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Original Research Article

Effect of Propolis Extract on Oxidative Stress Biomarker in Diabetic Wistar Rat (*Rattus novergicus*)

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ABSTRACT

Diabetes mellitus (DM), is a hyperglycemic condition, which increases oxidative stress, thereby leading to complications. One of the biomarkers of oxidative stress is malondialdehyde (MDA), whose levels can be used to predict oxidative stress levels. Propolis, an herbal medicinal plant extensively used to treat a variety of ailments, has been shown to lower oxidative stress. This study was conducted to determine the effect of Gunung Lawu propolis extract on MDA levels in a diabetic rat model. Ethanol extract of Gunung Lawu propolis was prepared and used in the treatment. Twenty-five (25) Wistar rats (*Rattus novergicus*) were randomly chosen and divided into five groups. The normal (healthy control; K1), DM (positive control; K2), and treatment groups, which consisted of DM rats that received different doses of propolis extract: P1 (100 mg/kg BW after 14 days of induction), P2 (200 mg/kg BW after 14 days of induction), and P3 (200 mg/kg BW immediately after induction). After 28 days, the rats were euthanized, and blood samples were collected and processed into the serum. The levels of MDA in the blood serum samples were measured. The results revealed that propolis significantly reduced fasting blood sugar ($p<0.01$) and MDA levels ($p<0.1$) in diabetic rats. Propolis administration did not show a significant difference ($p>0.01$) in reducing fasting blood sugar and MDA levels in early (P3) and advanced (P2) diabetes stages. The findings of this study revealed that propolis can lower MDA levels (an oxidative stress biomarker) in diabetic rats, thereby suggesting its potential in managing DM.

Keywords: Diabetes mellitus, MDA, Oxidative stress, Propolis.

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia as a result of abnormalities in insulin secretion, insulin action, or both.[1] Hyperglycemia in DM can lead to problems due to increased oxidative stress.[2] Oxidative stress occurs as a result of an imbalance between oxidants and antioxidants, resulting in an increase in reactive oxygen species (ROS). One of the biomarkers of oxidative stress is malondialdehyde (MDA), an end product of lipid peroxidation. MDA levels either in plasma or "tissue homogenates" can be used to predict levels of oxidative stress.[3] The amount of MDA concentration in the blood of a normal person is 0.45 µM/L, whereas if there is an increase in oxidative stress, the MDA level will increase. In patients with glaucoma due to DM, MDA levels increased to 0.97 µM/L.[3–6] Propolis, an herbal medicinal plant that is widely used as a treatment for several diseases, contains more than 300 chemical compounds. The most common compounds found in propolis samples are phenol compounds.[7] Caffeic acid phenethyl ester (CAPE) is a naturally occurring polyphenol compound ($C_{17}H_{16}O_4$), found in propolis, which has antioxidant properties.[8]

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The antioxidant mechanism of propolis is achieved by means of phenol compounds donating hydrogen ions to free radicals to protect cells from oxidation reactions. Propolis can protect DNA, lipid and protein damage from free radical compounds.[9]
Several studies on propolis in DM have been carried out. Propolis has been shown to have antihyperglycemic, antioxidant, and glomerular rate improving properties.[10–14] Furthermore, in streptozotocin-induced mice, propolis exerts a protective effect on pancreatic cells.[15] The composition of propolis varies depending on geographical conditions such as plant type, climate, environmental conditions and plant species.[16,17] The CAPE content in Gunung Lawu propolis extract was measured using the Prussion Blue method and found to be 30.24±3.53 x 10^{-6} g, while it was 12.40 ± 0.77 x 10^{-6} g in Sragen propolis and 17.00 ± 1.84 x 10^{-6} g in Wonogiri propolis.[17] However, there has not been any current research on how Gunung Lawu propolis affects DM. Therefore, the present study was aimed at determining the effect of Gunung Lawu propolis extract on MDA levels in diabetic rats.

