 5). Research subjects were taken randomly and divided into five groups: (1) Normal (negative control) (2) DM with no propolis (positive control) (3) (P1) DM 14 days + Propolis 100 mg/kg BW/day, (4) (P2) DM 14 days + Propolis 200 mg/kg BW/day, and (5) (P3) DM 0 day + propolis 200 mg/kg BW/day. The BWs were measured every day. We divided P1 and P2 groups to know the effect of Propolis in chronic hyperglycemia (DM with complication) and P3 in acute hyperglycemia (early DM with no complication).

Blood glucose measurement

Blood glucose level measurement with GOD-PAP (glucose–oxidase–peroxidase aminoantypirin). Glucose is oxidized by glucose oxidase to glucuronic acid and hydrogen peroxide, which, in conjunction with peroxidase, react with choro-4-phenol and 4-amino-antipyrine to form red quinoneimine. The absorbance of the color complex, proportional to the concentration of glucose in the specimen, is measured using a spectrophotometer at 500 nm.

Statistical analysis

The data (blood and BW) were collected in the D28 only. Data were analyzed using Shapiro Wilk to know the distributed normality and compare the mean blood glucose level and body weight between groups using One-way ANOVA with a significant difference $p < 0.05$.

RESULTS

As shown in Table 1, using the One Way ANOVA, there was a significant difference ($p < 0.01$) both in blood glucose level (BGL) and BW in between groups. The BGL in the standard group was normal (75.07 ± 2.56 mg/dL), while the DM group had a glucose level >250 mg/dL (272.07 ± 3.92 mg/dL). Some researchers said that BGL > 250 mg/dL in animal models indicates diabetes disease (El-Gohary and Said, 2016). In the treatment group (P1, P2, and P3), glucose level was decreased significantly (P1: 115.28 ± 4.7 mg/dL; P2: 98.36 ± 4.8 mg/dL; and P3: 87.36 ± 4, 2 mg/dL)

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compared with that in the DM group (272.07 ± 3.9 mg/dL). Normal group and P3 had similar BGL. It was the best treatment because, in this group (P3), Propolis was given immediately as diabetes status with an effective dose of 200 mg/kg BW/day. While acute hyperglycemia (early DM with no complication), Propolis effectively decreased BGL. In P1 and P2 groups (chronic hyperglycemia/ DM complication), the diabetes status was 14 days long, which could damage the body's organs and decrease the BGL, making it less effective than group P3. Propolis in P2 was more effective than P1. It means Propolis with doses 200 mg/ kgBW is better than 100 mg/kgBW/day. It can be concluded that 200 mg/kg BW/day propolis was more effective than 100 mg/kg BW/day for decreased BGL in hyperglycemia acute and chronic in DM. Moreover, Propolis given immediately in critical hyperglycemia conditions to diabetic model rats is a more effective treatment than the 14-day diabetes (chronic hyperglycemia) status for decreased BGL.

Table 2 that used One Way ANOVA shows the BW in the normal group wasnormal (272.07 ± 3.92 gr) as an inclusion criterion (200–300 g). Compared with the normal group, the BW decreased in the DM group (160.00 ± 3.24 gr). In the treatment group (P1, P2, and P3), the BW was increased significantly (P1: 180.60 ± 5.7 g; P2: 180.60 ± 4.2 g; P3: 208.00 ± 5.1 g) compared with that in the DM group (160.00 ± 3.2 g).

The treatment group P3 had BW similar to that in the control group. It was the best treatment because, in this group, Propolis was given immediately as diabetes status (acute hyperglycemia) with an effective dose of 200 mg/kg BW/day. In the treatment groups P1 and P2, the diabetes status was 14 days long (chronic hyperglycemia/ DM complication). This caused lipolysis and glycogenolysis to decrease BW, thus making them less effective in improving (increasing) BW. P2 was more effective than group P1. It can be concluded that propolis 200 mg/kg BW/day was more effective than 100 mg/kg BW/day for increased BW. Furthermore, Propolis given immediately to diabetic model rats (acute hyperglycemia/ no complication DM) was a more effective treatment than the 14-day diabetic status (chronic hyperglycemia / DM complication) for increased BW.

Table 1. Comparison of mean blood glucose levels (mg/dL) in sample groups

Group	N	Mean ± SD	p
Normal	5	75.07 ± 2.56	0.001*
DM	5	272.07 ± 3.92	
P1	5	115.28 ± 5.75	
P2	5	98.36 ± 4.78	
P3	5	87.36 ± 4.21	

*) significant differences at 1%.

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Table 2. Comparison of mean body weights (g) in sample groups

Group	N	Mean ± SD	p
Normal	5	219.40 ± 4.88	0.001*
DM	5	160.00 ± 3.24	
P1	5	180.60 ± 5.73	
P2	5	180.60 ± 4.16	
P3	5	208.00 ± 5.10	

*) significant differences, 1%.