Materials and Methods

Experimental design
This study was a laboratory experiment using a post-test control group design. It was conducted in the Experimental Animals Maintenance Unit at the UGM Inter-University Center (PAU), Yogyakarta, Indonesia. The research was carried out between August 2020 and April 2021. Purposive sampling was used to choose the sample. The independent variable was propolis, whereas the dependent variable was MDA content. Figure 1 depicts an outline of the experimental design.

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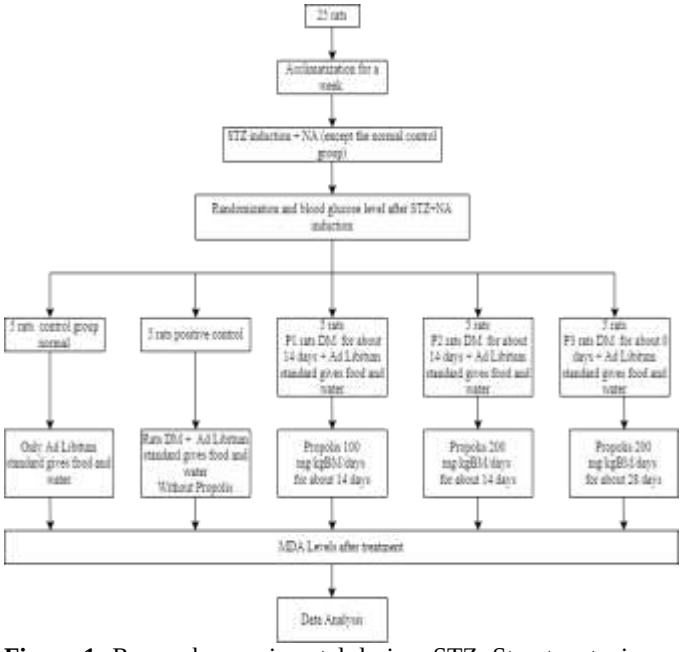

Figure 1: Research experimental design. STZ: Streptozotocin; NA: Nicotinamide

Ethical approval
Ethical approval was obtained from the Ethics Approval Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. An ethical approval ID No. KE/FK/0560/EC/2020 was issued on 11 May 2020.

Source of propolis
Propolis was obtained from the beekeepers in the sub-district of Kerjo, Karanganyar, Surakarta through the process of maceration and evaporation.

Source of experimental animals
The rats utilized in this investigation were 25 healthy male Wistar rat strains (*Rattus novergicus*), aged 8-10 weeks and weighing 200-250 grams. They were obtained from the experimental animal laboratory (Inter-University Center), Universitas Gadjah Mada, Yogyakarta.

Induction of diabetes in rat model
The rat was induced with DM using streptozotocin and nicotinamide (110 mg/kg BW) intraperitoneally. The streptozotocin was administered at a dose of 45-70 mg/kg BW, which was dissolved in 0.1M citrate buffer (2 ml/ 200 g at pH 4.5). Fasting blood sugar (FBS) level was measured three days later. If FBS > 250 mg/dl, the rats were classified as diabetic.[18–21]

Preparation of Gunung Lawu propolis extract
Maceration and evaporation procedures were used to prepare the Gunung Lawu propolis extract. At the maceration stage, up to 500 g of propolis were cleaned, dried, and blended before being placed in a beaker with 70% ethanol. The mixture was stirred twice daily with a stirring spatula and stored for 7 days. Then, it was filtered using filter paper and a Buchner funnel. At the evaporation stage, the filtrate was evaporated at 45^{o}C for 4 hours using a rotary evaporator under vacuum pressure (<1 atm) to generate a thick extract of 100 g. The extract was subsequently evaporated for 24 hours in a beaker to allow the ethanol to evaporate.

Experimental groupings and treatment
The Wistar rats were taken randomly and divided into 5 groups (based on Federer's formula) namely the normal group (healthy control; K1), DM group (positive control; K2) and three propolis treatment groups of P1 (14 days after diabetic induction + propolis extract [100 mg/kgBW/day] for 14 days), P2 (14 days after diabetic induction + propolis extract [200 mg/kgBW/day] for 14 days), and P3 (immediately after diabetic induction + propolis extract [200 mg/kgBW/day] propolis for 28 days).
Collection of blood samples
At the end of the experiments, all the rats were euthanized. Blood samples were obtained from the orbital vein and processed into serum in a tube. The tube holding all of the blood samples was placed on a tube rack and maintained at room temperature for about an hour until the blood clotted. The blood was frozen to avoid hemolysis and centrifuged at 3000 rpm for 10 minutes.

Measurement of malondialdehyde level
The amount of MDA in blood serum was estimated using the Thiobarbituris Acid (TBARS) method, which is a relatively simple and quick procedure. The standard solution used was 1,1,3,3-tetraethoxypropane. Thiobarbituric acid was added to the sample based on the color absorption produced by the reaction of TBA and MDA, which generated a red color, so that it could be analyzed using spectrophotometry (λmax = 532–535 nm).[22,23]

Statistical analysis
The Shapiro Wilk test was performed to determine whether the data were taken from a normally distributed sample. Numerical data (ratio scale), which included MDA levels were examined using the one-way analysis of variance (ANOVA) and post-hoc LSD test to determine the average difference between groups. The test results were considered significant if the p value was less than 0.05.

Results and Discussion

To investigate the effect of Gunung Lawu propolis extract on oxidative stress biomarker associated with DM, a diabetic rat model was created and administered with propolis extract. In this study, streptozotocin was administered intraperitoneally to induce DM in Wistar rats. Streptozotocin is a nitrosourea analogue, synthesized by *Streptomycetes achromogenes*. It is a source of free radicals that bind between N-methyl-N-nitrosorea and carbon-2 hexose, activating DNA alkylation and fragmentation. The drug functions by methylating cells, producing free radicals, and releasing nitric oxide. Its administration causes damage to pancreatic β cells, thereby resulting into hyperglycemia.[24,25] The use of solvents such as ethanol in the extraction of propolis has great impact on its biochemical composition and bioavailability.[26] Diabetes-induced rats achieved diabetic status (FBS levels > 250 mg/dl) in groups K2, P1, P2, and P3 and remained high after 14 days (Table 1). Fasting blood sugar levels in groups P1, P2, and P3 decreased significantly ($p<0.01$) following propolis extract administration after 14 days. There was a significant ($p<0.01$) difference in FBS levels between the study groups after 14 days of extract administration. The LSD test revealed no significant ($p>0.05$) difference between groups P2 and P3. As revealed by Table 2, administration of propolis extract in the early and later stages of DM had the same effect on FBS reduction. Hyperglycemia in diabetes causes an increase in oxidative stress. MDA levels are one of oxidative stress indicators. The results revealed that the data for each variable of MDA levels after 14 days of extract administration came from a normally distributed sample, as determined by the Shapiro-Wilk test. Table 3 shows that there were significant ($p<0.01$) differences in MDA levels at the 14th day after treatment among the study groups. In the P1, P2 and P3 treatment groups, MDA levels decreased more than the K2 group (positive control). The LSD test between P2 and P3 groups revealed no significant difference ($p>0.01$), indicating that administration of propolis with acid in the early and advanced stages of DM was effective in reducing MDA levels.
The administration of Gunung Lawu propolis extract to the treatment groups significantly ($p < 0.01$) lowered MDA levels (as a marker of oxidative stress) in diabetic rats. A reduction in MDA levels is associated with a reduction in oxidative stress.[27,28] Furthermore, a decrease in MDA levels has been linked to a decrease in FBS levels. The administration of propolis to reduce MDA levels in groups P2 (early stage of diabetes) and P3 (advanced stage of diabetes) showed no significant difference ($p>0.01$), indicating that propolis was equally

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beneficial in both early and advanced diabetic stages. Several investigations have shown that propolis from several countries such as Morocco, Iran, and Chihuahua has hypoglycemic activity.

Table 1: Fasting blood sugar levels (mg/dL) in diabetic-induced Wistar rats following administration of propolis extract after 28 days.