DISCUSSION

This study showed that Gunung Lawu propolis effectively decreased BGL and improved (increased) BW in diabetic model rats significantly. The 200 mg/kg BW/day propolis is more effective than 100 mg/kg BW/day in acute and chronic hyperglycemia for DM state. Moreover, Propolis given immediately in acute hyperglycemia conditions to diabetic model rats is a more effective treatment than chronic hyperglycemia status for decreased BGL and increased BW.

Diabetes patients are unable to metabolize glucose efficiently and cannot synthesize fatty acids and triglycerides from carbohydrates or amino acids due to insulin secretion or action failure. Since the cell cannot detect and absorb glucose in the blood, enzymes in the glycolytic, lipogenic, and pentose phosphate pathways are suppressed, while gluconeogenic, glycogenolytic, and lipolytic activities are elevated (Dillworth et al., 2021).

Hyperglycemia is the effect of uncontrolled diabetes that can damage the body system (Salsabila Z, 2022). This is responsible for glucose auto-oxidation, nonenzymatic protein glycation, and polyol pathway activation. Accumulation of advanced glycation end products can increase oxidative stress (Dillworth, 2021). Adipose tissue participates in body weight homeostasis and glucose and lipid metabolism (Garcia et al., 2020). In hyperglycemia conditions in DM, glucose transport to cells and muscle and fat tissues is disrupted, so energy is obtained via lipolysis and glycogenolysis in the body.

Consequently, muscle mass and fat tissue decrease, and body weight (BW) decreases (Rias and Sutikno, 2017). Besides this, weight loss might be caused by osmotic diuresis under high blood glucose levels. Weight loss had a higher risk of developing diabetes complications. The chronic hyperglycemic condition alters membrane permeability to cations and transmembrane potential. Due to constant oxidative stress in diabetic cells, hyperpolarization is responsible for the long-term complications of diabetes. Maintenance weight loss is associated with decreased complications of diabetes. So, it is necessary to strengthen the management and prevention of complications in patients DM with weight loss (Yang et al, 2016; Dillworth et al, 2021; Tomah S, 2022).

Propolis, as a preventive and curative therapy, can decrease hyperglycemia in DM. Phenolic compounds in Propolis, such as CAPE, have beneficial biological activities, such as having hypoglycemic effects in DM. The results of this study are like studies on Propolis from several countries, such as Morocco and Iran. In these countries, Propolis has hypoglycemic activity (El Menyiy et al., 2019; Rivera et al., 2018; Zakerkish et al., 2019). The studies showed that Propolis has decreased BGL. It has been

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suggested that the glycemic control achieved might be due to the reduction of the intestinal absorption of carbohydrates, increase in the level of glycolysis and utilization of glucose in the liver, glucose uptake by peripheral tissues like skeletal muscle cells by activating an insulin-sensitive glucose transporter, and inhibition of its release in circulation from the liver. Propolis extract has more potent inhibitory effects on α-glycosidase and intestinal sucrase when compared with synthetic α-glycosidase inhibitors such as acarbose. Additionally, propolis inhibition of glucose production from dietary carbohydrates strongly suggested the use of Propolis for controlling or delaying the postprandial glucose increase and improving insulin resistance (Zakerkish et al., 2014; Morales et al., 2022).

Studies in animal and cellular models have also indicated that Propolis manages oxidative stress, the accumulation of advanced glycation end products, and adipose tissue inflammation, all of which contribute to insulin resistance or defects in insulin secretion (Kitamura, 2019). The antioxidant mechanism of Propolis is by way of phenol compounds by giving hydrogen ions to free radicals to protect cells from oxidation reactions. Propolis can protect DNA, lipids, and proteins from damage due to free radical compounds (Jia et al., 2019; Anjum et al., 2019). As an antioxidant, Propolis may control blood glucose and modulate glucose and lipid metabolism, leading to decreased outputs of lipid peroxidation and scavenging of the free radicals in rats with diabetes (Sartori et al., 2019).

Propolis improves body weight profile in breast cancer models (Kusnul, 2019). In DM, body weight changes in relationship glycaemic control. If BGL is normal, body weight improves, too. In this study, Propolis can decrease blood glucose levels from hyperglycemia (both acute and chronic) and enhance the body weight loss condition significantly.

CONCLUSION

Propolis effectively reduced BGL and improved (increasing) BW in diabetic model rats. Propolis can improve the condition of diabetes status, so that Propolis can be an alternative therapy for DM. However, in animal models, several aspects that affect changes in BGL and BW, such as stress and physical activities, cannot be controlled.

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