Exp. group	Fasting blood level (mg/dL)		
	H0	H14	H28
K1	73.164 ± 2.771	74.014 ± 2.979	75.070 ± 2.557
K2	258.182 ± 3.608	270.832 ± 4.089	272.072 ± 3.921
P1	259.128 ± 2.381	268.560 ± 5.768	115.284 ± 5.746
P2	258.184 ± 3.591	268.028 ± 5.199	98.358 ± 4.782
P3	260.872 ± 4.732	267.576 ± 5.671	87.360 ± 4.207

K1: Control group without propolis extract treatment; K2: DM group without propolis extract treatment; P1: DM group administered with 100 mg/kg BW propolis extract after 14 days of diabetic induction; P2: DM group administered with 200 mg/kg BW propolis extract after 14 days of diabetic induction; P3: DM group administered with 200 mg/kg BW propolis extract immediately after diabetic induction; H0: 3 days after streptozotocin/ nicotinamide-induced DM (diabetic status); H14: 14 days after diabetic status; H28: 14 days after administration of propolis extract; DM: Diabetes mellitus.

Table 2: Differences in average variable levels of fasting blood sugar (mg/dL), 14 days after administration of propolis extract.

Group	N	Mean ± SD	p
K1	5	75.070 ± 2.557	
K2	5	272.072 ± 3.921	
P1	5	115.284 ± 5.746	<0,001
P2	5	98.358 ± 4.782	
P3	5	87.360 ± 4.207	

K1: Control group without propolis extract treatment; K2: DM group without propolis extract treatment; P1: DM group administered with 100 mg/kg BW propolis extract after 14 days of diabetic induction; P2: DM group administered with 200 mg/kg BW propolis extract after 14 days of diabetic induction; P3: DM group administered with 200 mg/kg BW propolis extract immediately after diabetic induction.

Table 3: Differences in mean variable serum malondialdehyde levels (nmol/dL), 14 days after administration of propolis extract.

Group	N	Mean ± SD	p
K1	5	1.690 ± 0.265	
K2	5	9.684 ± 0.411	
P1	5	4.890 ± 0.522	<0,001
P2	5	3.476 ± 0.332	
P3	5	2.758 ± 0.254	

K1: Control group without propolis extract treatment; K2: DM group without propolis extract treatment; P1: DM group administered with 100 mg/kg BW propolis extract after 14 days of diabetic induction; P2: DM group administered with 200 mg/kg BW propolis extract after 14 days of diabetic induction; P3: DM group administered with 200 mg/kg BW propolis extract immediately after diabetic induction.

In this study, propolis from Indonesia was used, which has different levels of content than other countries that can also be hypoglycemic. Propolis, as an antioxidant, can control blood sugar levels in diabetic rats. It can inhibit glucose production by stimulating the release of insulin in pancreatic cells, preventing insulin resistance and increasing insulin receptor sensitivity. Propolis can also decrease the absorption of carbohydrates in the intestine, inhibit the activity of intestinal maltase, increase glycolysis and glucose uptake in peripheral tissues (such as skeletal muscle cells), through the activation of insulin-sensitive glucose transporters, and inhibit the release of glucose from the liver and protect pancreatic tissue.[12–15,27,29]

Antioxidants such as CAPE can be found in phenolic compounds present in propolis. In our investigation, the ethanol extract of Gunung Lawu propolis contains 30.24 ±3.53 x 10^{-6} g of CAPE phenolic components and 4.42 ±0.50 x 10^{-6} g of quercetin. Propolis' antioxidant mechanism involves phenol components providing hydrogen ions to free radicals to protect cells from peroxidation events. Propolis can preserve DNA, lipids, and proteins from free radical damage.[8,9,17] Several studies have found that propolis from Iran and Malaysia can lower MDA levels in diabetic rats. This is due to the antioxidant activity and hypoglycemic effect of propolis. Decreased glucose levels due to propolis administration can reduce oxidative stress, which is characterized by a decrease in MDA levels as the end result of lipid peroxidation and is used as a biomarker or predictor of oxidative stress.[3,27,28]

Conclusion

The findings of this study revealed that propolis can reduce MDA levels (an oxidative stress biomarker) in diabetic rats. Therefore, propolis has a lot of potential for controlling DM. Further research is required on the activity of propolis other than as an antioxidant (anti-inflammatory, antiproliferative, and anticancer) and its relationship to DM complications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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